Interactive comment on “Biotic and abiotic transformation of amino acids in cloud water: Experimental studies and atmospheric implications” by Saly Jaber et al.

Answers to reviewer #1

Referee Comment:

The authors present a very interesting work, measuring biotic and abiotic transformation rates of amino acids under cloud water conditions. The topic is very relevant, the approach is innovative and the results are promising. The manuscript is written in an understandable way and reads very well. Some improvements on the Figures are needed. This work is suitable for the journal; however, some comments and questions should be addressed.

Authors’ Response:

We thank the reviewer for the very positive evaluation of our work and their constructive comments. We address all points in detail below.

Referee Comment:

I have some questions and comments about the analytical method: A concentration of 1 µmol of each amino acid was applied for the experiments. How does this concentration compare to ambient amino acid concentrations? And, even more important: how are typical compositions of amino acids in the ambient atmosphere? Is a uniform concentration of 1µmol for each amino acid realistic? This might strongly influence the different degradation pathways. Please comment on that and I’d recommend to include such discussions in the manuscript.

Authors’ Response:

The amino acid (AA) concentrations and their ratios to each other in atmospheric waters (rain, clouds, aerosol water) are extremely variable from one sample to another (Bianco et al., 2016b; Mopper and Zika, 1987; Triesch et al., 2020; Xu et al., 2019; Yan et al., 2015). It does neither seem feasible nor necessary to perform experiments that consider all possible concentration ranges and ratios. Our brief review below shows that in general AA concentrations are present in micromolar concentrations in cloud water; their distribution likely depends on sources, processing, dilution etc. It should be also noted that not all cloud condensation nuclei contain amino acids, while cloud water concentrations are based on the analysis of bulk water samples. Thus, individual cloud droplets might be much more highly concentrated in amino acids than the bulk cloud water. However, since there are no analytical techniques to date that can routinely determine the solute concentrations in single cloud droplets, we can only take average cloud water concentrations as guidance from our experiments.

Given the multitude of AA sources and distributions and variety in cloud properties, an exactly uniform concentration distribution may not be encountered in any cloud water sample. Our assumption of a uniform distribution could possibly slightly impact the rates of biodegradation, but they should be on the same order of magnitude as we express the rates of biodegradation in mol cell^{-1} h^{-1}.

In rain, the total amino acid concentrations vary from 1.1 to 15.5 µM (Mopper and Zika, 1987), from 0.023 to 4.250 µM (Yan et al., 2015), from 1.1 to 10.1 µM (Xu et al., 2019), while in cloud water, it is between 2.7 to 3.1 µM (Bianco et al., 2016b). Considering that between 13 to 18 AA were measured in general, our total AA concentration in this experiment would be around 19 µM as we have included 19 AAs in the solution. This concentration is consistent with what was reported in rain samples, and about five times higher, i.e. less than an order of magnitude, than the concentrations measured in cloud water.

To take this factor of five into account we used an artificial cloud water whose composition was multiplied by 5 compared to what is observed in bulk cloud water samples (Vaïtilingom et al., 2011) and we also used a five-fold concentration for bacteria (Vaïtilingom et al., 2012). So we have respected the concentration ratio of chemical compounds [(main organic and inorganic ions + AA) / number of cells] present in cloud water. In the past we have shown that if the ratio is constant, the rate of biodegradation remains constant in the experiments (Vaïtilingom et al., 2010).

We will modify the Materials and Methods section as follows:

2.1 Experiments in microcosms

The experiments of biotic and abiotic transformation of amino acids were performed in microcosms mimicking cloud conditions at the puy de Dôme station (1465 m). Solar light was fitted to that measured directly under cloudy conditions and the temperature (17°C) was representative of the average temperature in the summer. Rhodococcus enclensis PDD-23b-28, Pseudomonas graminis PDD-13b-3, Pseudomonas syringae PDD-32b-74 and Sphingomonas sp.PDD-32b-11 bacterial strains were chosen because they belong to the most abundant and active bacterial genera in cloud water (Amato et al., 2017; Vaïtilingom et al., 2012). In addition, the complete genome sequences of Rhodococcus enclensis PDD-23b-28, Pseudomonas graminis PDD-13b-3, Pseudomonas syringae PDD-32b-74 have been published recently giving access to their metabolic pathways in more detail (Besaury et al., 2017a, 2017b; Lallement et al., 2017). ~~*Incubations were performed in an artificial cloud water medium containing inorganic ions, carboxylic acids and amino acids within the same range of concentrations as those measured in clouds that were impacted by marine air masses collected at the puy de Dôme station (Table S1, pH = 6.0) (Bianco et al., 2016a; Deguillaume et al., 2014).*~~ *In this work the total AA concentration used for the incubations was 19 µM as we have included 19 AAs at a concentration of 1µM each in the solution. This concentration is about five times higher than the concentrations measured in cloud water collected as the puy de Dôme station by Bianco et al. (2016a)(the total AA concentration varied from 2.7 to 3.1 µM). To take this factor of five into account we used an artificial cloud water whose composition in inorganic ions, carboxylic acids and amino acids was multiplied by 5 compared to what is observed in clouds (*(Vaïtilingom et al., 2011)*). We also used a 5X concentration for bacteria (~5×10^5 cells mL^{-1}) (Vaïtilingom et al., 2012). So we have respected the concentration ratio of chemical compounds [(main organic and inorganic ions*

+ AA) / number of cells] present in cloud water. In the past we have shown that is the ratio is constant, the rate of biodegradation is constant (Vaïtilingom et al., 2010).

All experiments were performed in triplicates.

Referee Comment:

Concerning the analytical method; the authors used ESI. It is known that ESI is prone the matrix effects (ion suppression) especially in ambient samples containing salt. Therefore, a sample preparation method is often applied, to eliminate disturbing matrix compounds. Did the authors test such effects, as ion suppression for the individual amino acids, for example by comparison of the external calibration to standard addition?

Authors' Response:

We agree that matrix effect can occur using ESI on environmental samples, in which the salt composition and concentration can be very variable. However, in our microcosms experiments, we have used an artificial cloud water medium with a very well-defined composition (Table S1) and we have used exactly the same artificial medium for our external calibration. It is clear from Figure S1 that the signal intensity depends linearly on the AA concentration. The calibrations are performed during the same runs as the experiment analyses. As the salt concentration was identical in the various samples, the matrix effect is the same in all samples. We checked that there is no bias as we can measure the concentrations of the AA at time zero and compare it with the added concentration as we know it (1 µM).

We shall modify this sentence in the Materials and Methods section 2.2.2:

In order to quantify the amino acid concentrations, calibration curves were established for each experimental series of LC-HRMS analyses using the same artificial cloud medium than in the incubations.

Referee Comment:

The LOQs seem quite high. How do they compare to other analytical methods used for amino acid analytics? It seems that the LOQ are close to the applied concentration of 1µmol, so did this cause problems in the analytical accuracy? How was the precision (e.g. standard deviation) of the analytical method? As the authors introduce the an-alytical method as a new approach and an improved technique, some further method validation would be necessary in my opinion. How about contaminations? Did you measure blanks and if so, were they considered?

Authors' Response:

In our opinion LOQs are not too high, because we measure concentrations at the beginning of the kinetic experiments of the transformation (initial rates of transformation) and during that period the measured concentrations are above the LOQ. In addition, our experiments are not designed to measure "absolute concentrations", but we measure slopes of $\ln(Ct/C_0) = f(t)$ as demonstrated in Figure S2. As seen in figure S2, the relationship of $\ln(Ct/C_0)$ vs time is very well described by a linear approximation. If the measurements were not sufficiently accurate, the data points would be much

more dispersed. If we compare with the literature in the field of atmospheric sciences, our LOQs are within the same order of magnitude to those described using LC-MS (see table below):

Amino acid	LOQ[a](nmol L^{-1})	LOQ[b] (µg L^{-1})
ALA	20	0.2
ARG	30	ND
ASN	8	ND
ASP	20	0.2
GLN	5	1.0
GLU	8	0.2
GLY	40	0.2
HIS	160	ND
ILE+LEU	10	1.0/1.0
LYS	130	ND
MET	8	1.0
PHE	4	1.0
PRO	5	0.2
SER	70	0.2
THR	13	1.0
TRP	8	1.0
TYR	7	ND
VAL	7	1.0
CYS	20	ND

a) LOQ determined by LC-MS (direct injection) after extraction of aerosol samples (Helin et al., 2017),b) LOQ determined by UPLC-HRMS (derivatization and concentration by 44 fold) of cloud samples (Triesch et al., 2020). ND: Not determined.

We will add the following text and Table S3 into Section 2.2.2:

The obtained values of LOD and LOQ were considered to be fit-for-purpose (Table S2) and are consistent with data from the literature ((Helin et al., 2017).

We also have calculated the Relative Standard Deviation (RSD = Standard deviation/mean) for each AA based on calibration curves (3 technical replicates). As you can see in the Table S3 these RSD are rather low, ranging from around 0.5% to 10%, except for Valine and Glycine where it can reach 20%. It can be noticed that these RSD due to the LC-MS method are much lower than those due to the transformation experiments, especially for biotransformation where there are biological variations (see error bars in Figure 1 and 2)

Table S3: Relative standard deviation (RSD = Standard deviation/mean) for each AA based on calibration curves (3 technical replicates).

	Relative Standard Deviation (RSD = Standard deviation/mean)		
Amino acid	0.1 µM (n = 3)	0.5 µM (n = 3)	1 µM (n = 3)
ALA		0.71%	3.61%
ARG	0.83%	1.96%	1.56%
ASN	5.23%	4.92%	3.63%
ASP		10.77%	5.96%

GLN	4.19%	4.37%	3.20%
GLU	3.77%	2.89%	3.92%
GLY			21.39%
HIS	0.62%	0.89%	1.22%
ILE	4.48%	0.48%	0.59%
LYS	6.64%	1.96%	1.50%
MET	4.49%	4.35%	6.38%
PHE	4.63%	1.68%	1.02%
PRO	11.67%	5.08%	1.28%
SER	14.34%	3.06%	3.20%
THR	14.15%	3.67%	1.06%
TRP	7.00%	1.67%	1.75%
TYR	0.94%	1.81%	1.15%
VAL	17.94%	2.98%	11.41%

Of course, blanks were made for each series of runs. They consisted of using the artificial cloud medium without AAs. No signals corresponding to AA are detected under these conditions.

As we have introduced this new Table S3, the previous Tables S3 and S4 will be renamed Tables S4 and S5

Table S4*: Rate constants for 18 amino acids for the OH, O_3 and $^1O_2$ reactions*

Table S5: *Selected experimental studies of amino acid oxidation by various oxidants. Note that the experimental conditions were not necessarily atmospherically-relevant. Products are only listed to demonstrate the wide variety of possible reaction pathways and products.*

Referee Comment:

Finally; would your analytical method (without pre-concentration and sample preparation) be applicable for measuring amino acids in ambient marine samples?

Authors' Response:

Using this method for marine samples may cause some problems due to the much higher salt concentrations (~0.1 M) and the lower AA concentration than encountered in cloud water where these concentrations are in the range of milli- to micromolar, respectively. In addition, to prevent matrix effects, we recommend to use the addition of a standard method for calibration and not an external calibration.

Referee Comment:

Chapter 2.1.: The authors explained that the strains were chosen because they are the most abundant and active bacteria in cloud water. Are there more information on these strains available, that might be used to explain their different behaviour towards the individual amino acids?

Authors' Response:

We have used these strains in many previous studies to explore their biodegradation of a variety of organic compounds, e.g. small carboxylic acids (Vaïtilingom et al., 2010,2011) or phenol (Jaber et al., 2020). We could not observe such high difference between the biological activities of these strains towards these organics. Thus, the different behavior of the different strains towards AAs cannot be explained despite the fact that they belong to different genera (Figure S5).

Referee Comment:

Chapter 3.1.1: Interestingly, the efficiencies of the different strains are very variable among each other and concerning the different amino acids. The authors mentioned that all amino acids were mixed together in the experiment. I was wondering if you also performed these experiments with single amino acids? This might be interesting especially regarding the net production of GLY that is certainly a product from the degradation of other amino acids.

Authors' Response:

We chose to perform the experiments with this mixture of AA in a medium mimicking the cloud medium to be as close of possible to realistic atmospheric conditions. Working with single AA could be interesting but very time consuming and would not reflect real cloud conditions.

Referee Comment:

Chapter 3.1.2.: The manuscript often refers to the Figures S3 and S4 which seem to be crucial for following and understanding the text. As the manuscript does not contain many Figures, maybe transfer them to the main part? An alternative could be to highlight the amino acids that have the same metabolic pathway in Figure 1(instead of Figure S4). The statement that the "blue box" amino acids exhibit the same behaviour regarding their biodegradation is difficult to see in Figure S4 and a "zoom in" would be required. Actually, it seems that GLY shows quite a different behavior, not in line with the other "blue box" amino acids. Also the "green box" amino acids are difficult to see (Fig. S4). For the "purple box" amino acids; the mentioned strong similarities are not obvious from Fig. S4. The 23b-28 strain seems to be much stronger for ASN compared to ALA. Please re-think the way of showing the similarities and maybe find a clearer way to present similarities and differences for the metabolomic-groups amino acids and their response to the different strains.

