Referee Comments

Title: Mass-spectrometric identification of primary biological particle markers: indication for low abundance of primary biological material in the pristine submicron aerosol of Amazonia

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General Comments:

This paper describes aerosol mass spectra of several amino acids, carbohydrates, peptides, and proteins, in an attempt to determine tracer m/z peaks to identify primary biological aerosol particles (PBAP) in the Central Amazon Basin. The authors chose m/z 30 and 42 for amino acid markers and m/z 60, 61, and 73 as carbohydrate markers. Schneider et al. used the inverses of the mean fraction of the marker peaks in the amino acids and carbohydrate standards as scaling factors for the marker peak intensities in the ambient signal to determine the ambient fraction of amino acids and carbohydrates (7.5% and 5.6%). Assuming that amino acids and carbohydrates account for 2/3 of the mass of a biological cell, the authors attribute that PBAP account for up to 20% of the mass fraction of the submicron organic aerosol in the Central Amazon Basin. However, the calculation determining the mass fraction of PBAP needs to be reevaluated. This paper should be published after some revisions.

Specific Comments:

1. Free amino acids have been found in atmospheric precipitation, cloud water droplets, and aerosols (Mopper and Zika, *Nature,* 1987) (Matsumoto and Uematsu, *Atmos. Environ*., 2005). Previous studies have found that free amino acids contribute up to 20% of the total amino acids found in PM2.5 aerosol (Zhang and Anastasio, *Atmos. Environ.,* 2003). Such free amino acids have been suggested to be the product of photooxidation and photodegredation of biological material (Milne and Zitke, *J. Atmos. Chem.,* 1993). In tropical forests, the majority of PBAP have been found in the supermicron fraction, where up to 80% of the number concentration can be categorized as PBAP (Graham et al., *J. Geophys. Res.,* 2003). Considering the majority of PBAP is confined to the supermicron fraction, one would speculate that the majority of free amino aerosols would be from the photooxidation and photodesociation of larger biological aerosols. However, the method described by the authors does not distinguish free amino acids from submicron PBAP. Thus it appears that the calculation describing the quantification of amino acids in the ambient atmosphere quantifies amino acids from submicron PBAP, in addition to free amino acids, which could have originated from submicron PBAP, but most likely from supermicron PBAP. The authors should include in the manuscript that the calculation of amino acids is not limited to submicron PBAP. This is only a

minor point if the authors suggest that 20% of submicron aerosol is attributed to bacteria and viruses in the atmosphere.

2. In calculating the scaling factor for the amino acid class, the authors chose not to include glycine and tryptophan. However, glycine has been found to be the most or second-most abundant amino acid in ambient PM2.5 aerosol, fog-water, and rain-water samples (Zhang and Anastasio, *Atmos. Environ.,* 2003) (Milne and Zitke, *J. Atmos. Chem.,* 1993). Using an average scaling factor that excludes glycine (as well as tryptophan) appears to not accurately describe the amino acids present in the atmosphere. The authors should consider including a range for the scaling factor that both includes and excludes glycine and tryptophan. In addition the authors should consider using a weighted average for the scaling factor for amino acids that would more accurately describe the distribution of amino acids found in the atmosphere and/or bacteria and viruses.

3. The upper limits of the fractions of amino acids and carbohydrates were calculated as: $$fraction = SF_{class} \sum_i \left(f_i - f_i^{background} \right)$$ However, the authors used a value of 0 to describe $f_{i,background}$, even though background values for other campaigns were presented in the manuscript. Since background values are known for some regions, including this information into the calculation could produce at most, a lower bound for the percentage of PBAP in the Amazon Rainforest.

4. On page 19158, the authors state that the carbohydrate fraction peaked during the night on multiple occasions. According to this manuscript and Chen et al., 2009, the remaining organic aerosol presented here is primarily biogenic SOA. Since the biogenic SOA had a diel pattern (Chen et al., *GRL,* 2009), which peaked during the day due to photochemical production, is the increase in PBAP at night due to a decrease in photochemical biogenic SOA production?

5. The authors describe submicron PBAP as primarily bacteria and viruses. Vegetative detritus has been found to be a constituent of fine organic aerosol (Rogge et al., *Environ. Sci. Technol.,* 1993). Do the authors feel vegetative detritus and vegetative matter from wind-blown leaf abrasions do not attribute to the organic mass of submicron aerosols?

6. On page 19160, the authors claim, "Because amino acids in the form of proteins and carbohydrates together account for about 2/3 of a biological cell (Munk, 2000), we can conclude that the upper limit of the total contribution of PBAPs to the submicron organic mass concentration was no more than 20%." It appears that the authors calculated the PBAP organic mass contribution as follows:

To get the mass fraction of biological material of organic aerosol ($f_{B,A}$=$Mass_B$/$Mass_A$) the equation applied by the authors is:

$$f_{B,A} = \frac{f_{P,A} + f_{C,A}}{f_{P,B} + f_{C,B}}$$

Where a biological cell has been represented as,

$$f_{P,B} + f_{C,B} = \frac{2}{3}$$

Since the percentage of proteins and carbohydrates within the dry mass of a cell is not equivalent, the mass fraction of biological material of organic aerosol should be instead written as,

$$f_{B,A} = \frac{f_{P,A}}{f_{P,B}} + \frac{f_{C,A}}{f_{C,B}}$$

Knowing that for a biological cell, the dry mass contains 50% proteins and 15% carbohydrates then,

$$\phi = \frac{f_{\mathrm{C,B}}}{f_{\mathrm{P,B}}} = \frac{.15}{.50}$$

Since ϕ is fixed for the majority of biological cells, then $f_{\mathrm{C,A}} / f_{\mathrm{P,A}}$ (ambient ratio of .056/.075) should also be equivalent to ϕ. This raises the question as to why the ambient ratio is not equal to ϕ, if the organic matter measured is similar to PBAP, and thus biological cells. This could suggest that the photooxidation and photodegredation is faster for amino acids, than carbohydrates. However, if the ratio of carbohydrates to proteins is fixed, the fraction of biological material of organic aerosol should be calculated as follows (equation 3 rewritten),

$$f_{B,A} = f_{P,B}^{-1}(f_{P,A} + \phi^{-1} f_{C,A})$$

When the correct apportionment of protein and carbohydrates are considered (.50 and .15) with the ambient fractions (.075 and .056), the actual upper bound of PBAP should be 52% and not 20%.

Specific Comments:

1. Page 19151, Line 18: What was the justification for averaging over 12 hours?
2. Page 19161, Lines 3-5: Sentence appears to be out of context. Consider removing or moving to a different section.