



Dissolved hydrolyzed amino acids in precipitation in suburban Guiyang, southwestern China: Seasonal variations and potential atmospheric processes

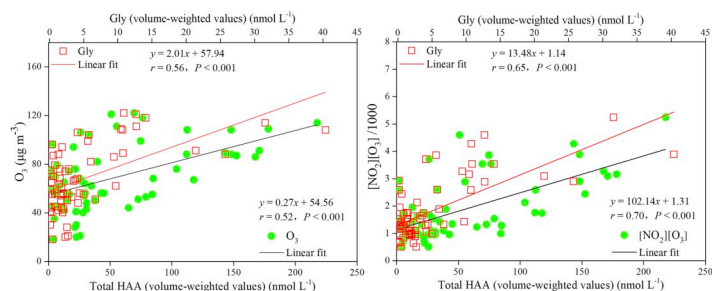


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GRAPHICAL ABSTRACT



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ABSTRACT

Proteinaceous compounds are particularly interesting because of their ubiquity and importance in many atmospheric processes. We investigated hydrolyzed amino acid (HAA), dissolved organic nitrogen (DON), nitrate (NO_3^-) and ammonium (NH_4^+) concentrations in precipitation samples collected in a suburban site in Guiyang over a 12 month period. Annually averaged total HAA, DON, NO_3^- and NH_4^+ concentrations were $3.7 \mu\text{mol L}^{-1}$, $151.1 \mu\text{mol L}^{-1}$, $68.9 \mu\text{mol L}^{-1}$ and $117.3 \mu\text{mol L}^{-1}$, respectively. Regarding the HAAs in precipitation, glutamic acid, glycine and proline were present in relatively high concentrations, followed by aspartic acid and alanine. The concentrations of total HAAs in precipitation showed a clear seasonal cycle, with a minimum level in winter and a maximum level in spring. Based on seasonal variations of total HAAs together with back-trajectory analysis, the air mass origins did not significantly impact the precipitation HAA levels. The NO_3^- concentrations recorded a better positive correlation ($P < 0.01$) with both the DON and total HAA concentrations than the NH_4^+ concentrations, possibly revealing that the sources for precipitation amino acids in suburban Guiyang were more linked with NO_3^- sources (from biomass burning, microbial activities and agricultural activities) than with NH_4^+ sources (from biomass burning and agricultural activities). In particular, in some specific periods, such as spring, abundant pollen releases may have been responsible for the relatively high precipitation amino acid concentrations. The average air temperature and the highest air temperature showed a positive correlation with the total HAA levels in precipitation. Clearly, the increase in precipitation total HAAs with higher air temperatures may indicate the enhanced temperature-induced degradation of high molecular weight atmospheric proteinaceous matter. Moreover, the volume-weighted precipitation glycine and total HAA levels were positively correlated with the product of atmospheric ozone and nitrogen dioxide,

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indicating that atmospheric proteinaceous matter may be inextricably bound up with both ozone- and nitrogen dioxide-related atmospheric processes. In conclusion, this study improves current knowledge on the origins and atmospheric processes of atmospheric proteinaceous compounds.

1. Introduction

Recently, in some areas of Asia, such as China, a relatively high proportion of organic nitrogen (ON) aerosols has been observed during haze episodes, suggesting the nonnegligible importance of ON aerosols in haze pollution chemistry (Ho et al., 2016). Proteinaceous compounds, namely free or combined amino acids (peptides and proteins), are important components of the total ON and organic carbon (OC) in aerosols (Zhang et al., 2002; Pöschl, 2005; Matos et al., 2016). Thus, the functional properties of proteinaceous compounds are of interest to atmospheric scientists (Matos et al., 2016). Several studies have demonstrated that the proteinaceous compounds in aerosols impact the formation, hygroscopicity, microstructural rearrangement, chemical transformations, and acidity/basicity of organic aerosols (Szyrmer and Zawadzki, 1997; Zhang and Anastasio, 2003; Mikhailov et al., 2004; Cape et al., 2011). Some aerosol amino compounds can even alter the climate by controlling the processes of cloud formation (acting as cloud condensation nuclei) (Chan et al., 2005; Kristensson et al., 2010). Additionally, some proteinaceous compounds that exist in atmospheric aerosols are regarded as important allergens, which could be a direct cause of inflammatory, respiratory, and eye diseases (Miguel et al., 1999; Pöschl, 2005; Gruijthuijzen et al., 2006). Thus, a better understanding of the possible sources of atmospheric proteinaceous matter in urban environments is of great significance for management strategies designed to reduce their adverse impacts on the health of atmospheric environments and human beings.

Increased anthropogenic and natural organic matter emissions can be removed through dry and wet deposition and eventually return to the terrestrial and aquatic environments (Hallquist et al., 2009; Cape et al., 2011; Cornell, 2011). Although a large uncertainty remains in our understanding of the biogeochemical role and fate of atmospheric organic matter in receiving ecosystems, atmospheric organic matter has increasingly been considered to constitute an indispensable part of the global N and C cycles (Wedyan and Preston, 2008; Zamora et al., 2011). In particular, the water-soluble fraction of the organic matter in aerosols has drawn special attention because of its direct linkages to bioavailable nutrients. Proteinaceous compounds are characterized by certain water-solubilities (Matos et al., 2016), thereby suggesting that water-soluble proteinaceous compounds are extremely important in atmosphere-biosphere N nutrient cycling. Further, a better comprehending of the eco-environment effects of atmospheric organic matter necessitates investigations on atmospheric proteinaceous matter.

The sizes and compositions of amino acid pools in atmospheric aerosol particles have been widely investigated worldwide (e.g., South America, North America, Asia, Europe, the Arctic and the Antarctic) (Mace et al., 2003a; Kuznetsova et al., 2005; Barbaro et al., 2011, 2017; Scalabrin et al., 2012; Song et al., 2017) and in different environmental situations (e.g., urban, rural, suburban, marine, and polar areas) (Zhang et al., 2002; Mace et al., 2003a; Mandalakis et al., 2011; Di Filippo et al., 2014; Barbaro et al., 2015, 2017; Ho et al., 2015). In contrast, the identification of amino acids in other condensed phases such as precipitation has been conducted infrequently, except for four studies on rainwater in non-inland areas (marine and coastal areas) by Mopper and Zika (1987), Mace et al. (2003b), Kieber et al. (2005) and Yan et al. (2015); the authors deduced that the presence of those rainwater amino acids was partly related to marine sources. As described, an increasing number of studies have been performed on atmospheric amino acids (particularly aerosol amino acids); however, the focus of these studies has mainly been on free amino acids, which are relatively non-dominant forms of amino acids in most environments. The determined

amount of combined amino acids in atmospheric aerosols was more than four times higher than that of free amino acids (Wedyan and Preston, 2008; Mandalakis et al., 2011; Samy et al., 2013). To date, no study on precipitation amino acids has been conducted in inland suburban regions far from the sea; likely, the origins and atmospheric processes of dissolved hydrolyzed amino acids in precipitation in inland areas are different from those in ocean-related rainwater. Indeed, detailed analyses on each individual amino acid in precipitation are extremely limited, particularly for combined amino acids.