Authors' Response:

We agree with the referee that it is not easy to look at the different figures in the main text and in the SI. Actually we really thought at the various possibilities and decided that the one we chose was the clearest. We prefer to keep the main manuscript concise and only show the essential results and only provide additional details in the SI.

Referee Comment:

Chapter 3.1.3 Are there any more detailed explanation theories why these different strains exhibit such different behaviours? To what properties could that be related? On page 9, line 24 the authors mention that the AA biodegradation could be linked to the phylogeny of the bacterial strains. Could you give some more explanation (to non-biologists) about this?

Authors' Response:

As explained in the text, metabolic pathways are rather similar for all the living organisms, however the metabolic fluxes (i.e. rates of transformation of metabolites by each enzyme) can be modulated by the environmental conditions and the type of organisms (namely their phylogeny). In our experiments, the environmental conditions are the same for all the four studied strains, so the observed differences are only due to their phylogeny. We can see in Figure S3 that the two *Pseudomonas* strains (closely related from a phylogenetic point of view as they belong to the same genus "*Pseudomonas*", and same class "*γ-Proteobacteria* ") have a closer behavior that the other strains. However we are not able to understand what is the direct connection between the phylogeny and the biological activity towards AA, and thus we are not able to predict the activity of a strain looking at its phylogeny.

To make clearer the notion of phylogeny we propose to add this text in the SI under Figure S5

An example of phylogenetic classification is given bellow

Phylum-- →Class →Genus →species →strain number

Proteobacteria →γ-Proteobacteria →Pseudomonas →graminis → PDD-13b-3

Referee Comment:

Chapter 3.3.1: I wonder how relevant singlet oxygen is for diluted systems. (lifetime?) Is the sink for singlet oxygen considered in the rates (Fig 3)?

Authors' Response:

There are several studies that have reported a steady state singlet oxygen concentration in fog and cloud waters on the order of 10^{-14} – 10^{-12} M (Faust and Allen, 1992; Kaur and Anastasio, 2017), similar to concentrations found in surface water (Faust and Allen, 1992). This is about two orders of magnitude higher than steady-state concentrations OH radical in the atmospheric aqueous phases. OH is considered the main oxidant in the atmospheric multiphase (gas + aqueous) system because of its high reactivity towards many organic and inorganic compounds. The lifetime of singlet oxygen is longer than that of the OH radical in water as it is more selective towards reactants (Kaur and Anastasio, 2017).

Given the high rates of production and loss processes of the radical species (OH and $^1O_2$) that result in stable steady-state concentrations, we only considered these concentrations to estimate the loss rates of the amino acids. This approach implies (pseudo) first order kinetics as it has been used in

many previous studies that estimated the chemical lifetime of various compounds in the atmosphere (and other media), e.g (McGregor and Anastasio, 2001; Triesch et al., 2020), or more general in standard atmospheric chemistry books (Seinfeld and Pandis, 2006). As explained in the text already, we refrained from presenting our results in terms of lifetimes as production rates would result in negative values which are clearly meaningless.

Figure 2 shows that degradation and formation happens for the individual amino acids. As a general question and also related to Fig. 2: Can any mechanisms for the formation/degradation of the individual amino acids be derived from that?

Authors' Response:

We cannot give any additional reliable information on the mechanism of the amino acid decay and formation as currently there is no sufficient mechanistic information available. The studies summarized in Table S4 give some hints that the oxidation of amino acids can possibly lead to the formation of other amino acids. However, since these studies were neither performed under conditions similar to those in our experiments (and thus to those as relevant for cloud water), nor were any yields or branching ratios reported, any conclusions on the transformation of AAs would be speculative. We hope that our study motivates laboratory experiments in the future that investigate in detail the mechanisms, yields, branching ratios and time scales of such conversions so that ultimately a figure as the 'chemical equivalent' to Figure S3 could be created, i.e. with the chemical instead of metabolic routes included.

Chapter 3.3.2 and Figure 4: This chapter deals with the comparison of the biotic and the abiotic pathway. They are shown in Fig.3. While some exemplary comparisons areC3made between both pathways (Page 11, Line 30 - Page 12 Line 6) I miss some real conclusions here. In addition, Figure 3 is difficult to understand and not well discussed; some more details might be helpful to understand the outcome of Fig. 3.

Authors' Response:

We assume that the referee's comment refers only to Figure 3 here as there is no Figure 4 in the original manuscript. We agree with the referee that the description and discussion of Figure 3 was rather short. We will modify Section 3.3.2 as follows:

- We add an index 'bio' to the left-hand term in Equation 2 so it reads

$$\left(\frac{d[AA]}{dt}\right)_{bio} = -0.063\, R_{23b28} \cdot 1.91 - 0.162\, R_{32b11} \cdot 1.91 - \frac{29.8}{2} R_{23b28b} \cdot 1.91 - \frac{29.8}{2} R_{13b2} \cdot 1.91$$

- We modify the last paragraph in Section 3.3.2 (new text in green):

The three rates, i.e. the biodegradation (Eq.-2) and photochemical (Eq.-3) rates as derived from the experiments, and the kinetic loss rates based on chemical kinetics (Eq.-4), respectively, are compared in Figure 3 for teach of the 18 amino acids. For some of the acids (ALA, GLU, THR) the predicted losses by OH from both approaches (photochemical experiments (red dashed bars) and based on OH kinetic data (solid dark red bars)) are similar. Thus, we can conclude that these acids are oxidized to products other than amino acids and that the approximation of their loss rates by Equation 4 is justified, as it has been done in previous studies, e.g. (McGregor and Anastasio, 2001; Triesch et al., 2020). *For*

several other amino acids (e.g. ARG, GLN, LYS, SER, and THR) there is a large discrepancy in the observed trends of the predicted chemical loss rates and the ones observed in the photochemical experiments. The latter ones have positive values, i.e. they indicate a net production rather than a net loss. While we cannot conclude on the exact conversion and formation mechanisms of these acids based on our experiments, it is evident that the assumption of a net loss underestimates the lifetime of these acids as they do not only have chemical sinks but also sources in the atmospheric aqueous phase. As also reflected in Figure 1, such net production is only seen for ASP and GLY for biotic processes.

~~*The comparison between the rates calculated by Equations 2 - 4 is shown in Figure 3.*~~ *The comparison of the predicted role of the three oxidants in cloud water (OH, O_3, $^1O_2$) reveals for some AAs, the oxidation by ozone might contribute significantly more to their loss than the other two oxidants (light red bars; note the logarithmic scale, i.e. the contributions of the ozone reactions to the total predicted loss exceeds those by other oxidants by far).*

For several of the acids (e.g. ALA, ASN, GLU, PRO, VAL), biotransformation is predicted to exceed the loss by chemical reactions ~~*(e.g. ALA, ASN, GLU, PRO, VAL)*~~*, for the bacteria cell and oxidant concentrations considered here. Given that the ratios of bacteria cells/radicals in our estimate here are similar to those as encountered in cloud water, it may be concluded that both types of pathways might compete in the atmosphere. Similar conclusions were qualitatively drawn based on ambient measurement in a recent study* (Zhu et al., 2020)*. However, the exact contributions of biotic and abiotic pathways to the loss and conversion of amino acids will depend on the cell concentrations of the different bacteria strains, their distribution among cloud droplets, and oxidant levels.*

~~*Note that the loss rates calculated by Equation 4 cannot reproduce the observed production of the various acids as observed in the experiments with the mixture of all amino acids.*~~

Chapter 4: The conclusions are well written. The authors summarize that the so far only degradation (losses) of amino acids but not production (transformation into each other) was considered. However, I was struggling with the following sentence: “Our study qualitatively suggests that the sources and distribution of amino acids in the atmospheric particle and aqueous phases can be modified by metabolic and chemical transformation pathways.” -> Could the authors derive more precise conclusions here? I understood it was the aim to show HOW the two pathways (biotic, abiotic) contribute. I was wondering if the authors could finally comment on the relative importance of the biotic and the abiotic pathway e.g. which seems to be the more important way?

Authors’ Response:

We thank the referee for this comment. Our study is the first one to suggest based on lab studies the formation and conversion of amino acids by not only biotic but also by chemical processes. Overall, we can conclude that both types of processes might be similarly important for many of the amino acids as shown in Figure 3 under atmospheric conditions. The exact rates will depend on the distribution of the radicals and bacteria cells throughout the cloud droplet population. Based on the analysis of cloud water samples (Vaïtilingom et al., 2013) and recent model studies (Khaled et al., 2020), it can be hypothesized that the low fraction of cloud droplets that contain bacteria cells might translate into very non-linear overall loss rates of non-volatile compounds (such as amino acids).

However, given the large variability in the atmosphere of cloud properties, bacteria diversity and cell concentrations, oxidant concentrations (e.g. depending on air mass characteristics, photochemical activity etc) and amino acid sources and distributions (cf e.g. references cited in the introduction of our manuscript), we cannot perform a global estimate of the relative importance of biotic versus abiotic amino acid processes.

Minor referee comments:

- There are several typos e.g. page 2 line 11 (C.L-1), sometimes the chemicals / amino acids are written with capital letter, sometimes with small letters (e.g.Table S4).

Authors' Response: We removed the '.' After the C in the unit on page 2 and everywhere else in the manuscript. We also corrected the chemical names in Table S4 (now Table S5) for consistent upper and lower case use.

- Empty spaces are missing and the formulas in eq. 2-4 are not represented right.

Authors' Response: We will fix the formatting of the equations and will make sure that are correct in the uploaded pdf files.

- In addition, the reference style needs revisions (e.g. page 16, line 44-45, page 17, line11, page 19, line 25.

Authors' Response: We Carefully went through the reference list and corrected all references where needed.

- Table S3: There are missing references (for GLU, GLY, SER...).

Authors' Response: We added the missing references

- At what temperature was the rate constant obtained?

Authors' Response: Whenever possible we chose rate constants at or near room temperature. We will add this information to the table caption.

- Concerning the Data availability I'd strongly recommend to upload the data in a public database such as PANGAEA or similar.

Authors' Response: Data are available upon request

- Author contributions: I was surprised that "SJ", as the first author, did not "write the manuscript"?

Authors' Response: In our team, the first author is the one who made the largest contribution to the work, here it is considered for the experimental work which is very demanding. She also read and corrected the manuscript (as noticed in the text).

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Changes in the revised manuscript:

P3 line 3

P4 lines 4-10

P5 lines 2-16

P6 line 31

P7 lines 25-26

P8 lines 3-10

P8 lines 25-28

P9 line10

P10 line 29

P13 lines 1-32

P14 line 2

Changes in the Supplementary Material

P S4

P S5

P S13

P S14

P S15

Biotic and abiotic transformation of amino acids in cloud water: Experimental studies and atmospheric implications.

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Abstract

The interest for organic nitrogen and particularly for quantifying and studying the fate of amino acids (AA) has been growing in the atmospheric science community. However very little is known about biotic and abiotic transformation mechanisms of amino acids in clouds.

In this work, we measured the biotransformation rates of 18 amino acids with four bacterial strains (*Pseudomonas graminis* PDD-13b-3, *Rhodococcus enclensis* PDD-23b-28, *Sphingomonas* PDD-32b-11 and *Pseudomonas syringae* PDD-32b-74) isolated from cloud water and representative of this environment. At the same time, we also determined the abiotic (chemical, OH radical) transformation rates within the same solutions mimicking the composition of cloud water. We used a new approach by UPLC-HRMS to quantify free AA directly in the artificial cloud water medium without concentration and derivatization.

The experimentally-derived transformation rates were used to compare their relative importance under atmospheric conditions and compared to the chemical loss rates based on kinetic data of amino acid oxidation in the aqueous phase. This analysis shows that previous estimates overestimated the abiotic degradation rates, and thus underestimated the lifetime of amino acids in the atmosphere as they only considered loss processes but did not take into account the potential transformation of amino acids into each other.

1. Introduction

The organic matter (OM) content of the cloud water phase is very complex; it has been described using Fourier-Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR MS) (Bianco et al., 2018; Zhao et al., 2013) . These global analytical methods revealed a very large number of organic carbon, organic sulfur and organic nitrogen compounds. For instance, in cloud water at the puy de Dôme, 5258 monoisotopic molecular formulas were assigned to

CHO, CHNO, CHSO, and CHNSO (Bianco et al., 2018). Organic nitrogen compounds contribute a significant fraction to the total nitrogen in cloud water (18%) (Hill et al., 2007) and in aerosol particles 7 – 10% in urban areas (Xu et al., 2017) or even exceed other nitrogen contributions in marine aerosol (Miyazaki et al., 2011). Among these organic nitrogen molecules, amino acids (AA) have been recently analyzed and quantified in cloud droplets collected at the puy de Dôme station and on the Cape Verde Islands (Triesch et al., 2020). AA were also quantified in rain collected in marine and sub-urban sites (Mace et al., 2003b, 2003a; Mopper and Zika, 1987; Sidle, 1967; Xu et al., 2019; Yan et al., 2015), and in fog samples in Northern California (Zhang and Anastasio, 2003). In cloud water, free AA concentrations range from 2.4±2.0 to 74.3±43.8 µg C. L^{-1} at the rural site of the puy de Dôme (Bianco et al., 2016a) and from 17 to 757 µg C. L^{-1} at the marine site of Cape Verde (Triesch et al., 2020). These AA are from biological origin and are building blocks of peptides (also called 'combined AA') and proteins. They are initially present in aerosols which are further dissolved in atmospheric waters (Matos et al., 2016). Primary and secondary atmospheric sources of AAs are discussed in previous reviews (Cape et al., 2011; Sutton et al., 2011). Biomass burning (Zhu et al., 2020b), grassland (Scheller, 2001), ocean (Triesch et al., 2020) and agricultural activities (Song et al., 2017) were identified as major emission sources of amino acids.