The origin, composition and formation mechanisms of amino acids in the atmosphere are quite complicated, and many remain unknown (Matos et al., 2016). Existing researches have shown that variations in amino acid concentrations in atmospheric aerosol particles can be controlled by different sources and atmospheric conditions; these include biological sources (e.g., pollen, plant debris, viruses, bacteria, fungi and spores), anthropogenic sources (e.g., biomass burning, fossil-fuel combustion, crop cultivation and soil dust from construction and traffic), levels of ozone and hydroxyl radicals in the atmosphere, wind speed, temperature, humidity, atmospheric pressure, etc. (Milne and Zika, 1993; Miguel et al., 1999; Mandalakis et al., 2011; Bianco et al., 2017; Liu et al., 2017; Song et al., 2017). Actually, the factors affecting the levels of aerosol amino acids remain poorly understood, and even fewer studies have been conducted on the precipitation amino acids. In addition, based on investigations of aerosol amino acids, the free amino acids in aerosols have been well documented to have significant seasonal variation. For example, Rathnayake et al. (2016) reported lower concentrations of aerosol free amino acids in cold seasons (e.g., autumn and winter) than in warm seasons (e.g., spring and summer) in Iowa in the midwestern United States. A study by Di Filippo et al. (2014) revealed that the concentration percentages of free amino acids in coarse, fine and ultrafine particulate matter were 0.6%, 1.0% and 7.4% in summer and 0.3%, 0.7% and 3.9% in winter in the area surrounding Rome, respectively. Differently, higher levels of aerosol free amino acids have been measured in the cold season in both Xi'an and Guangzhou in China (Ho et al., 2015; Song et al., 2017). Clearly, the different seasonal patterns of aerosol amino acid concentrations may also be closely linked to different sources and atmospheric conditions, as previously illustrated. However, seasonal trends of precipitation amino acids have not yet been widely characterized, which may provide useful insights into the season-partitioned eco-environmental effects and origins of the dissolved ON in precipitation.

The present study underscores the role of hydrolyzed amino acids in precipitation in the biogeochemical N cycle at a suburban site in Guiyang city (southwestern China) by collecting and analyzing precipitation samples across four seasons in 2017–2018. On this basis, we expect to further improve our understanding of atmospheric amino acid-N from the perspective of biogeochemistry; specifically, we attempt (1) to evaluate the concentration levels and seasonal variations in the hydrolyzed amino acids (HAAs) in precipitation in suburban Guiyang; (2) to investigate the major potential sources of the HAAs in precipitation in suburban Guiyang; and (3) to reveal the possible atmospheric processes associated with the precipitation HAAs in the atmosphere.

2. Materials and methods

2.1. Study site description and sample collection

Guiyang city is located in a wide karst valley basin (an inland area far from the sea) in southwest China; Guiyang city is a typical developing

city with a large and dense urban population and heavy traffic. A more detailed description about this city has been exhibited in our previous studies (Xu et al., 2018a, 2018b). The sampling site (26.65°N, 106.62°E) was selected at the Institute of Geochemistry, Chinese Academy of Sciences, and is characterized by a suburban area with large inputs of primary biological aerosol particles and few impacts from urban pollution emissions. There are four urban atmospheric environmental monitoring sites within four km of the sampling site, making this area ideal for assessing atmospheric conditions in a timely manner (e.g., data of air pollutants and some important meteorological observations) and continuously observing atmospheric processes. In particular, the daily precipitation amount and average air temperature are shown in Fig. 1a. The sample equipment, which consisted of a large polyethylene funnel connected to a polyethylene bottle, was placed on the spacious rooftop of a five-storey building without pollution sources. Both the funnels and bottles were soaked in a 20% hydrochloric acid solution for over 24 h. Prior to deployment (two sets of equipment were placed at a time), the funnels and bottles were rinsed at least four times with ultrapure water and then dried. In order to minimize the influences of dry deposition on the samples, the samplers were retrieved after the collection of rainwater was completed (less than 5 h of exposure to dry atmospheric conditions in overnight events and no more than 1 h for daytime) and manually placed before the next rain event; moreover, the sampling duration was strictly controlled to be less than 24 h in most cases, with the exception of shower events (the sampler was covered with a large clean polyethylene sheet until the next rainfall event) in summer. The precipitation samples were continuously collected at the research site previously mentioned from 23 May 2017 to 6 April 2018. Since the collection site was in close proximity to the laboratory, a portion of sample at each collection timepoint was immediately filtered using pre-combusted 0.7 μm glass fiber filters. Each filtered sample was divided into two parts: 50 mL of each sample was transferred to a clean glass bottle, and the rest was placed in a clean polyethylene bottle. All rainwater samples were stored at -30°C until analysis.

2.2. Chemical analyses

An Elementar Vario total organic carbon (TOC)/total nitrogen (TN) analyser (Elementar, Frankfurt, Germany) was used to detect the

concentrations of total dissolved nitrogen (TDN) in the samples. The detection limit of the analytic methods was 0.1 mg L^{-1} for TDN. Dissolved organic nitrogen (DON) was defined as the difference between TDN and dissolved inorganic nitrogen (the sum of NO_3^- , NO_2^- and NH_4^+). The NO_3^- and NO_2^- concentrations in rainwater were analysed using an ICS-90 ion chromatography system (Dionex, California, USA) with detection limits of 0.08 mg L^{-1} and 0.02 mg L^{-1} , respectively. However, less than 1% of the NO_x^- concentrations were from NO_2^- in most of the samples, and therefore, we neglected the contribution of NO_2^- to NO_x^- . Regarding the concentrations of NH_4^+ , spectrophotometry based on Nessler's reagent was used, and the detection limit was 0.1 mg L^{-1} . The relative standard deviations of replicate measurements of the standard samples were less than 2%, 5% and 2% for NO_3^- , NH_4^+ and TDN, respectively.