Although organic carbon has been studied for a long time by atmospheric scientists, the interest for organic nitrogen and particularly for quantifying and studying the fate of AAs has been growing these last decades due to their specific properties. Some AA can act as ice nuclei, for instance L-leucine nucleates ice at -4.5°C (Szyrmer and Zawadzki, 1997). Their mass can also add to the hygroscopic fraction of cloud condensation nuclei due to their high water-solubility (Kristensson et al., 2010). Another point concerns the participation of AA in the global nitrogen and carbon cycles. For example, it has been estimated that organo-nitrogen compounds are a significant fraction (28%) of the total nitrogen deposited (Zhang et al., 2012). Their ubiquity in living organisms makes their presence in atmospheric deposition very important for both terrestrial and aquatic ecosystems as AA represent the most bioavailable form of nitrogen (Cornell, 2011).

Finally, as part of the atmospheric OM, AA are expected to undergo chemical processes in the atmospheric water phase (clouds, fog, aerosol). Due to their low volatility, it can be assumed that they are not present in the gas phase. However little is known on their transformation processes occurring in the atmospheric compartments, and particularly in clouds.

Concerning abiotic transformation (phototransformation and radical chemistry) in atmospheric waters, some studies determined kinetic rate constants (k) of AAs with radicals (e.g. OH) (Scholes et al., 1965;(Motohashi and Saito, 1993; Prütz and Vogel, 1976; Reasoner and Geldreich, 1985) , singlet oxygen ($^1O_2$) (Kraljić and Sharpatyi, 1978; Matheson and Lee, 1979; McGregor and Anastasio, 2001; Michaeli and Feitelson, 1994; Miskoski and García, 1993); (McGregor and Anastasio, 2001) or ozone (O_3) (Ignatenko and Cherenkevich, 1985) Pryor et al., 1984). Based on such kinetic data, some studies have reported the time of life of amino acids in fog (McGregor and Anastasio, 2001) or in cloud water (Triesch et al., 2020). From these studies it is clear that some amino acids are transformed very rapidly, while others are almost never transformed within the time scale of fog or cloud life. When additional effect of $^1O_2$ was considered, MET, TRP, TYR and HIS remained the most degraded AA (McGregor and Anastasio, 2001). Among other mechanisms, this fast degradation could explain why these AA are usually among the less concentrated in aerosols (Barbaro et al., 2015; Matsumoto and Uematsu, 2005; Barbaro et al., 2011; Helin et al., 2017; Mashayekhy Rad et al., 2019; Mace et al., 2003b; Samy et al., 2013; Yang et al., 2004), in rain (Mace et al., 2003b; Xu et al., 2019; Yan et al., 2015) or in clouds (Triesch et al., 2020). The characterization of amino acids in dew showed differences depending on seasons, meteorological parameters and irradiation conditions (Scheller, 2001).

Even less is known about the abiotic transformation pathways of these amino acids, as only some AA have been studied in detail. Most mechanistic studies are limited to the transformation of AA (GLY, TRP, ASP, SER) into small carboxylic acids such as acetic, oxalic, malonic or formic acids (Berger et al., 1999; Bianco et al., 2016b; Marion et al., 2018). In some cases, an amino acid can be converted into another one or into very different molecules (Bianco et al., 2016b; Mudd et al., 1969; Prasse et al., 2018; Stadtman, 1993; Stadtman and Levine, 2004). The main concern with these mechanistic studies, is that they were performed under conditions rather far from atmospheric conditions. Incubation media did not contain a mixture of AA or real atmospheric samples. More they were sometimes measured with proteins in which the peptidyl bond might change the reactivity compared to free AA (Pattison et al., 2012).

Another missing aspect concerns the potential biotransformation of these AAs in atmospheric waters. The microbial community which is present in cloud waters is metabolically active (Amato et al., 2017, 2019; Vaïtilingom et al., 2012) and has been shown ~~the~~ to biotransform mono and dicarboxylic acids, methanol, formaldehyde, phenol and catechol (Ariya et al., 2002; Husárová et al., 2011; Jaber et al., 2020a; Vaïtilingom et al., 2010a, 2011, 2012). It is

well-known that microorganisms have enzymatic networks able to biodegrade or biosynthesize amino acids. These pathways are complex and very interconnected (KEGG pathway database, n.d.). In cloud water, the biodegradation and biosynthesis of AAs is suspected to occur as i) it was shown that bacteria can use AA as substrates in incubations with real cloud water containing endogenous bacteria and AAs because they can produce proteins and other cellular component allowing their growth in this medium (Amato et al., 2007), iii) a recent metatranscriptomic study performed directly in cloud water, showed the presence of transcripts of genes coding for AA biodegradation and synthesis (Amato et al., 2019). This is a proof of *in situ* activity of bacteria in clouds. However, no data exist about the biotransformation rates ~~and metabolic pathways~~ of AAs in cloud water.

The aim of the present study is thus to measure biotic and abiotic rates of transformation of free AA in microcosms mimicking cloud water with an incubation medium containing 19 AA, other major carbon (acetate, succinate, formate, oxalate) and nitrogen sources (NH_4^+, NO_3^-) as well as major salts (e.g., Na^+, Cl^-, SO_4^{2-}) present in cloud water collected at the puy de Dôme station (Deguillaume et al., 2014). In addition, abiotic transformation rates are calculated based on rate constants of oxidation reactions with OH, $^1O_2$ and O_3 as reported in the literature. These experimental and theoretical rates of transformation are compared with each other and to previous literature studies and are discussed in terms of their atmospheric implications.

2. Materials and Methods

2.1 Experiments in microcosms

The experiments of biotic and abiotic transformation of amino acids were performed in microcosms mimicking cloud conditions at the puy de Dôme station (1465 m). Solar light was fitted to that measured directly under cloudy conditions and the temperature (17°C) was representative of the average temperature in the summer. Incubations were performed in an artificial cloud water medium containing inorganic ions, carboxylic acids and amino acids within the same range of concentrations as those measured in clouds that were impacted by marine air masses collected at the puy de Dôme station (Table S1, pH = 6.0) (Bianco et al., 2016a; Deguillaume et al., 2014). *Rhodococcus enclensis* PDD-23b-28, *Pseudomonas graminis* PDD-13b-3, *Pseudomonas syringae* PDD-32b-74 and *Sphingomonas* sp.PDD-32b-11 bacterial strains were chosen because they belong to the most abundant and active bacterial genera in cloud water (Amato et al., 2017; Vaïtilingom et al., 2012). In addition, the complete genome sequences of *Rhodococcus enclensis* PDD-23b-28, *Pseudomonas graminis* PDD-13b-

3, *Pseudomonas syringae* PDD-32b-74 have been published recently giving access to their metabolic pathways in more detail (Besaury et al., 2017b, 2017a; Lallement et al., 2017). ~~Incubations were performed in an artificial cloud water medium containing inorganic ions, carboxylic acids and amino acids within the same range of concentrations as those measured in clouds that were impacted by marine air masses collected at the puy de Dôme station (Table S1, pH = 6.0).~~ In this work the total AA concentration used for the incubations was 19 µM as we have included 19 AAs at a concentration of 1 µM each in the solution. This concentration is about five times higher than the concentrations measured in cloud water collected as the puy de Dôme station by Bianco et al. (2016a)(the total AA concentration varied from 2.7 to 3.1 µM). To take this factor of five into account we used an artificial cloud water whose composition in inorganic ions, carboxylic acids and amino acids was multiplied by 5 compared to what is observed in clouds ((Vaïtilingom et al., 2011)). We also used a 5X concentration for bacteria (~5×10^5 cells mL^{-1}) (Vaïtilingom et al., 2012). So we have respected the concentration ratio of chemical compounds [(main organic and inorganic ions + AA) / number of cells] present in cloud water. In the past we have shown that is the ratio is constant, the rate of biodegradation is constant (Vaïtilingom et al., 2010b). -All experiments were performed in triplicates.

2.1.1 Cell preparation for further incubations

Rhodococcus enclensis PDD-23b-28, *Pseudomonas graminis* PDD-13b-3, *Pseudomonas syringae* PDD-32b-74 and *Sphingomonas* sp. PDD-32b-11 were grown in 10 mL of R2A medium for 16 h at 17°C, 130 rpm (Reasoner and Geldreich, 1985). Then 1 mL of cultures were centrifuged at 12500 rpm for 3 min. Bacteria pellets were rinsed two times with 1 mL of artificial marine cloud water, previously sterilized by filtration under sterile conditions using a 0.22 µm PES filter. The bacterial cell concentration was estimated by optical density at 600 nm using a spectrophotometer UV3100 to obtain a concentration close to 5×10^5 cell mL^{-1}. Finally, the concentration of cells was precisely determined by counting the colonies on R2A Petri dishes or by flow cytometry technique.

2.1.2 Biotransformation of amino acids

Rhodococcus enclensis PDD-23b-28, *Pseudomonas graminis* PDD-13b-3, *Pseudomonas syringae* PDD-32b-74 and *Sphingomonas* sp. PDD-32b-11 cells were each resuspended in a 50 mL flask of 1 µM amino acids (19 amino acids namely alanine (ALA, SIGMA), arginine (ARG SIMAFEX), asparagine (ASN, SIGMA), aspartate (ASP, Aldrich-Chemie), glutamine (GLN, SIGMA), glutamic acid (GLU), glycine (GLY, MERCK), histidine (HIS, SIGMA),

isoleucine(ILE SIGMA-ALDRICH,), lysine(LYS, SIGMA-ALDRICH), methionine (MET, SIGMA), phenylalanine (PHE, ACROS organics), proline (PRO, SIGMA-ALDRICH), serine (SER, SIGMA,), threonine (THR, SIGMA), tryptophan (TRP, SIGMA), tyrosine (TYR, SIGMA-ALDRICH), valine (VAL, SIGMA-ALDRICH), cysteine (CYS, SIGMA-ALDRICH)/ 1 µM of each amino acid), prepared in artificial cloud water (Table S1) and incubated at 17 ° C,130 rpm agitation for 7 hours in the dark. A control experiment was performed by incubating amino acids without bacteria; AA concentration remained stable over time (1 µM for each amino acid was obtained at the end of the experiment).

2.1.3 Abiotic transformation of amino acids

The same 19 amino acids, at a concentration of 1 µM each in the artificial cloud medium (Table S1) were incubated at 17°C, 130 rpm agitation for 7 hours in photo-bioreactors designed by (Vaïtilingom et al., 2011).OH radicals were generated by photolysis adding 0.5 mM Fe-Ethylenediamine-*N*,*N*′-disuccinic acid (EDDS) complex solution. The Fe(EDDS) solution (iron complex with 1:1 stoichiometry) was prepared from iron(III) chloride hexahydrate ($FeCl_3$, $6H_2O$; Sigma-Aldrich) and (S,S)-ethylenediamine-N,N'-disuccinic acid trisodium salt (EDDS, 35% in water). A complementary experiment was also performed consisting of incubation of this solution in the presence of light without Fe(EDDS) complex. The experimental conditions of the irradiation experiments (Sylvania Reptistar lamps; 15 W; 6500 K) and the mechanism of the $^{\bullet}OH$ radical production under light irradiation are described by (Jaber et al., 2020b). Assuming steady-state conditions for $^{\bullet}OH$ at the beginning of the experiments (i.e., equal $^{\bullet}OH$ production and loss rates), an $^{\bullet}OH$ concentration of $8.3 \cdot 10^{-13}$ M was calculated as described by (Jaber et al., 2020b). This concentration is at the upper limit of $^{\bullet}OH$ concentrations in cloud water as derived from various model studies (Arakaki et al., 2013; Lallement et al., 2018).

2.2 Analytical methods

2.2.1 Amino acid UPLC-HRMS Analyses

During the experiments in microcosms, 600 µL of the incubation medium were sampled regularly and centrifuged at 10 500 x g for 3 min and the supernatants were kept frozen until analyses. In order to quantify the amino acid concentrations in the incubations we developed here a new approach using a LC-HRMS technique based on a direct measurement by injection of the incubation medium without derivatization. The volume of injection was 5µL.

All AAs could be quantified under these conditions, except cysteine.

LC-HRMS analyzes of amino acids were performed using an UltiMate™ 3000 (Thermo Scientific™) UHPLC equipped with a Q Exactive™ Hybrid Quadrupole-Orbitrap™ Mass Spectrometer (Thermo Scientific™) ionization chamber. Chromatographic separation of the analytes was performed on BEH Amide/HILIC (1.7 µm, 100 mm x 2.1 mm) column with column temperature of 30°C. The mobile phases consisted of 0.1% formic acid and water (A) and 0.1% formic acid and acetonitrile (B) with a flow rate 0.4 mL min^{-1}. A four-step linear gradient of 10% A and 90% B in 8 min, 42% A and 58% B in 0.1 min, 50% A and 50% B for 0.9 min, 10% A and 90% B for 3 min was used throughout the analysis.

The Q Exactive ion source was composed of an electrospray ionization (ESI+) and the Q-Orbitrap™. Flow injection analyses were performed for individual amino acid solutions in order to obtain the mass spectra, from which ions were selected using the SIM (Selected Ion Monitoring) mode. The instrument was set for maximum ion throughput, the automatic gain control target or the number of ions to fill C-Trap was set to 10^5 for a maximum injection time of 100 ms. Gas (N_2) flow rate and sheath gas (N_2) flow rate were set at 13 a.u. and 50 a.u. respectively. Other parameters were as follows: 2 a.u for the sweep gas flow rate, 3.2 kV for the spray voltage in positive mode, 320°C and 425°C for the capillary temperature and the heater temperature, respectively. Under these conditions the mass resolution was 35000 fwhm. Analysis and visualization of the mass data were performed using Xcalibur™ 2.2 software (Thermo Scientific™).

Table S2 presents the retention times and values of m/z for the ions [M+H] measured under these conditions for each amino acid.

2.2.2 Calibration curves, LOD and LOQ determination

In order to quantify the amino acid concentrations, calibration curves were established for each experimental series of LC-HRMS using the same artificial cloud medium than in the incubations.

In standard solutions, six concentrations of amino acids (0.01, 0.05, 0.1, 0.5, 1.0, 5.0 µM) were used for these external standard multipoint calibrations. This range of concentrations is appropriate considering that the initial amino acid concentration in the biotic and abiotic transformation experiments is 1µM. Figure S1 presents an example of calibration curves for the 18 amino acids. The limits of detection (LOD) and quantification (LOQ) were calculated based on the standard deviation of the response (Sa) and on the slope of the calibration curves (b) (technical triplicate).

LOD=3Sa/b µM

LOQ=6Sa/b µM

The obtained values of LOD and LOQ were considered to be fit-for-purpose (Table S2) and are consistent with data from the literature ((Helin et al., 2017).

We also have calculated the Relative Standard Deviation (RSD = Standard deviation/mean) for each AA based on calibration curves (3 technical replicates). As you can see in the Table S3 these RSD are rather low, ranging from around 0.5% to 10%, except for Valine and Glycine where it can reach 20%. It can be noticed that these RSD due to the LC-MS method are much lower than those due to the transformation experiments, especially for biotransformation where there are biological variations (see error bars in Figure 1 and 2)

.

2.2.3 Calculation of amino acids degradation rates in microcosms

The degradation rates of amino acids were calculated after normalization based on the ratio of the concentration at time t (Ct) and the concentration at time t = 0 (C_0). The pseudo-first-order rate constants ($k_{isoleucine}$, k_{valine} $k_{proline}$…) were determined using Equation 1:

$$Ln(Ct/C_0) = f(t) = - k_{amino\ acid}\ t \qquad [Eq\text{-}1]$$

The slopes at the origin were used to calculate the corresponding degradation rates. For biotransformation, the rates were corrected by the precise number of bacterial cells present in the incubations and are expressed in the form of mol $cell^{-1}$ h^{-1}. An example is given in Figure S2a and b for the case of the biodegradation of GLN.

3. Results and discussion

3.1 Biotransformation of amino acids in microcosms

3.1.1 Biotransformation rates of the 18 amino acids by the different bacterial strains

The biotransformation of the amino acids ~~alanine, arginine, asparagine, aspartate, glutamic acid, glycine, histidine, isoleucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine and glutamine~~ by four different bacterial strains isolated from cloud water at the puy de Dôme station in a marine artificial cloud medium was monitored in four independent microcosms containing only one of the strains. Figure 1 shows the results obtained for each amino acid and each bacterial strain (*Rhodococcus enclensis* PDD-23b-28, *Pseudomonas graminis* PDD-13b-3, *Pseudomonas syringae* PDD-32b-74 and

Sphingomonas sp. PDD-32b-11). The standard error bars reflect significant biological variability measured from three triplicates (independent incubations). Note that the biotransformation rates of valine, isoleucine and glycine could be obtained only for one replicate due to technical problems. Table 1 summarizes the average values of the biodegradation rates of the 18 amino acids for the four bacterial strains. These average values for biodegradation (negative values) range from -1.03 10^{-14} mol cell^{-1} h^{-1} to -8.0210^{-17} mol cell^{-1} h^{-1}, i.e. spanning a range of almost two orders of magnitude depending on the amino acid and the bacterial strain. Note that in the case of glycine and the strain *Pseudomonas graminis* PDD-13b-3, and of aspartate and the strain *Sphingomonas* sp. PDD-32b-11, the values are positive, indicating a net ~~synthesis~~ production and not a net loss. The incubations were performed in a complex medium containing all AA, and as a consequence the rate values are actually net values as all the AA are connected through metabolic pathways corresponding to both biodegradation and biosynthetic pathways (Figure S3).

Overall *Pseudomonas graminis* PDD-13b-3 appears to be the most active strain followed by *Rhodococcus enclensis* PDD-23b-28 (Figure 1, Table 1). However, for some amino acids, this order is reversed, *Rhodococcus enclensis* degrades alanine, asparagine, phenylalanine and tryptophan more efficiently than *P. graminis* does. For all amino acids, *Pseudomonas syringae* PDD-32b-74 is less active than *R. enclensis* and *P. graminis* followed by *Sphingomonas* sp. PDD-32b-1.1

Considering the best degrading strains (Figure 1 and Table 1), the most efficiently biodegraded amino acids are in the order valine >alanine > arginine > glutamate > glutamine > lysine > proline > asparagine > arginine > serine > tryrosine > aspartate, with biodegradation rates within the range of 10^{-14} to 10^{-15} mol cell^{-1} h^{-1}. A second group of AA have lower biodegradation rates in the range of 10^{-16} to 10^{-17} mol cell^{-1} h^{-1} in the following order: phenylalanine > threonine > histidine > methionine > glycine >isoleucine > tryptophan.

3.1.2 Link of the biodegradation rates with metabolic pathways

In bacteria many amino acids are connected within the same metabolic pathways *via* the enzymatic activities of their biosynthesis or biodegradation. Figure S3 presents a simplified network of the AA metabolic pathways as described in K(KEGG pathway database, n.d.) where the AA belonging to the same pathway are shown in the same color. We investigated the hypothesis of a potential link between the rates of biodegradation for each amino acid by the four strains with their connection in specific metabolic pathways (Figures S3 and S4). Glutamate, glutamine, proline and arginine metabolic pathways are closely linked (blue boxes

in Figure S3) and in parallel their biodegradation rates are on the same order of magnitude (Figure S4). This is also true for the group of serine, threonine glycine and methionine (yellow boxes in Figure S3), and for the group tyrosine, phenylalanine and tryptophan (green boxes in Figure S3), respectively. Alanine, asparagine and aspartate (purple boxes in Figure S3) are also related in the network, although the rate of biodegradation of aspartate is lower compared to the other two. Valine and isoleucine biodegradation rates are quite different; this can be explained by two divergent routes: valine is produced from pyruvate, while isoleucine is formed from 2-oxobutanoate. Histidine has a unique metabolic pathway, while lysine is also a special case as two metabolic routes exist: one is linked to 2-oxoadipate, the other is connected to alanine, aspartate and asparagine. To conclude, the rates of biodegradation can be grouped according to their presence in common metabolic pathways. This could explain, as suggested by (Scheller, 2001), why in dew, the concentrations ARG, PRO and GLU, three AA belonging to the same pathway and connected to the urea cycle (Figure S3), were increasing simultaneously.

3.1.3 Dependence of the selectivity of AA biodegradation on the bacterial phylogeny

The rates of biodegradation of the different amino acids expressed as a percentage of the highest rate for each strain are presented in the form of a radar plot in Figure S5. A clear difference is observed between *Rhodococcus enclensis* PDD-23b-28 belonging to Actinobateria (Figure S5a) and the other strains belonging to Proteobacteria (Figure S5b and c). Within Proteobacteria, it is possible to distinguish *Sphingomonas* sp PDD-32b-11 (Figure S5b) belonging to α-Proteobacteria from *Pseudomonas graminis* PDD-13b-3 (grey, Figure S5c) and *Pseudomonas syring*ae PDD-32b-74 (yellow, Figure S5c) belonging to γ-Proteobacteria. In addition, the two *Pseudomona*s strains share very similar trends. So, although the biodegradation rates of *P. syringae* are much lower than those of *P. graminis*, they seem to transform preferentially the same type of amino acids. This should be confirmed with a larger set of isolates. It suggests that the selectivity of AA biodegradation could be related to the phylogeny of the bacterial strains.

3.2 Abiotic transformation of amino acids in microcosms

The abiotic transformation rates of the amino acids measured ~~in the experiments~~ in our microcosms are shown in Table 2 and Figure 2. The first important result is that some amino acids are degraded (TYR, THR, MET, TRP, SER, GLU, VAL, HIS, ALA, ILE) while others are produced (ASN, PRO, GLY, ARG, LYS, GLN, ASP). Abiotic degradation rates (negative values of the transformation rates) were within the range of -7.98 10^{-8} to -9.70 10^{-7} mol h^{-1} L^{-1}.

Net abiotic production rates (positive values) were within the range of 7.69 10^{-8} to 1.05 10^{-6} mol h^{-1} L^{-1}, except for ASP whose rate was very high (3.79 10^{-5} mol h^{-1} L^{-1}). As mentioned in the context of biotic transformations (Section 3.1.1), the incubations are performed in artificial cloud media containing the mixture of the 19 AA, and, thus, the measured rates of abiotic transformations are net values, integrating various mechanisms.

3.3 Comparison of amino acid biotic and abiotic transformation rates

3.3.1 Kinetic rate constants for chemical oxidation reactions

In order to assess the atmospheric importance for the transformation of individual amino acids, we make the following assumptions. Loss by OH reactions occur with the rate constants listed in Table S3 and an OH(aq) concentration of $1 \cdot 10^{-14}$ M (Arakaki et al., 2013). For the oxidation by ozone, ozone has a concentration in cloud water of 0.5 nM which corresponds to a gas phase mixing ratio of 50 ppb, using $K_H(O_3) \sim 10^{-3}$ M atm^{-1} (Sander, 1999). It has been shown previously that the rate constants of amino acids with ozone are strongly pH dependent, with smaller values for the protonated amino form (McGregor and Anastasio, 2001). Since the first acid dissociation constants (pKa_1) for all amino acids are in the range of 2 – 2.5 and the second acid dissociation constants (pKa_2) (de/protonation of the amino group) in the range of 9 – 9.5 (Lide, 2009), it can be assumed that at cloud-relevant pH values (3 < pH < 6) the amino acids are present as carboxylates with protonated amine groups. In addition, we also consider the oxidation by singlet oxygen $^1O_2$. Kinetic rate constants for only about half of the amino acids are available (Table S3). The estimates for $^1O_2$ concentrations in the atmospheric aqueous phase are much sparser and less constrained than for the other oxidants. However, several studies agree that its concentration may be two to three orders of magnitude higher than the OH radical in clouds, fogs and aerosol particles, respectively (Faust and Allen, 1992; Manfrin et al., 2019). Therefore, we assume an aqueous concentration of $[^1O_2(aq)] = 10^{-12}$ M here. Other oxidants (e.g. HO_2/O_2^-, NO_3) are not included in our analysis as based on the few available kinetic data, it can be estimated that reaction rates may be too slow to represent an efficient sink (McGregor and Anastasio, 2001).

3.3.2 Comparison of biotic and abiotic transformation rates

In order to compare the relative importance of biotic (microbial) and abiotic (chemical) transformations under atmospheric conditions, we weight the experimentally derived biotransformation rates by the relative abundance of the various bacteria strains as found in

cloud water. An average concentration of $6.8 \cdot 10^7$ bacterial cells per liter of cloud water was identified in cloud water samples at the puy de Dôme (France) (Vaïtilingom et al., 2012). Further characterization of these samples showed that Actinobacteria (*Rhodococcus enclensis* PDD-23b-28), α-Proteobacteria (*Sphingomonas* sp PDD-32b-1) and γ-Proteobacteria (*Pseudomonas graminis PDD-13b-3 and Pseudomonas syringae PDD-32b-74*) contributed to 6.3%, 16.2% and 29.8%, respectively, to the total cell concentration (Amato et al., 2017); the remaining 47.7% belonged to other phyla or classes (Bacteroidetes, beta-Proteobacteria, Firmicutes…).

Using these relative contributions, the loss rates as observed in our experiments (Section 3.1 and 3.2) were used to compare the loss rates under atmospheric conditions. For this comparison, we calculated the biotransformation rates in cloud water as

$$\frac{d[AA]}{dt} = -0.063\, R_{23b28} \cdot 1.91 - 0.162\, R_{32b11} \cdot 1.91 - \frac{29.8}{2} R_{23b28b} \cdot 1.91 - \frac{29.8}{2} R_{13b2} \cdot 1.91 \qquad \text{[Eq-2]}$$

We scaled each contribution by a factor 1.91 (= 100/52.3) implying that the four bacteria types are representative for the remainder (47.7%) of the bacteria population.