The amino acids were measured using the developed HPLC-based amino acid analysis method described by Xu and Xiao (2017) (details in the Supporting Information). Prior to analysis, the procedures of amino acid extraction were performed according to modified existing methods (Wedyan and Preston, 2008; Mandalakis et al., 2010). Briefly, 40 mL of filtered samples were freeze-dried and subsequently re-dissolved in 2 mL of 0.1 mol L^{-1} hydrochloric acid (HCl) solution. After the addition of a freshly prepared ascorbic acid solution and internal standards (α -aminobutyric acid and sarcosine), the re-dissolved samples were hydrolyzed with 2 mL of 12 mol L^{-1} HCl in a N_2 -bubbled and tightly sealed hydrolysis tube at 110°C for 24 h. Later, the hydrolysate that underwent N_2 blowing (HCl levels were thus decreased) was freeze-dried. Finally, the samples were re-dissolved in $400\text{ }\mu\text{L}$ of HCl (mol L^{-1}). The samples were directly analysed using HPLC after filtration through a $0.22\text{ }\mu\text{m}$ membrane. A typical chromatogram for the amino acid standard solution is exhibited in Fig. S1. Generally, the detectable amino acids based on the HPLC method included glutamic acid (Glu), glutamine (Gln), aspartic acid (Asp), theanine (Thea), α -aminobutyric acid (AABA), serine (Ser), tryptophan (Trp), leucine (Leu), glycine (Gly), norvaline (Nva), threonine (Thr), γ -aminobutyric acid (GABA), arginine (Arg), lysine (Lys), isoleucine (Ile), citrulline (Cit), hydroxyproline (Hyp), ornithine (Orn), alanine (Ala), proline (Pro), phenylalanine (Phe), valine (Val), histidine (His), cystine (Cy2), sarcosine (Sar), tyrosine (Tyr), and methionine (Met). In present study, the relative standard deviations (RSD), method determination limits (MDLs),

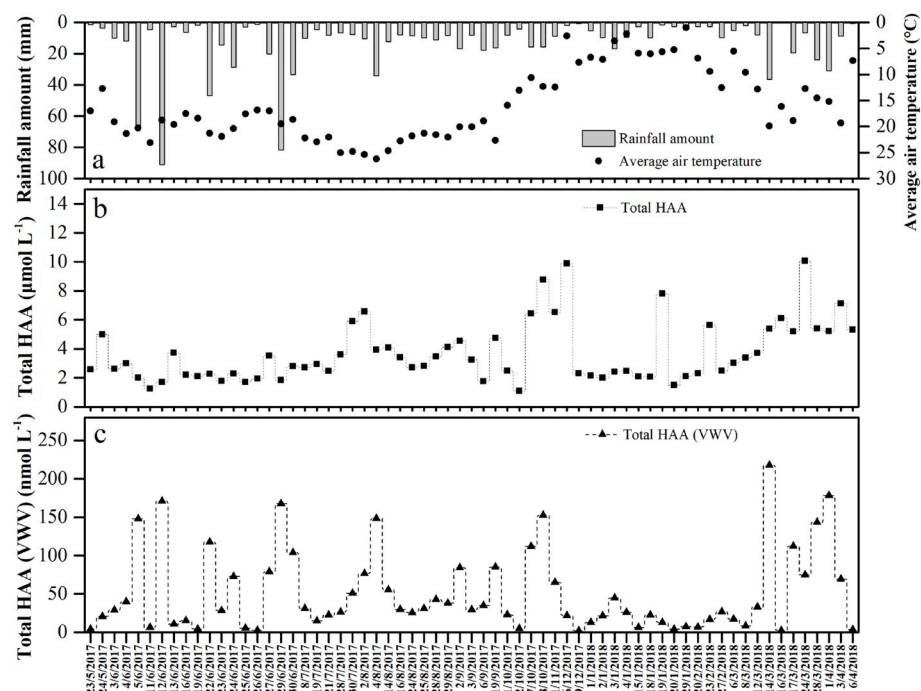


Fig. 1. Daily precipitation amounts and average air temperatures (a), total HAA concentrations (b), and volume-weighted values (VWV, which was calculated via the individual rainfall amounts in every investigated precipitation event and the total rainfall amount throughout the whole investigation period, namely $(C_{\text{actual}} \text{ concentration in each sample} \times M_{\text{individual rainfall amount}}) / M_{\text{annually total rainfall amount}}$) of the total HAA concentrations (c) in the daily integrated precipitation collected in 65 days from May 2017 to April 2018 in suburban Guiyang, China.

recoveries and correlation coefficients of the amino acid analysis are also detailed in Table S1. All data used in the study represent the mean of at least two measurements of each sample.

2.3. Auxiliary meteorological information

To determine the long-distance transport pathways of the air masses arriving at the sampling site in every investigated precipitation event, a program of TrajStat (version 1.4.4R5) run in MeteInfoMap software (version 1.4.9R2) was used to produce 5-day (120 h) back trajectories of these air masses beginning at 500 m above sea level. The meteorological data (GDAS dataset) used for calculating the back trajectories were obtained from the database of NOAA's Air Resources Laboratory (<https://ready.arl.noaa.gov/archives.php>). Other meteorological data (e.g., humidity, temperature, atmospheric pressure, precipitation, wind speed and ozone concentration) during the sampling period were collected from local environmental monitoring stations.

2.4. Statistical analyses

Regression analysis was conducted with Origin software (version 9.0; OriginLab Corporation, Massachusetts, USA) to analyse correlations between the response and explanatory variables. Moreover, all graphs were also plotted via Origin software (version 9.0; OriginLab Corporation, Massachusetts, USA). Differences in the concentrations of chemicals between the investigated groups were examined using SPSS software (version 19.0; SPSS Science, Chicago, USA).

3. Results and discussion

3.1. Compositions and variation patterns of the HAAs in precipitation

Eighteen amino acids were detected in the precipitation samples that underwent acid hydrolysis process. These detectable HAAs included Glu, Asp, Ser, Leu, Gly, Thr, GABA, Arg, Lys, Ile, Ala, Pro, Phe, Val, His, Met, Trp and Tyr. Among them, His was only measured in a few samples, with a negligibly low concentration. Thus, His was excluded from the further analysis. Moreover, due to Met and Trp could not be efficiently recovered during acidic hydrolysis, these compounds are not further discussed and were only included in the total amino acids. Considering the conversion of Gln and Asn into the

corresponding Glu and Asp during hydrolysis, the concentrations of Glu and Asp in the hydrolyzed samples represented the sum of the Glu and Gln sample concentrations and the sum of the Asp and Asn sample concentrations, respectively. As exhibited in Fig. 2, Glu (46.5–1468.9 nmol L⁻¹), Gly (2.3–1468.2 nmol L⁻¹) and Pro (94.3–1297.1 nmol L⁻¹) represented relatively high fractions of the total amino acids; subsequently, Asp, Ala, Lys and Ser accounted for 7.7%, 6.8%, 6.5% and 6.3% of the total HAAs, respectively. Minor species, such as GABA, Phe, Ile, Lue, Arg and Val, contributed 2.0–5.8% of the total HAAs. In contrast, Tyr and Thr were observed in the smallest amounts, contributing less than 1% and less than 2% to the total HAA concentrations, respectively.

In a study conducted by Yan et al. (2015), higher levels of precipitation Glu and Gly were also detected in a coastal rural area of Korea (4.3–60.8 nmol L⁻¹ for Glu; 3.4–462.0 nmol L⁻¹ for Gly) as well as in an urban site near the coast of the Korea peninsula (6.9–756.0 nmol L⁻¹ for Glu; 6.4–653.0 nmol L⁻¹ for Gly); similarly, the Tyr levels measured in the abovementioned two areas were the least abundant and accounted for less than 1%. Many previous studies regarding aerosol organic N also showed a significant dominance of Gly in both the total free amino acids and combined amino acids (Zhang and Anastasio, 2003; Mandalakis et al., 2011; Scalabrin et al., 2012; Zangrando et al., 2016; Song et al., 2017). It is noteworthy that Gly is not only an essential component of animal fibrous proteins (collagen) but is also enriched in elastin and certain keratins (Voet et al., 2008; Samy et al., 2013). The universal existence of Gly in the environment may thus be an important factor resulting in the high Gly levels in aerosols and precipitation. Furthermore, the enrichment of Gly may also be partially explained by the following two aspects. First, the absence of a side chain for Gly resulted in low rate constants for further reactions with oxidants in the atmosphere. Low photochemical reactivity was observed in the aqueous phase, with a half-life over 2000 h (Mcgregor and Anastasio, 2001). On the other hand, the low steric hindrance of the initial radical generation on the peptide backbone caused a higher release of Gly from the combined amino acids, particularly when Gly was at the end of the carbon chain (Liu et al., 2017). Regarding Tyr, its half-life has only 23 h in the aqueous phase following exposure to sunlight (Mcgregor and Anastasio, 2001). Other important individual amino acids, including Pro, Asp, Ser, Ala, Lys, Lue, Pro and Val, have all been observed to be relatively dominant contributors to the amino acid pool in some freshwater and surface seawater together

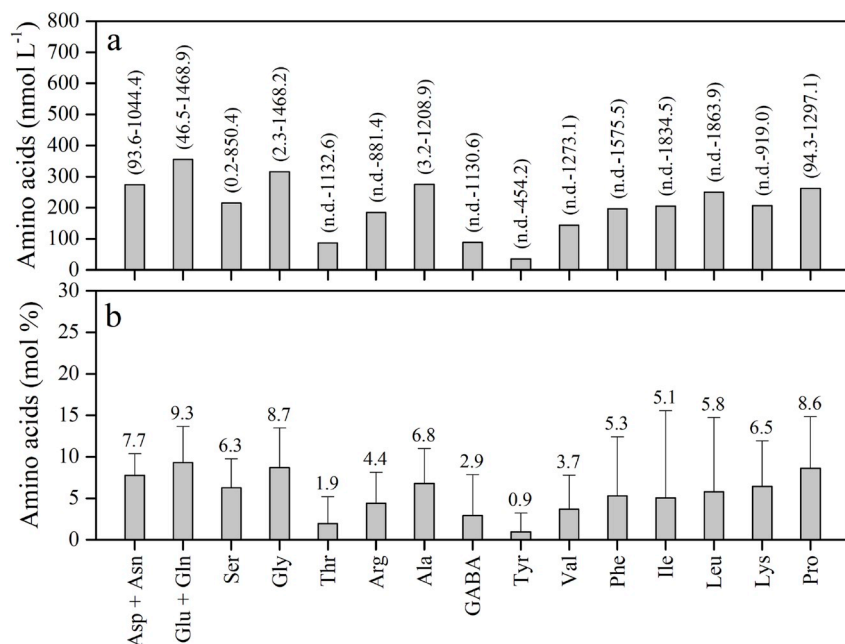


Fig. 2. Concentrations of HAAs (a) and average molar compositions (%) of the total HAAs (b) in the precipitation samples collected from a suburban site in Guiyang. The values above the bars represent the ranges in the HAA concentrations (a) and the average results of the proportional contributions (b), respectively. The vertical lines represent the standard deviations. The abbreviation “n.d.” indicates that analytes were not detected.

with snow and ice environments (Thomas, 1997; Wedyan and Preston, 2008; Tsukasaki and Tanoue, 2010; Barbaro et al., 2017). The relative compositions of amino acids in the present case were essentially consistent with the results found in the atmospheric aerosol particles and precipitation from different areas; moreover, the main species between them were similar (Mandalakis et al., 2011; Di Filippo et al., 2014; Yan et al., 2015; Barbaro et al., 2017; Song et al., 2017). However, there were still a few exceptions. For example, Mace et al. (2003b) found a marked contribution of Arg (51.0%) instead of Gly (4.8%), Glu (3.0%), Ser (2.0%) and Asp (1.4%) to the total free amino acid-N in rainwater collected on the island of Tasmania (Australia). This discrepancy was possibly explained by (1) high regional differences associated with different meteorological conditions; additionally (2), the degradation of Arg-rich pollen may be a vital source for precipitation free amino acids. Indeed, currently available reports on precipitation HAAs are too sparse to make extensive comparisons, showing an urgent need for further research on the precipitation amino acids.