We compare these rates to the photochemical rates derived in the experiments (Section 3.2). However, since the experiments where conducted with OH concentrations likely higher than ambient ones in cloud water, we correct these rates to OH(aq) concentrations in clouds by

$$\left(\frac{d[AA]}{dt}\right)_{photo,exp} = -R_{photo,exp} \cdot \frac{[OH(aq)]_{photo.exp}}{[OH(aq)]_{cloud}} \qquad \text{[Eq-3]}$$

with $[OH(aq)]_{photo,exp} = 8.3 \cdot 10^{-13}$ M and $[OH(aq)]_{cloud} = 1 \cdot 10^{-14}$ M.

Finally, these experimentally-based abiotic transformation rates based on the experiments are compared to those calculated based on kinetic data only.

$$\left(\frac{d[AA]}{dt}\right)_{cloud} = -k_{OH}[OH(aq)]_{cloud} - k_{O3}[O_3(aq)]_{cloud} - k_{1O2}[\,^1O_2(aq)]_{cloud} \qquad \text{[Eq-4]}$$

In previous studies, the reactivity towards the OH radical and/or other oxidants was compared in terms of half-lives τ. However, we chose not to present half-lives here because net production terms as observed in the experiments cannot be represented and would result in unphysical, negative values for τ.

The three rates, i.e. the biodegradation (Eq.-2) and photochemical (Eq.-3) rates as derived from the experiments, and the kinetic loss rates based on chemical kinetics (Eq.-4), respectively, are compared in Figure 3 for each of the 18 amino acids. For some of the acids (ALA, GLU, THR) the predicted losses by OH from both approaches (photochemical experiments (red dashed bars) and based on OH kinetic data (solid dark red bars)) are similar. Thus, we can conclude that these acids are oxidized to products other than amino acids and that the approximation of their loss rates by Equation 4 is justified, as it has been done in previous studies, e.g. (McGregor and Anastasio, 2001; Triesch et al., 2020). For several other amino acids (e.g. ARG, GLN, LYS, SER, and THR) there is a large discrepancy in the observed trends of the predicted chemical loss rates and the ones observed in the photochemical experiments. The latter ones have positive values, i.e. they indicate a net production rather than a net loss. While we cannot conclude on the exact conversion and formation mechanisms of these acids based on our experiments, it is evident that the assumption of a net loss underestimates the lifetime of these acids as they do not only have chemical sinks but also sources in the atmospheric aqueous phase. As also reflected in Figure 1, such net production is only seen for ASP and GLY for biotic processes.

~~The comparison between the rates calculated by Equations 2 – 4 is shown in Figure 3.~~ The comparison of the predicted role of the three oxidants in cloud water (OH, O_3, $^1O_2$) reveals for some AAs, the oxidation by ozone might contribute significantly more to their loss than the other two oxidants (light red bars; note the logarithmic scale, i.e. the contributions of the ozone reactions to the total predicted loss exceeds those by other oxidants by far).

For several of the acids (e.g. ALA, ASN, GLU, PRO, VAL), biotransformation is predicted to exceed the loss by chemical reactions, ~~(e.g. ALA, ASN, GLU, PRO, VAL),~~ for the bacteria cell and oxidant concentrations considered here. Given that the ratios of bacteria cells/radicals in our estimate here are similar to those as encountered in cloud water, it may be concluded that both types of pathways might compete in the atmosphere. Similar conclusions were qualitatively drawn based on ambient measurement in a recent study (Zhu et al., 2020a). However, the exact contributions of biotic and abiotic pathways to the loss and conversion of amino acids will depend on the cell concentrations of the different bacteria strains, their distribution among cloud droplets, and oxidant levels.

~~Note that the loss rates calculated by Equation 4 cannot reproduce the observed production of the various acids as observed in the experiments with the mixture of all amino acids.~~

3.3.2 Amino acid conversions

The oxidation of amino acids by a variety of oxidants has been performed in lab experiments. Results of such experiments are summarized in Table S4. ~~It is obvious that~~ Ggenerally most oxidation reactions lead to smaller fragmentation products and not to amino acids, independent of the oxidant. A detailed discussion of the previously suggested reaction mechanisms of OH and/or HO_2/O_2^- initiated amino acid oxidation has been given by (Stadtman and Levine, 2004). The studies summarized in Table S4 were not motivated by the investigation of amino acid oxidation pathways in the atmospheric aqueous phase. However, our experimental results suggest that some of the amino acids may be the product of oxidation reactions from precursor amino acids, in qualitative agreement with some of the experiments listed in Table S4. The products and their distributions, however, are different than in the metabolic pathways shown in the KEGG mechanism (Figure S3). There are some similarities between the biotransformation and oxidation products, such as the formation of aspartic acid and asparagine from histidine, tyrosine formation from phenylalanine and glutamic acid formation from proline. However, as the yields in the oxidation reactions were not reported, the efficiency of the various pathways for the formation of these acids cannot be estimated.

Our experiments suggest that amino acids can not only be chemically degraded in cloud water but also produced. While such transformation cycles are known from biological systems (KEGG mechanism, Figure S3), the production of amino acids by oxidation reactions in cloud water has not been discussed in the literature. Previous model studies of amino acids in the atmospheric aqueous phase only compared the half-life times of the acids to each other or for different oxidants, solely based on kinetic data (McGregor and Anastasio, 2001; Triesch et al., 2020). Our study suggests that such estimates underestimate the concentrations of amino acids in the atmosphere since they ignore any production. These findings are qualitative as the product yields and distributions are not known. Many of the experiments listed in Table S4 were performed under conditions that are not necessarily atmospherically relevant.

4. Summary, conclusions and atmospheric implications

We measured the biotic (microbial) transformation rates of 18 amino acids with four bacteria strains (*Pseudomonas graminis* PDD-13b-3, *Rhodococcus enclensis* PDD-23b-28, *Sphingomonas* sp. PDD-32b-11 and *Pseudomonas syringae* PDD-32b-74) that have been previously identified as being representative of the microbial communities in cloud water. At the same time, we also determined the abiotic (chemical, OH radical) transformation rates within the same solutions that resembled the composition of cloud water. We used a new approach by UPLC-HRMS to quantify free AA directly in the artificial cloud water medium

without concentration and derivatization, improving the technique used in cloud water by (Triesch et al., 2020). This direct MS method avoids time-consuming and potential biases.

We used our experimentally-derived transformation rates to compare their relative importance under atmospheric conditions, i.e., for atmospherically relevant bacteria cell and OH concentrations in cloud water. These rates were compared to the chemical loss rates based on kinetic data of oxidation reactions of amino acids in the aqueous phase, as they were used previously to derive lifetimes of amino acids in the atmosphere. Our experiments show that previous estimates overestimated the degradation rates, and thus underestimated the lifetime of amino acids in the atmosphere as they only considered kinetic data describing loss processes but did not take into account the transformation of amino acids into each other. While such transformation cycles are well known for metabolic pathways (KEGG pathways), the mechanisms for chemical transformations are poorly constrained.

Our study qualitatively suggests that the sources and distribution of amino acids in the atmospheric particle and aqueous phases can be modified by metabolic and chemical transformation pathways. The distribution and abundance of specific amino acids in particles has been used in previous studies to conclude on aerosol sources (Barbaro et al., 2014, 2015). However, efficient abiotic and or biotic amino acid transformations during aerosol transport might alter the distribution and concentrations of amino acids so that source contributions might be more complex.

Free amino acids can represent up to 5% of WSOC in submicron sized particles but only 0.04% of WSOC in supermicron sized particles (Triesch et al., 2020) , or 9.1% of DOC in cloud water (Bianco et al., 2016a). Free AA can also represent 0.4% and 0.05% of WSON in submicron and supermicron sized particles (Triesch et al., 2020). Total hydrolysed AA (THAA) can account for 0.7 to 1.8% of DOC and from 3.8 to 6.0% of DON in rain samples (Yan et al., 2015) and from 6.2 to 23 % of DOC in fog sample (Zhang and Anastasio, 2003). Considering that WSON contributes to 25% of TDN of ambient aerosols (Lesworth et al., 2010) and WSOC contributes to 20% of TOC (Saxena and Hildemann, 1996), the understanding of the lifetime and transformation rates of amino acids are essential, in order to characterize their atmospheric abundance and residence time. Our study highlights the need for further mechanistic investigations of the biotic (metabolic) and abiotic (chemical) transformations of amino acids under conditions relevant for the atmospheric aqueous phases (clouds, fogs, aerosols). Such data should be used in atmospheric multiphase models to explore the role and competition of biotic and abiotic processes for the transformation and loss of amino acids and related compounds.

Data availability: All data can be obtained from the authors upon request.

Ethics statements: This work does not involve human or animal subject. There is no ethical problem.

Author contributions: AMD designed the experiments in microcosms. SJ, MB, MJ performed the experiments. BE and AK made the calculations for the abiotic and biotic transformations. AMD, MJ and BE wrote the manuscript. All the authors read and corrected the manuscript.

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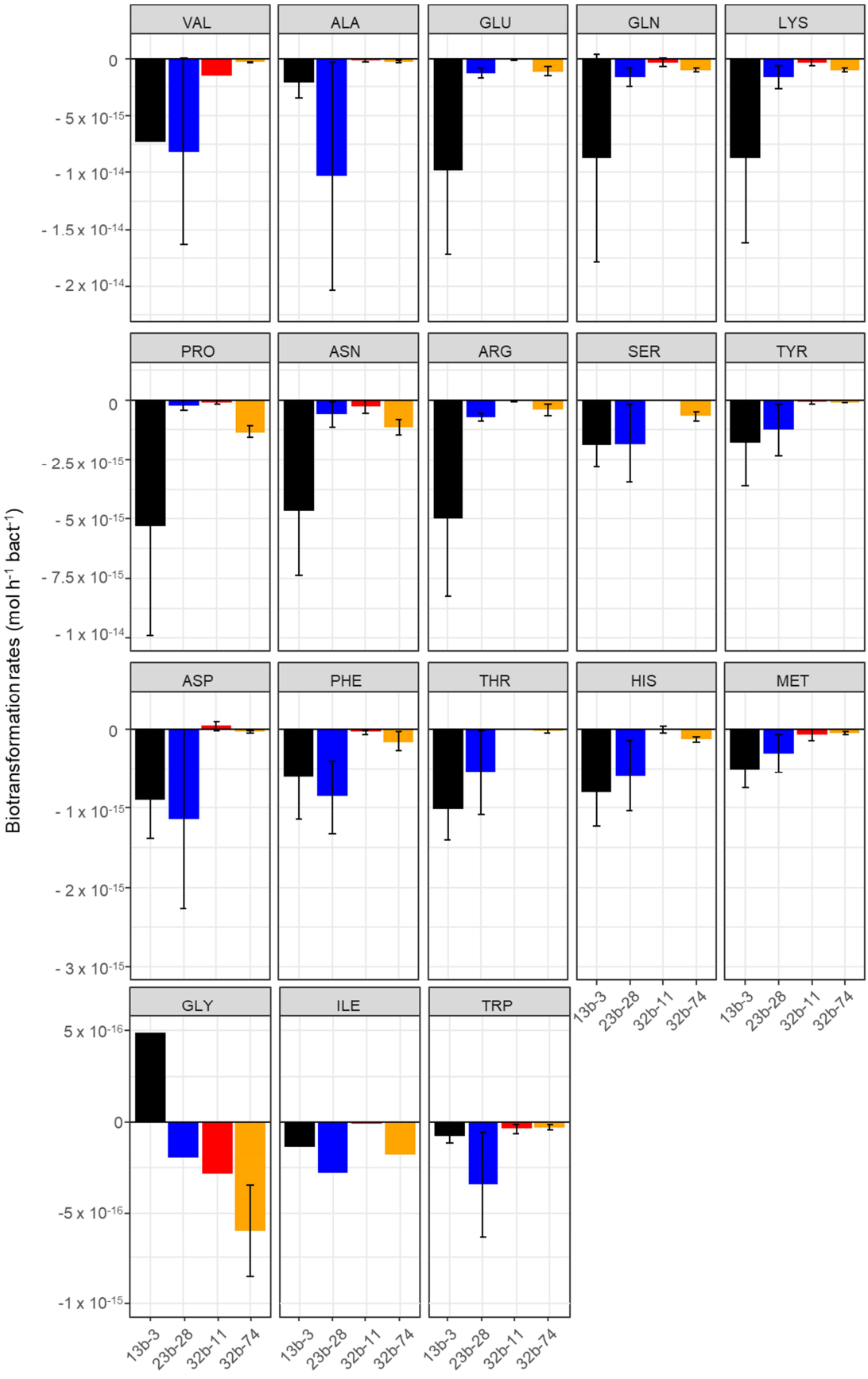
Figure captions

Figure 1: Biotransformation rates obtained for each amino acid and each bacterial strain (*Pseudomonas graminis* PDD-13b-3 (black), *Rhodococcus enclensis* PDD-23b-28 (blue), *Sphingomonas* sp. PDD-32b-11 (red) and *Pseudomonas syringae* PDD-32b-74 (orange). The experiments were performed in microcosms containing the mixture of the 19 AA in a cloud artificial medium. The standard error bars reflect the significant biological variability measured from 3 triplicates (independent incubations).

Figure 2: Abiotic transformation rates (mol h^{-1} L^{-1}) obtained for each amino acid in microcosms containing the mixture of the 19 AA in a cloud artificial medium under irradiation in the presence of Fe(EDDSS) as source of OH radicals. The standard error bars reflect the variability measured from 3 triplicates (independent experiments). Negative values represent abiotic degradation while positive values represent abiotic production.