Fig. 1 exhibits the seasonal cycles of the total HAA concentrations together with the corresponding patterns of rainfall amounts and air temperatures over a 12 month period (from May 2017 to April 2018). The average concentrations of total HAAs in precipitation in each season are also summarized in Table 1. To our knowledge, this is the first investigation on the characteristic variations in a full annual cycle of total HAAs in inland suburban regions far from a coastline. The total HAA concentrations in precipitation varied from 1.1 to 10.1 $\mu\text{mol L}^{-1}$, with an average of 3.7 $\mu\text{mol L}^{-1}$ (volume-weighted average = 3.3 $\mu\text{mol L}^{-1}$). The concentrations of total HAAs in precipitation showed a distinct seasonal cycle, with minimum levels in cold seasons (during December 2017 and early March 2018; Fig. 1). Because precipitation amounts can have a marked impact on the abundance of HAAs, the seasonal patterns of total HAAs in precipitation were better reflected following a transformation of the direct volume concentrations to volume-weighted values (the VWV was calculated via the individual rainfall amounts in every investigated precipitation event and the total rainfall amount throughout the entire investigation period) (Fig. 1c). It is observed that the total HAAs represented less than 6% of the DON in different seasons on average. The minimum average contributions of HAAs to the DON were also recorded in cold seasons, which were close to the level of 6% reported in a coastal rural area (Yan et al., 2015). Previous studies also analysed the seasonal cycle of amino

acids in atmospheric aerosols. It is indicated that lower protein and free amino acid concentrations occurred in cold seasons (e.g., late autumn and winter) than in warm seasons (e.g., late spring and summer) in some South American, North American and European sites (Mace et al., 2003a; Kuznetsova et al., 2005; Barbaro et al., 2011; Di Filippo et al., 2014; Rathnayake et al., 2016), and conversely, an enrichment of aerosol proteins and free amino acids was found in cold seasons in many sites in China (Kang et al., 2012; Ho et al., 2015; Song et al., 2017). In addition, the only available research on precipitation HAAs in coastal areas (the Korea peninsula) did not discern a significant dominance of the total HAAs in precipitation in relatively cold months (Yan et al., 2015). For the different seasonal patterns of atmospheric proteinaceous compounds, a more plausible explanation is that these compounds can be influenced by different environmental behaviours and sources in different areas. Thus, a better understanding of the possible sources of atmospheric proteinaceous compounds (particularly the combined amino acids in precipitation) is of great significance and will likely provide insights into the potential formation processes and environmental effects of atmospheric amino acids in investigated regions.

3.2. The major potential sources of the HAAs in precipitation

To evaluate whether the precipitation amino acid concentrations relied on the air masses arriving at the sampling site, 5-day air mass back trajectories were calculated seasonally for different sampling events (Fig. S2). In general, the air mass trajectories mainly originated from the southwestern and southeastern ocean areas in summer and from the southeastern ocean areas and northern China in spring but from the local region with a relatively short pathway in autumn and winter. The seasonal variations (excluded winter) in the total HAA levels (volume-weighted concentrations based on the total precipitation amount in 65 days) in precipitation were not remarkably different ($P > 0.05$); additionally, there was no significant difference between the total HAA levels (volume-weighted concentrations based on the total precipitation amount in 65 days) in the summer and winter precipitation (Table 2). Further, considering that the concentrations of total HAAs in summer (likely related to marine sources) primarily fell within those concentration ranges in autumn and winter (air mass trajectories were limited in the local region with a relatively short

Table 1

Descriptive statistics for the DON, NH_4^+ , NO_3^- and total HAA concentrations in the precipitation samples collected at the suburban site in Guiyang city.

	DON ($\mu\text{mol L}^{-1}$)	NO_3^- ($\mu\text{mol L}^{-1}$)	NH_4^+ ($\mu\text{mol L}^{-1}$)	Total HAA ($\mu\text{mol L}^{-1}$)
2017 Jun - 2017 Aug (summer)				
(Min.); (Max.) ($n = 29$)	(1.0); (365.6)	(3.5); (146.4)	(1.9); (185.8)	(1.3); (6.6)
Median	36.2	17.6	39.1	2.7
(Mean)/(VWA ^a)	(57.7)/(34.6)	(33.4)/(20.1)	(53.7)/(39.3)	(2.9)/(2.5)
2017 Sep - 2017 Nov (autumn)				
(Min.); (Max.) ($n = 9$)	(58.0); (490.6)	(5.7); (357.6)	(24.2); (251.5)	(1.1); (8.8)
Median	222.6	26.0	45.4	4.5
(Mean)/(VWA ^a)	(249.7)/(250.2)	(95.4)/(90.7)	(88.2)/(91.9)	(4.4)/(4.8)
2017 Dec - 2018 Feb (winter)				
(Min.); (Max.) ($n = 14$)	(44.5); (516.1)	(26.0); (420.7)	(49.2); (381.3)	(1.5); (9.9)
Median	172.9	75.8	187.1	2.3
(Mean)/(VWA ^a)	(198)/(135.4)	(97.3)/(66.7)	(186.0)/(170.6)	(3.4)/(2.7)
2018 Mar–Apr and 2017 May (spring)				
(Min.); (Max.) ($n = 13$)	(79.8); (447.9)	(21.8); (231.7)	(80.5); (280.7)	(2.6); (10.1)
Median	199.7	89.3	204.1	5.2
(Mean)/(VWA ^a)	(240.7)/(235.6)	(99.1)/(102.4)	(205.3)/(199.0)	(5.2)/(5.4)
2017 May - 2018 Apr (all samples)				
(Min.); (Max.) ($n = 65$)	(1.0); (516.1)	(3.5); (420.7)	(1.9); (381.3)	(1.1); (10.1)
Median	112.6	43.9	80.6	2.9
(Mean)/(VWA ^b)	(151.1)/(104.0)	(68.9)/(47.3)	(117.3)/(84.1)	(3.7)/(3.3)

^a Volume-weighted average (based on the precipitation amount (mm) in different seasons, namely the sum of (C_{actual} concentration in each sample $\times M_{\text{individual}}$ rainfall amount)/ $M_{\text{seasonally total}}$ rainfall amount).

^b Volume-weighted average (based on the precipitation amount (mm) in 65 days from May- 2017 to April 2018 in suburban Guiyang, namely the sum of (C_{actual} concentration in each sample $\times M_{\text{individual}}$ rainfall amount)/ $M_{\text{annually total}}$ rainfall amount).

Table 2Seasonal concentration averages for DON, NH_4^+ , NO_3^- and the total HAA in the precipitation samples collected at the suburban site.

	DON (nmol L ⁻¹) ^a	NO_3^- (nmol L ⁻¹) ^a	NH_4^+ (nmol L ⁻¹) ^a	Total HAA (nmol L ⁻¹) ^a
2017 Jun - 2017 Aug (summer)	745.5 (b)	434.3 (b)	847.6 (b)	55.0 (ab)
2017 Sep - 2017 Nov (autumn)	3443.4 (a)	1247.6 (a)	1265.3 (ab)	65.7 (a)
2017 Dec - 2018 Feb (winter)	918.7 (b)	476.3 (b)	1063.5 (ab)	16.6 (b)
2018 Mar - Apr and 2017 May (spring)	2967.4 (a)	1289.5 (a)	2555.4 (a)	68.0 (a)

The same lowercase letters indicate no seasonally significant difference between groups of samples ($P > 0.05$).

^a Arithmetic averages were calculated via the volume-weighted concentrations ($(C_{\text{actual concentration in each sample}} \times M_{\text{individual rainfall amount}}) / M_{\text{annually total rainfall amount}}$).

pathway) (Table 1), it can be indicated that the impact of marine sources was not predominant. Thus, although the air mass trajectories presented clear seasonal variations, these variations did not appear to reflect a significant impact from the air mass origins on the concentrations of precipitation HAAs.

Previous studies have shown that biomass burning, marine biogenic emissions, continental biogenic releases (including viruses, protozoa, algae, fungi, bacteria, pollen, spores, epithelial cells of humans and animals, and fragments of insects and plants), and agricultural activities are the main sources of aerosol proteinaceous matter (Cape et al., 2011; Kang et al., 2012; Matos et al., 2016; Song et al., 2017). Commonly, these sources are also the primary contributors of atmospheric NO_x , NH_3 and atmospheric ON (Cape et al., 2011; Pan et al., 2016; Liu et al., 2017; Xu et al., 2018a). Table 1 shows the descriptive statistics for the DON, nitrate (NO_3^-) and ammonium (NH_4^+) concentrations in the precipitation samples collected at the suburban site. The concentrations of DON, NO_3^- and NH_4^+ in precipitation ranged from 1.0 to $516.1 \mu\text{mol L}^{-1}$ for DON, from 3.5 to $420.7 \mu\text{mol L}^{-1}$ for NO_3^- , and from 1.9 to $381.3 \mu\text{mol L}^{-1}$ for NH_4^+ . DON accounted for 44.8% of the precipitation TDN deposition during the study period, similar to the value of 41% reported in the Guiyang area (Liu et al., 2017). In addition, clear seasonal variations in the inorganic N species (NO_3^- and NH_4^+) were recorded, with relatively low levels in summer and winter,

which is consistent with the variation patterns of the DON and total HAA concentrations in precipitation (Tables 1 and 2). Further, to investigate the possible sources of precipitation amino acids, Fig. 3 compared precipitation DON and total HAA concentrations with both the nitrate and ammonium concentrations in individual precipitation samples (a more detailed comparison of their season-partitioned concentrations is exhibited in Fig. S3). The nitrate concentrations exhibited a better positive correlation with the total HAA concentrations than the ammonium concentrations, likely suggesting that the sources for precipitation amino acids in suburban Guiyang are preferentially linked with NO_3^- sources relative to NH_4^+ sources. Similarly, the DON concentrations were also obviously correlated with both the nitrate and ammonium concentrations.

According to the $\delta^{15}\text{N}$ signatures of the nitrate (NO_3^-) and ammonium (NH_4^+) in precipitation in the Guiyang area, Liu et al. (2017) revealed that non-fossil N oxides contributed around 50% of the N in precipitation on average, with $24 \pm 12\%$ from biomass burning and $26 \pm 5\%$ from the microbial N cycle. The NH_4^+ in Guiyang precipitation originated more from agricultural activities ($22 \pm 12\%$ from livestock waste and $22 \pm 10\%$ from fertilizers) and biomass burning ($17 \pm 8\%$) than from the combustion of fossil flues ($19 \pm 11\%$ from vehicle exhaust and $19 \pm 12\%$ from coal combustion) (Liu et al., 2017). Fossil-derived N compounds attributed to vehicle exhaust and

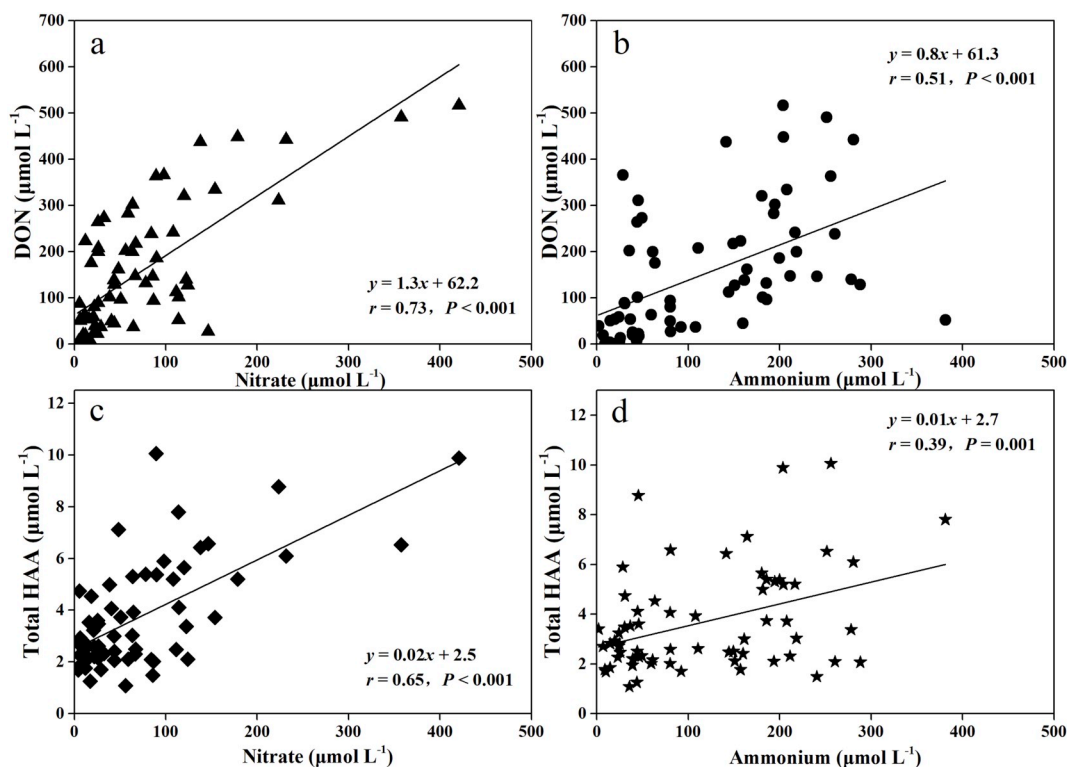


Fig. 3. Relationships of the DON and total HAA concentrations with both the nitrate and ammonium concentrations in individual precipitation samples collected in suburban Guiyang from May 2017 to April 2018.