Figure 3: Reaction rates for 18 amino acids as observed based on experiments in the present study, scaled to atmospheric conditions (Eqs. 2 and 3) and rates for loss reactions by OH(aq), O_3(aq) and $^1O_2$(aq) (Eq-4)

Figure 1


VAL
ALA
GLU
GLN
LYS
PRO
ASN
ARG
SER
TYR
ASP
PHE
THR
HIS
MET
GLY
ILE
TRP
Biotransformation rates (mol h-1 bact-1)
0
- 5 x 10-15
- 1 x 10-14
- 1.5 x 10-14
- 2 x 10-14
- 2.5 x 10-15
- 7.5 x 10-15
- 3 x 10-15
5 x 10-16
-5 x 10-16
-1 x 10-15
13b-3
23b-28
32b-11
32b-74

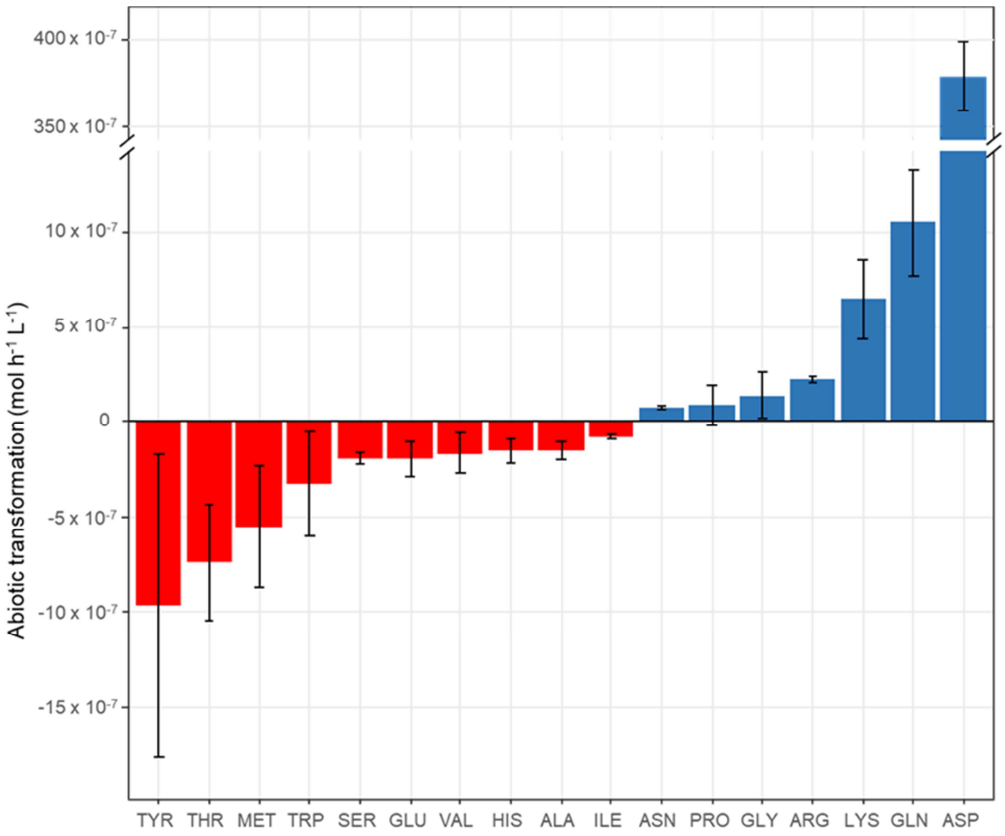

400 x 10-7
350 x 10-7
10 x 10-7
5 x 10-7
0
-5 x 10-7
-10 x 10-7
-15 x 10-7
Abiotic transformation (mol h-1 L-1)
TYR
THR
MET
TRP
SER
GLU
VAL
HIS
ALA
ILE
ASN
PRO
GLY
ARG
LYS
GLN
ASP

Figure 2

Figure 3

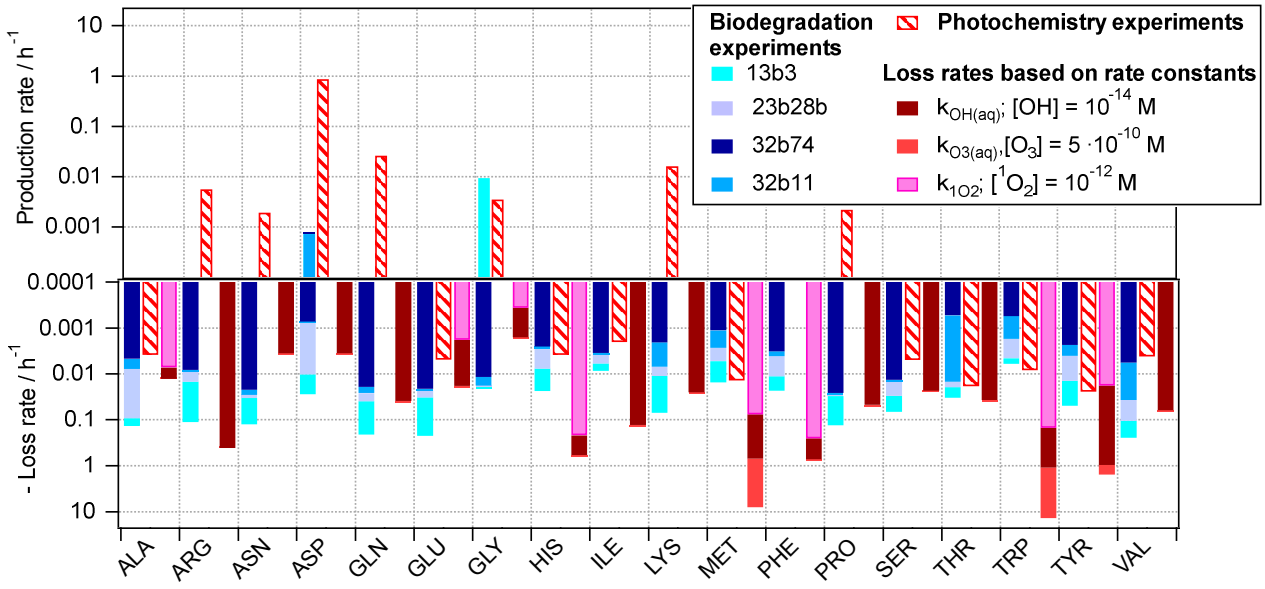

Biodegradation experiments
Photochemistry experiments
13b3
23b28b
32b74
32b11
Loss rates based on rate constants
$k_{OH(aq)}$; [OH] = 10^{-14} M
$k_{O3(aq)}$,[O_3] = 5 ·10^{-10} M
k_{1O2}; [$^1O_2$] = 10^{-12} M
Production rate / h^{-1}
- Loss rate / h^{-1}
10
1
0.1
0.01
0.001
0.0001
ALA
ARG
ASN
ASP
GLN
GLU
GLY
HIS
ILE
LYS
MET
PHE
PRO
SER
THR
TRP
TYR
VAL

Table 1: Average values of the biotransformation rates (mol $bact^{-1}$ h^{-1}) of 18 amino acids by the four bacterial strains (*Pseudomonas graminis* PDD-13b-3, *Rhodococcus enclensis* PDD-23b-28, *Pseudomonas syringae* PDD-32b-74 and *Sphingomonas* sp. PDD-32b-11) and by the combination of these strains as representative of the biodiversity in a real cloud (named "Cloud") as described in section 3.3.2.

Positive values correspond to a net biosynthesis, while negative ones correspond to a net biodegradation.

	VAL	ALA	GLU	GLN	LYS	PRO	ASN	ARG	SER
13b-3 *Pseudomonas graminis*	-7.29 x 10^{-15}	-2.19 x 10^{-15}	-9.89 x 10^{-15}	-8.72 x 10^{-15}	-3.05 x 10^{-15}	-5.29 x 10^{-15}	-4.67 x 10^{-15}	-4.99 x 10^{-15}	-1.90 x 10^{-15}
23b-28 *Rhodococcus enclensis*	-8.11 x 10^{-15}	-1.03 x 10^{-14}	-1.27 x 10^{-15}	-1.62 x 10^{-15}	-5.07 x 10^{-16}	-2.33 x 10^{-16}	-6.14 x 10^{-16}	-7.03 x 10^{-16}	-1.81 x 10^{-15}
32b-11 *Sphingomonas* sp.	-1.48 x 10^{-15}	-1.46 x 10^{-16}	-3.91 x 10^{-17}	-3.22 x 10^{-16}	-2.31 x 10^{-16}	-1.08 x 10^{-16}	-2.89 x 10^{-16}	-3.98 x 10^{-17}	-6.82 x 10^{-17}
32b74 *Pseudomonas syringae*	-2.91 x 10^{-16}	-2.40 x 10^{-16}	-1.09 x 10^{-15}	-9.87 x 10^{-16}	-1.05 x 10^{-16}	-1.33 x 10^{-15}	-1.14 x 10^{-15}	-4.21 x 10^{-16}	-6.88 x 10^{-16}
Cloud	-3.60 x 10^{-15}	-1.97 x 10^{-15}	-3.29 x 10^{-15}	-3.06 x 10^{-15}	-1.03 x 10^{-15}	-1.95 x 10^{-15}	-1.82 x 10^{-15}	-1.64 x 10^{-15}	-9.75 x 10^{-16}

	TYR	THR	ASP	HIS	PHE	MET	GLY	ILE	TRP
13b-3 *Pseudomonas graminis*	-1.79 x 10^{-15}	-6.83 x 10^{-16}	-9.07 x 10^{-16}	-8.08 x 10^{-16}	-6.19 x 10^{-16}	-5.14 x 10^{-16}	4.85 x 10^{-16}	-1.35 x 10^{-16}	-7.48 x 10^{-17}
23b-28 *Rhodococcus enclensis*	-1.23 x 10^{-15}	-5.53 x 10^{-16}	-1.14 x 10^{-15}	-5.91 x 10^{-16}	-8.57 x 10^{-16}	-3.12 x 10^{-16}	-1.96 x 10^{-16}	-2.78 x 10^{-16}	-3.45 x 10^{-16}
32b-11 *Sphingomonas* sp.	-8.02 x 10^{-17}	-6.76 x 10^{-16}	3.41 x 10^{-17}	-1.42 x 10^{-17}	-4.42 x 10^{-17}	-7.40 x 10^{-17}	-2.89 x 10^{-16}	-7.50 x 10^{-18}	-5.50 x 10^{-17}
32b74 *Pseudomonas syringae*	-1.19 x 10^{-16}	-2.71 x 10^{-17}	-3.69 x 10^{-17}	-1.31 x 10^{-16}	-1.64 x 10^{-16}	-5.78 x 10^{-17}	-6.01 x 10^{-16}	-1.78 x 10^{-16}	-2.85 x 10^{-17}
Cloud	-7.16 x 10^{-16}	-4.78 x 10^{-16}	-3.96 x 10^{-16}	-3.43 x 10^{-16}	-3.40 x 10^{-16}	-2.23 x 10^{-16}	-1.46 x 10^{-16}	-1.25 x 10^{-16}	-8.80 x 10^{-17}

Table 2: Abiotic transformation rates of the 17 AA (mole h^{-1} L^{-1}) measured in microcosms containing the mixture of the all AAs in a cloud artificial medium under irradiation in the presence of Fe(EDDSS) as source of OH radicals. Positive values represent degradation while negative values represent production. Mean values were calculated from 3 triplicates (independent experiments) except for ASN, GLN, GLY and PRO. No value could be obtained for PHE (technical problem).

Degradation	**TYR**	**THR**	**MET**	**TRP**	**SER**	**GLU**	**VAL**	**HIS**	**ALA**	**ILE**
	-9.70 x 10^{-7}	-7.41 x 10^{-7}	-5.55 x 10^{-7}	-3.29 x 10^{-7}	-1.97 x 10^{-7}	-1.96 x 10^{-7}	-1.67 x 10^{-7}	-1.53 x 10^{-7}	-1.53 x 10^{-7}	-7.98 x 10^{-8}
Production	**ASN**	**PRO**	**GLY**	**ARG**	**LYS**	**GLN***	**ASP**			
	7.69 x 10^{-8}	8.82 x 10^{-8}	1.40 x 10^{-7}	2.25 x 10^{-7}	6.47 X 10^{-7}	1.05 x10^{-6}	3.79 X 10^{-5}			

SUPPLEMENTARY MATERIAL

Biotic and abiotic transformation of amino acids in cloud water: Experimental studies and atmospheric implications.

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Table S1: Composition of the artificial cloud medium used for biotic and abiotic transformation of amino acids in microcosms.

Chemical species	Concentration (µM)	Chemical species	Concentration (µM)
Acetate	100	cysteine	1
Formiate	72.5	glutamic acid	1
Succinate	7.5	glycine	1
Oxalate	15	histidine	1
Cl^-	200	isoleucine	1
NO_3^-	400	lysine	1
SO_4^{2-}	25	methionine	1
Na^+	1000	phenylalanine	1
NH_4^+	400	proline	1
K^+	25	serine	1
Mg^{2+}	50	threonine	1
Ca^{2+}	200	tryptophan	1
alanine	1	tyrosine	1
arginine	1	valine	1
asparagine	1	glutamine	1
aspartic acid	1	pH[a]	6

[a] The pH of the artificial cloud medium was adjusted to 6 (a few drops of NaOH at 1.38 M and of 0.39M H_2SO_4) and the medium was sterilized by filtration on a polyethersulphone (PES) membrane of 0.20 µm porosity (Fisher Scientific) before use. Note that cysteine was present in the medium but it could not be assayed by LC-HRMS, neither leucine that cannot be distinguished from isoleucine by LC-HRMS.