coal combustion are emitted mostly in urban centres. Xu et al. (2018a) reported that pollution signals (NH_3 and NO_x) were significant in urban areas but virtually disappeared in suburban areas over a 25 km distance from the urban centre. This finding suggested that non-fossil-derived N compounds (biomass burning, continental biogenic releases, and agricultural activities) were the dominant forms of N in the precipitation in non-urban centres (similar to current study site). Due to the chemical complexity of atmospheric ON, it is difficult to quantify the proportional contribution of each type of source. However, based on the previous discussion, biomass burning, marine biogenic emissions, continental biogenic releases, and agricultural activities were still the dominant sources of atmospheric ON in the current study. This result can also be partly reflected by the significant positive relationships of the precipitation DON concentrations with both the nitrate and ammonium concentrations in precipitation. Thus, from what has been previously discussed, it would be reasonable to deduce that a large amount of biomass burning (e.g., straw, because straw is burned locally after crop harvests) in autumn and enhanced agricultural activities in spring may lead to the increase in DON, HAA, NO_3^- and NH_4^+ levels in precipitation. In particular, during spring, the degradation of pollen (biogenic category) was a possible source for the high amino acid concentrations measured in the precipitation (in some spring rainfall events, yellow pollen particles can be observed directly on the surface of precipitation). As suggested by Scheller (2001), pollen grains were responsible for the large levels of amino acids in dew sampled in spring in Germany. In contrast, decreases in agricultural activities and biomass burning in summer and winter in suburban Guiyang could be responsible for the relatively lower DON, HAA, NO_3^- and NH_4^+ levels in the precipitation. Moreover, due to low temperatures in winter, which causes biogenic activities to decrease (Millard et al., 2006; Li et al., 2016), continental biogenic releases may not be a critical factor for determining the N compound levels in precipitation. Further, we can conclude a strong influence of non-fossil-derived N compounds

(biomass burning, continental biogenic releases, and agricultural activities) on precipitation N in suburban Guiyang. Consequently, the significant correlations of the total HAA concentrations with both the nitrate ($r = 0.65$) and ammonium (despite the r value being relatively low) concentrations indicated that biomass burning, continental biogenic releases, and agricultural activities may have also been the dominant contributors of precipitation amino acids.

3.3. Potential atmospheric processes related to the HAAs in precipitation

We investigated the possible impacts of meteorological conditions on the HAA levels in precipitation. Atmospheric pressure, relative humidity and wind speed were not observed to exert any remarkable impacts on the total HAA concentrations (VWV) in precipitation ($P > 0.05$) (Fig. S4), whereas the influences of the average air temperature and the highest air temperature were statistically significant ($P < 0.05$) (Fig. S5). The positive correlations of the logarithm of the total HAAs (VWV) with both the average air temperature and the highest air temperature may imply an enhancement of the enzymatic or photocatalytic hydrolysis of aerosol high molecular weight proteinaceous matter (e.g., pollen and fungal spores with a diameter greater than $1 \mu\text{m}$; Matos et al. (2016)) with appropriate increases in temperature (Milne and Zika, 1993; Zhang and Anastasio, 2003; Mandalakis et al., 2011). It is worth noting that the precipitation samples in this study were filtered using $0.7 \mu\text{m}$ filters. Therefore, when a great deal of degraded aerosol proteinaceous matter was removed via wet deposition, the levels of low molecular weight proteinaceous matter in precipitation increased directly.

On the other hand, previous studies have exhibited that ozone can facilitate the release of proteinaceous matter derived from atmospheric vegetable matter (e.g., pollen) (Beck et al., 2013; Ribeiro et al., 2017). This release is an important factor driving increases in biological aerosol levels. Under the impact of ozone, proteinaceous matter can

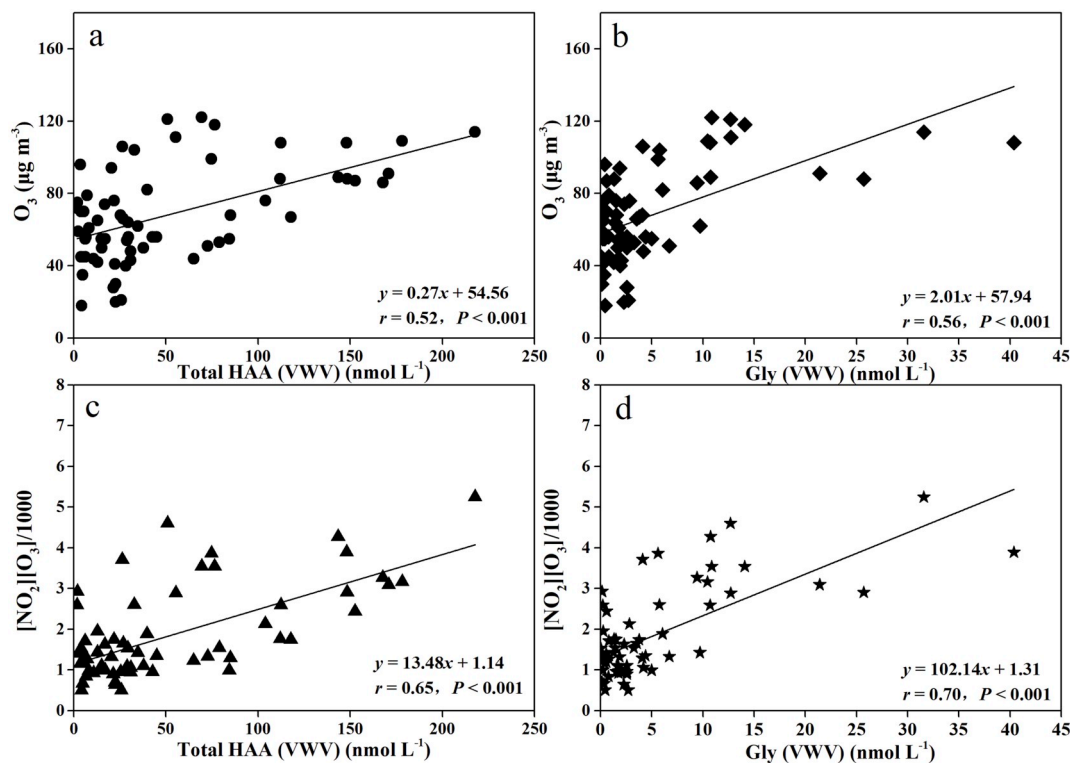


Fig. 4. Relationships of the ozone concentrations (8 h) and $[\text{NO}_2]/[\text{O}_3]$ with both the total HAA and Gly concentrations (VWV) in individual precipitation samples collected in suburban Guiyang from May 2017 to April 2018.

also undergo oxidation, oligomerization and nitration, which enhances the allergenic possibility induced by proteinaceous matter (Mcgregor and Anastasio, 2001; Franze et al., 2005; Shiraiwa et al., 2012; Kampf et al., 2015). Recent analyses on aerosol proteins and free amino acids have provided indirect evidence for the release of low molecular weight amino acids following ozone-induced oxidation of proteins and peptides (Song et al., 2017). It has been suggested that a close link exists between atmospheric proteinaceous matter and ozone. In order to further confirm this conclusion, the atmospheric ozone (8 h) concentrations recorded during the entire sampling period were compared with the concentrations of precipitation HAAs. As shown in Fig. 4 a and b, the total HAA concentrations (VWV) presented a positive correlation with the atmospheric ozone concentrations ($P < 0.01$); furthermore, the correlation of Gly concentrations (VWV) with the atmospheric ozone concentrations was also obvious ($P < 0.01$). Laboratory simulation experiments have suggested that low molecular weight amino acids, especially Gly, can be either nitrified or preferentially released from proteins when proteinaceous matter reacts with hydroxyl radicals ($\cdot\text{OH}$), nitrogen dioxide and ozone in the aqueous phase or the atmosphere (Mcgregor and Anastasio, 2001; Yang et al., 2010; Shiraiwa et al., 2011; Shiraiwa et al., 2012; Liu et al., 2017). A similar chemical process between proteinaceous matter and ozone may also occur in precipitation with continuous exposure to ozone, which caused an increased abundance of low molecular weight proteinaceous matter. In addition, the low molecular weight proteinaceous matter in aerosol particles (with a diameter less than $0.7\ \mu\text{m}$) can be dissolved into rainwater following an ozone-induced conversion of high molecular weight aerosol proteinaceous matter to low molecular weight proteinaceous matter. Accordingly, the total HAA and Gly concentrations in the atmosphere would be characterized by an interdependency with atmospheric ozone.

Regarding the relationships between atmospheric nitrogen dioxide and atmospheric amino acid compounds, Shiraiwa et al. (2012) reported a reactive uptake of nitrogen dioxide by aerosolized protein based on a model experiment. The results indicated that nitrogen dioxide was involved in protein nitration processes; briefly, proteins on the surface of the aerosol particles underwent fast nitration with continuous exposure to nitrogen dioxide. This finding was also reflected in the work of Franze et al. (2005), revealing that nitrogen dioxide can enhance the nitration of proteins in polluted urban atmospheres. In present study, both the total HAA and Gly concentrations (VWV) showed significant correlations with $[\text{NO}_2][\text{O}_3]$ ($P < 0.01$) (Fig. 4 c and d). Apparently, partial proteinaceous nitration or nitrogen dioxide-dependent atmospheric processes may directly lead to an increase in low molecular weight aerosol proteinaceous matter, implying that when it rains, low molecular weight aerosol proteinaceous matter is partly transferred into the precipitation. Combined with the previous discussion about the oxidation of proteinaceous matter by ozone and nitrogen dioxide, it can be concluded that proteinaceous matter in the atmosphere may be involved in both ozone- and nitrogen dioxide-related atmospheric processes. This would be likely to cause a further increase in bioavailable N.

4. Conclusions

This study investigated the seasonal variations, main sources and potential atmospheric processes of precipitation HAAs in a suburban site in Guiyang city. On the basis of the temporal trends in the concentrations of HAA, DON, NO_3^- and NH_4^+ in precipitation, a visible seasonal cycle with relatively low levels in summer and winter was identified for the species mentioned above. However, back-trajectory analysis together with seasonal patterns of the total HAA concentrations suggested that the air mass origins did not significantly affect the precipitation HAA levels. This result reflected that the seasonally different total HAA levels in precipitation may be attributed to differences in the contributing sources with seasonal cycles. The NO_3^- concentrations

exhibited a better positive correlation with both the DON and total HAA concentrations than the NH_4^+ concentrations, revealing that sources for precipitation amino acids in suburban Guiyang may be more associated with NO_3^- sources (from biomass burning, microbial activities and agricultural activities) than with NH_4^+ sources (from biomass burning and agricultural activities). Additionally, among a variety of meteorological parameters (atmospheric pressure, relative humidity, wind speed, average air temperature and the highest air temperature), only the average air temperature and the highest air temperature showed a potential influence on the total HAA levels in precipitation. The enrichments of precipitation HAAs with increasing air temperatures may be due to the enhanced degradation of high molecular weight atmospheric proteinaceous matter under conditions of relatively high air temperatures. Furthermore, the precipitation Gly and total HAA levels exhibited significant correlations with the product of atmospheric ozone and nitrogen dioxide, indicating that atmospheric proteinaceous matter may be involved in both ozone- and nitrogen dioxide-related atmospheric processes. Therefore, this study enhances current understanding of the origins and related atmospheric processes of atmospheric proteinaceous matter; moreover, it may provide valuable insights into the fate and role of atmospheric proteinaceous matter in subsequent biogeochemical cycling of N. Further work should focus on in situ chemical transformations of atmospheric proteinaceous matter, including oxidation, particle/liquid conversions and polymerization.

Declaration of interest statement

The authors declare that there are no competing financial and academic interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.atmosenv.2019.05.011>.

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