Table S2: Retention times, exact masses and LODs and LOQs measured for the 18 AA measured by UPLC-HRMS

Amino acid	Molecular formula	Retention time (min)	m/z [M+H]	LOD (µM)	LOQ (µM)
ALA	$C_3H_7NO_2$	4.25	90.0550	0.237	0.474
ARG	$C_6H_{14}N_4O_2$	7.41	175.1190	0.072	0.143
ASN	$C_4H_8N_2O_3$	5.57	133.0608	0.143	0.286
ASP	$C_4H_7NO_4$	5.09	134.0448	0.148	0.295
GLN	$C_5H_{10}N_2O_3$	5.35	147.0764	0.234	0.468
GLU	$C_5H_9NO_4$	4.8	148.0526	0.111	0.222
GLY	$C_2H_5NO_2$	4.6	76.0393	0.242	0.483
HIS	$C_6H_9N_3O_2$	7.47	156.0768	0.094	0.188
ILE	$C_6H_{13}NO_2$	2.59	160.1081	0.179	0.359
LYS	$C_6H_{14}N_2O_2$	7.62	147.1128	0.069	0.139
MET	$C_5H_{11}NO_2S$	2.69	150.0584	0.072	0.144
PHE	$C_9H_{11}NO_2$	2.64	166.0863	0.061	0.122
PRO	$C_5H_9NO_2$	3.05	116.0706	0.140	0.281
SER	C_3H7NO_3	5.36	106.0499	0.135	0.268
THR	$C_4H_9NO_3$	4.87	120.0655	0.163	0.327
TRP	$C_{11}H_{12}N_2O_2$	2.65	205.0972	0.058	0.117
TYR	$C_9H_{11}NO_3$	7.46	182.0812	0.072	0.143
VAL	$C_5H_{11}NO_2$	2.61	118.0863	0.237	0.475

30

Table S3: Relative standard deviation (RSD = Standard deviation/mean) for each AA based on calibration curves (3 technical replicates).

	Relative Standard Deviation (RSD = Standard deviation/mean)		
Amino acid	0.1 µM (n = 3)	0.5 µM (n = 3)	1 µM (n = 3)
ALA	.	0.71%	3.61%
ARG	0.83%	1.96%	1.56%
ASN	5.23%	4.92%	3.63%
ASP	.	10.77%	5.96%
GLN	4.19%	4.37%	3.20%
GLU	3.77%	2.89%	3.92%
GLY	.	.	21.39%
HIS	0.62%	0.89%	1.22%
ILE	4.48%	0.48%	0.59%
LYS	6.64%	1.96%	1.50%
MET	4.49%	4.35%	6.38%
PHE	4.63%	1.68%	1.02%
PRO	11.67%	5.08%	1.28%
SER	14.34%	3.06%	3.20%
THR	14.15%	3.67%	1.06%
TRP	7.00%	1.67%	1.75%
TYR	0.94%	1.81%	1.15%
VAL	17.94%	2.98%	11.41%

Table S4: Rate constants for 18 amino acids for the OH, O_3 and $^1O_2$ reactions. As most rate constants are only available at or near room temperature, we chose this temperature for all constants.

	k_{OH} / $M^{-1} s^{-1}$	Reference	k_{O3} / $M^{-1} s^{-1}$	Reference	k_{1O2} / $M^{-1} s^{-1}$	Reference
ALA	$7.7 \cdot 10^{7}$	(Scholes et al., 1965)	$2.5 \cdot 10^{1}$	(Ignatenko and Cherenkevich, 1985)	$2 \cdot 10^{6}$	(Matheson and Lee, 1979)
ARG	$3.5 \cdot 10^{9}$	(Buxton et al., 1988)	$2.8 \cdot 10^{2}$	(Ignatenko and Cherenkevich, 1985)	$< 1 \cdot 10^{6}$	(Kraljić and Sharpatyi, 1978)
ASN	$4.9 \cdot 10^{7}$	(MASUDA et al., 1973)	$7.0 \cdot 10^{1}$	(Ignatenko and Cherenkevich, 1985)		
ASP	$4.9 \cdot 10^{7}$	(MASUDA et al., 1973)	$5.0 \cdot 10^{1}$	(Ignatenko and Cherenkevich, 1985)		
GLN	$5.4 \cdot 10^{8}$	(MASUDA et al., 1973)	$8.0 \cdot 10^{1}$	(Ignatenko and Cherenkevich, 1985)		
GLU	$1.6 \cdot 10^{8}$	(Scholes et al., 1965)	$2 \cdot 10^{-1}$	(Ignatenko and Cherenkevich, 1985)	$5.0 \cdot 10^{5}$	(McGregor and Anastasio, 2001)
GLY	$1.7 \cdot 10^{7}$	(Scholes et al., 1965)	$2.1 \cdot 10^{1}$	(Ignatenko and Cherenkevich, 1985)	$< 1 \cdot 10^{5}$	(Michaeli and Feitelson, 1994)
HIS	$5 \cdot 10^{9}$	(Motohashi and Saito, 1993)	$3.9 \cdot 10^{3}$	(Ignatenko and Cherenkevich, 1985)	$6 \cdot 10^{7}$	(McGregor and Anastasio, 2001)
ILE	$1.8 \cdot 10^{9}$	(MASUDA et al., 1973)				
LYS	$3.5 \cdot 10^{8}$	(MASUDA et al., 1973)	$1.2 \cdot 10^{2}$	(Ignatenko and Cherenkevich, 1985)		
MET	$8.5 \cdot 10^{9}$	(Adams et al., 1965)	$4\ 10^{6}$	(Pryor et al., 1984)	$2.1 \cdot 10^{7}$	(Miskoski and García, 1993)
PHE	$6.5 \cdot 10^{9}$	(Buxton et al., 1988)	$1.3 \cdot 10^{3}$	(Ignatenko and Cherenkevich, 1985)	$7 \cdot 10^{5}$	(Michaeli and Feitelson, 1994)
PRO	$6.5 \cdot 10^{8}$	(MASUDA et al., 1973)	$4.8 \cdot 10^{2}$	(Ignatenko and Cherenkevich, 1985)		
SER	$2.5 \cdot 10^{8}$	(Scholes et al., 1965)	$1.8 \cdot 10^{2}$	(Ignatenko and Cherenkevich, 1985)		
THR	$5.1 \cdot 10^{8}$	(MASUDA et al., 1973)	$2.6 \cdot 10^{2}$	(Ignatenko and Cherenkevich, 1985)		

TRP	$1.3 \cdot 10^{10}$	(Buxton et al., 1988)	$5.6 \cdot 10^{4}$	(Ignatenko and Cherenkevich, 1985)	$4.1 \cdot 10^{7}$	(McGregor and Anastasio, 2001)
TYR	$1.3 \cdot 10^{10}$	(Solar et al., 1984)	$4.8 \cdot 10^{3}$	(Ignatenko and Cherenkevich, 1985)	$5 \cdot 10^{6}$	(McGregor and Anastasio, 2001)
VAL	$8.5 \cdot 10^{8}$	(Prütz and Vogel, 1976)	$4 \cdot 10^{1}$	(Ignatenko and Cherenkevich, 1985)		

Table S5: Selected experimental studies of amino acid oxidation by various oxidants. Note that the experimental conditions were not necessarily atmospherically-relevant. Products are only listed to demonstrate the wide variety of possible reaction pathways and products.

Amino acid	Oxidant	Main product(s)	Reference
ARG	Fenton chemistry	glutamic semialdehyde	(Stadtman, 1993; Stadtman and Levine, 2003) and reference therein
ASP	OH	NH_3,Malonic, oxalic and formic acids	(Marion et al., 2018)
CYS	Fenton chemistry	-S-S-disulfide cross-links	(Stadtman, 1993; Stadtman and Levine, 2003)
GLY	OH	Oxalic, formic, oxamic acids	(Berger et al., 1999)
GLY	O_3	Nitrate, nitrites	(Berger et al., 1999)
GLY-ALA-VAL-PRO peptides	OH	Hydroperoxides, alcohols	(Morgan et al., 2012)
HIS	O_3	PRO	(Mudd et al., 1969)
HIS	Fenton chemistry	ASP, ASN, 2-oxoimidazoline	(Stadtman, 1993; Stadtman and Levine, 2003) and references therein
LEU	OH	Isovaleric acid and other carbonyl compounds	
LYS	Fenton chemistry	2-amino-adipicsemialdehyde	(Stadtman, 1993; Stadtman and Levine, 2003) and references therein
MET	O_3	Methionine sulfoxide	(Mudd et al., 1969)
PHE	ROS	TYR	(Stadtman, 1993; Stadtman and Levine, 2003)
PHE	Direct UV absorption	TYR	(Pattison et al., 2012)
PRO	Fenton chemistry	GLU, pyroglutamate, Cis/trans-4-hydroxyproline, 2-pyrrolidone, glutamic semialdehyde	(Stadtman, 1993; Stadtman and Levine, 2003) and reference therein
SER	OH	carbonyl and carboxylic acid	
THR	Fenton chemistry	2-amino-3-ketobutyric acid	(Stadtman, 1993; Stadtman and Levine, 2003) and references therein
THR	O_3	Dihydroxyphenylalanine	(Mudd et al., 1969)
TRP	OH	Formic and acetic acids, many aromatic	(Bianco et al., 2016)

Mis en forme : Anglais (États Unis)

Mis en forme : Anglais (États Unis)

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Mis en forme : Anglais (États Unis)

		intermediates	
TRP	$^1O_2$	3α-hydroxypyrroloindole; *N*-formylkynurenine, kynurenine, 3α-dihydroxypyrroloindole.	
TYR	OH	Enedial	(Prasse et al., 2018)
TYR	Fenton chemistry	Tyr-Tyr cross-links	(Stadtman, 1993; Stadtman and Levine, 2003) and references therein
TYR	$^1O_2$ or direct UV absorption	3a-hydroxy-6-oxo-2,3,3a,6,7,7a-hexahydro-1H-indol-2-carboxylic acid	

Mis en forme : Anglais (États Unis)

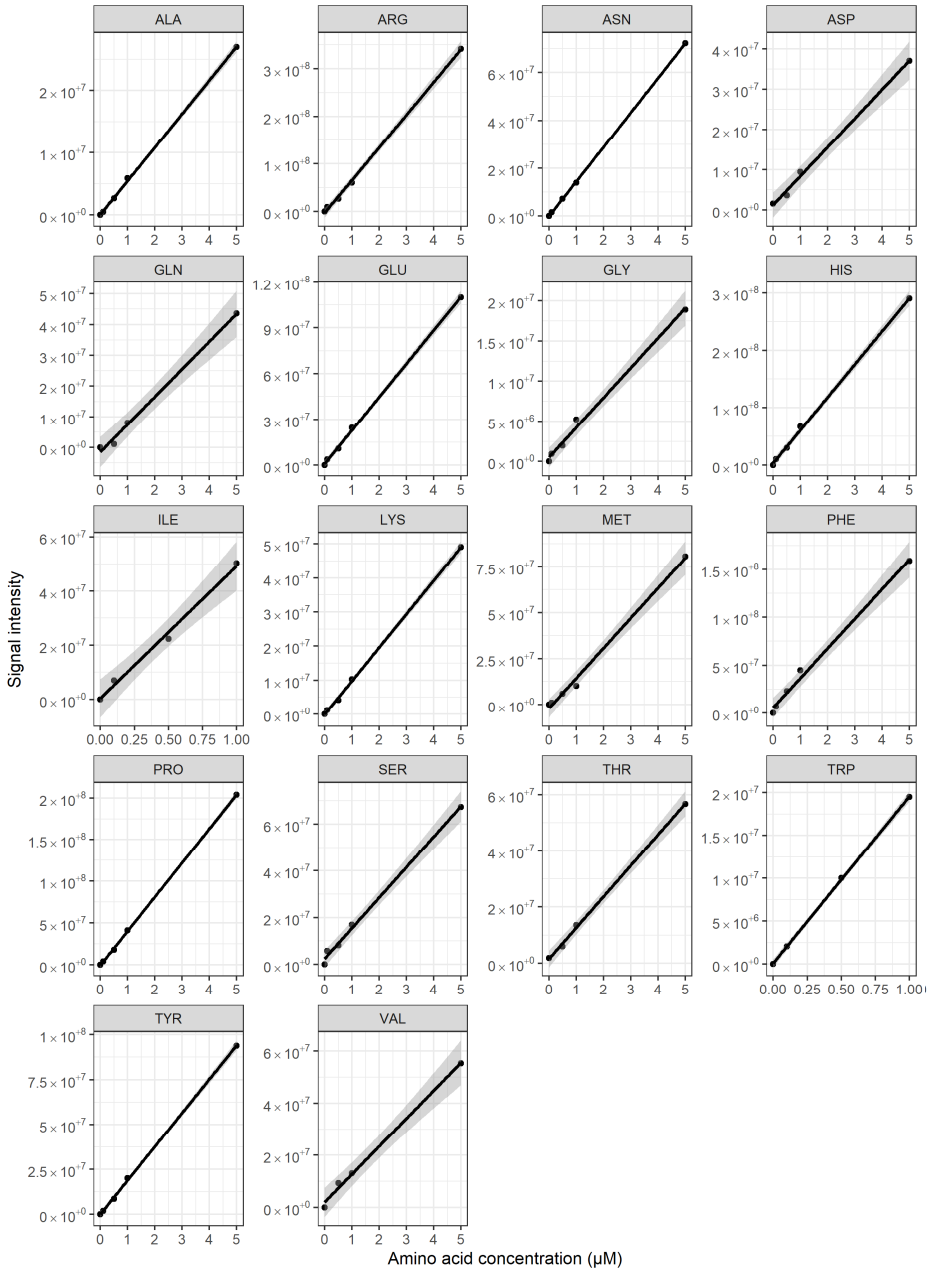


Figure S1: Calibration curves for LC-HRMS experiments

Example of biodegradation rate calculation:

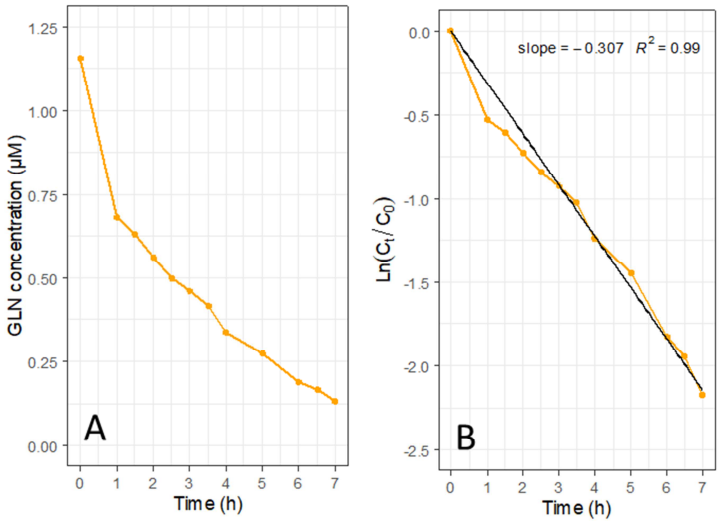
Bacterial degradation of amino acids follows a first order decay equation as $C_t = C_0 . e^{-kt}$ with t the incubation time, C_0 and C_t the initial concentration and concentration at t respectively and k the first order decay constant.

First, concentration of each amino acid is followed through time (Figure S2A) by LC-HRMS as described in the Materials and Methods section. Values are converted to determine k corresponding to the slope of $Ln(C_t/C_0) = f(t)$ (Figure S2B)

Biodegration rates are then calculated as follows: $V_b = \frac{k \times C_0}{N_{cell}}$ with V_b the biodegradation rate (in mol h^{-1} $cell^{-1}$), k the first order decay constant (in h^{-1}), C_0 the initial concentration in aminoacid (in mol L^{-1}) and N_{cell} the bacterial concentration (in cell L^{-1}).

In this example, N_{cell} = 4 10^8 cell L^{-1} and C_0 = 1.16 10^{-6} mol L^{-1} so V_b = 8.88 10^{-16} mol h^{-1} $cell^{-1}$

.



Figures S2: Example of calculation of the biodegradation rate of GLN. A) time dependence of GLN concentration with time measured by LC-HRMS. B) $ln(Ct/C_0)=f(t)$, degradation rates are calculated from the slope at the origin.

C_t: GLN measured concentration at time = t , C_0 :GLN measured concentration at time zero.

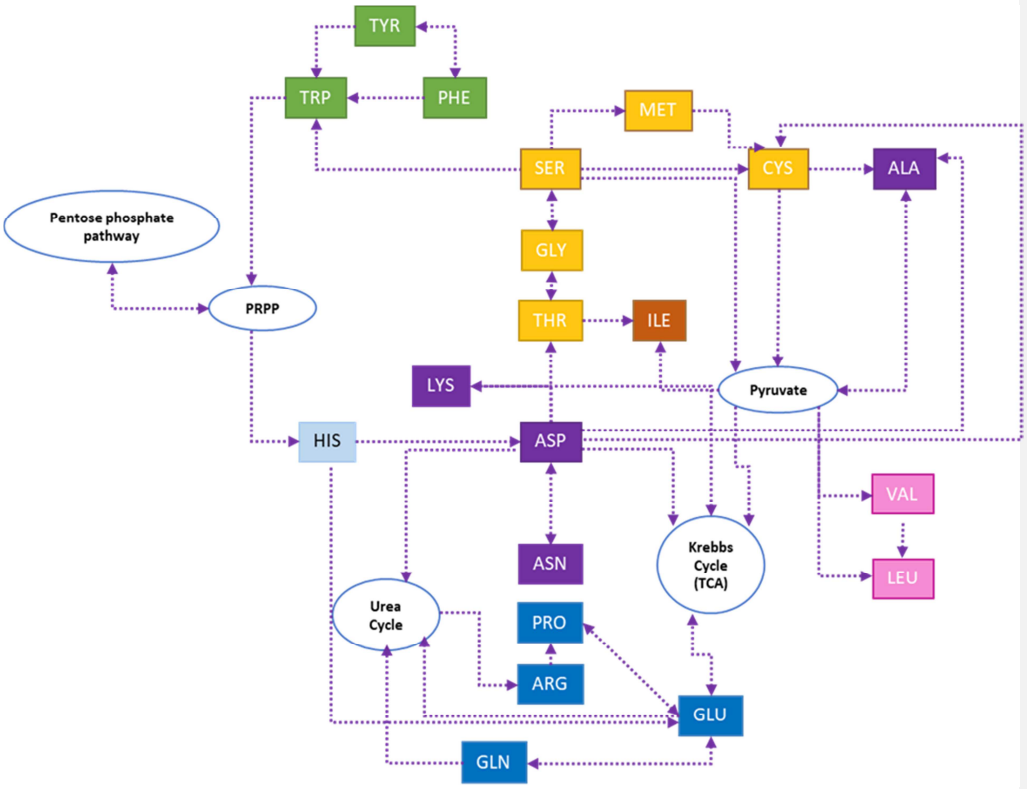


Figure S3: Main metabolic routes for AA metabolism according to (KEGG pathway database, n.d.)

Mis en forme : Anglais (États Unis)

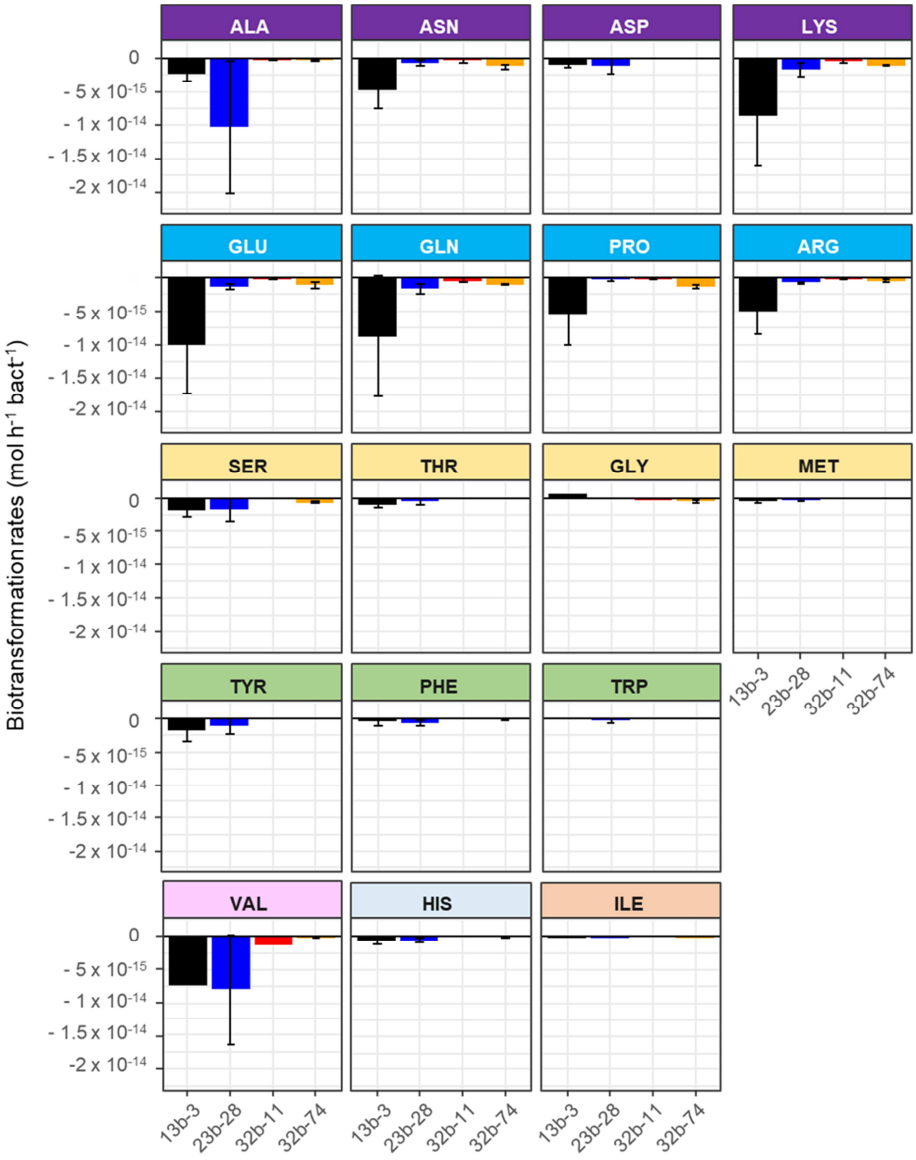


Figure S4: Biotransformation rates (mol $bact^{-1}$ h^{-1}) of AA by the four bacterial strains grouped according the metabolic pathways of the AA (see Figure S3). *Pseudomonas graminis* PDD-13b-3 in black, *Rhodococcus enclensis* PDD-23b-28 in blue, *Sphingomonas* sp. PDD-32b-11 in red and *Pseudomonas syringae* PDD-32b-74 in orange). The standard error bars reflect the rather important biological variability measured from 3 triplicates (independent incubations). Positive values correspond to a biosynthesis process, negative values to a biodegradation process.

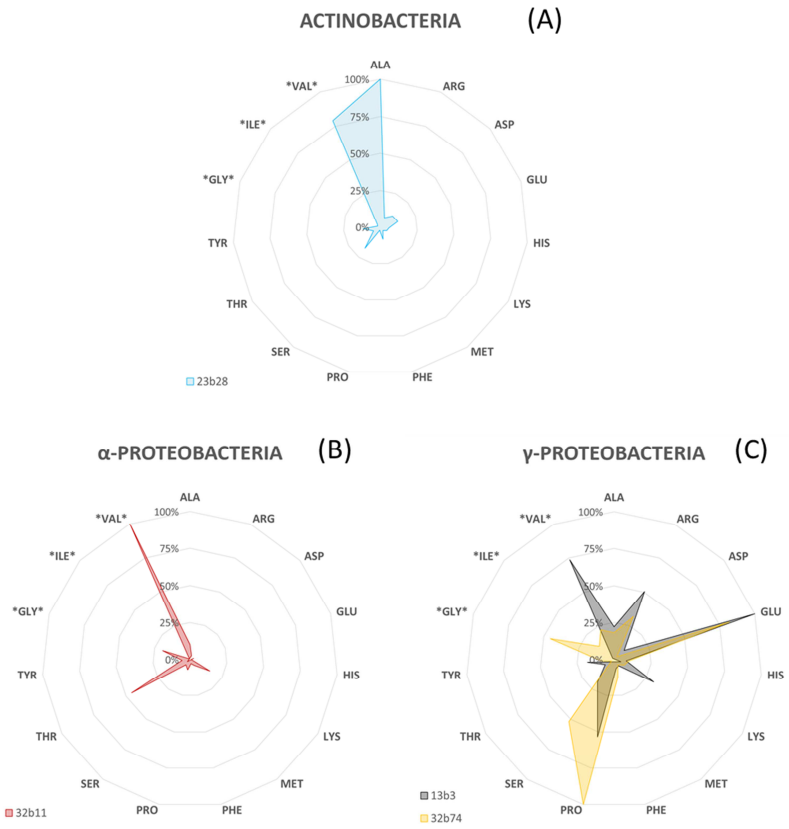


Figure S5: Comparison of the ability of the different strains to metabolize amino acids according to their phylogeny: (A) Actinobacteria (*Rhodococcus enclensis* PDD-23b-28 in blue), B) α-Proteobacteria (*Sphingomonas* sp PDD-32b-11 in red), (C) γ-Proteobacteria (*Pseudomonas graminis* PDD-13b-3 in black and *Pseudomonas syringa*e PDD-32b-74 in yellow). The rates of biodegradation (average from 3 replicates) are presented as a % for each amino acid in the form of a radar plot. For each strain the highest rate is put at 100%. (* only one replicate value was available).

An example of phylogenetic classification is given bellow

Phylum--→Class→Genus→species→strain number

Proteobacteria→γ-Proteobacteria→*Pseudomonas*→*graminis*→ PDD-13b-3

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