The authors would like to thank all reviewers for their insightful comments which we believe helped to significantly improve this paper. When Reviewers cited each other and/or in the case of similar comments we grouped these by topic first to make the responses easier to follow.

Journal placement/atmospheric implications

- [T]he manuscript is focused more on the method development instead of the atmospheric application, which seems only a very minor addition to the paper and doesn't provide much new insight beyond a proof-of-concept. My feeling therefore is that the manuscript would be more suited for a journal more dedicated to the technical aspects of aerosol measurements, like Atmospheric Measurement Techniques. (Reviewer #1)
- My main concern, like the referees, is that the journal chosen is not the best fit for what is essentially an instrumentation paper to my mind. AMT would be a better fit. (Reviewer #4)

The authors agree that the single field dataset in the original manuscript could be expanded. We have added a different location and season - the 2010 CARES campaign - in order to make a stronger case. See Figure 7 and associated descriptions of the CARES dataset.

We feel strongly that ACP is the right destination for this paper because the topic is of significant interest to our community, there is significant laboratory and field data, and the results affect conclusions of many previously published papers in this area. We note in particular Pratt et al., (2009) and Creamean et al., (2013) which are widely cited studies where the conclusions hinge on the use of SPMS spectra to identify biological particles. Our paper expands upon this work and suggests the urgent need for expanded analysis.

In further defending inclusion of this paper in ACP and not AMT we note that calling this "an instrumentation paper" is not correct as it includes significant laboratory work, data analysis to elucidate a contemporary issue in aerosol studies and now multiple field data sets. One could take this argument further to say a paper centered on field or lab data with incomplete analysis would be applicable to ACP but a paper with significant data analysis would not; this is not in keeping with the ACP guidance "The main subject areas comprise atmospheric modeling, field measurements, remote sensing, and laboratory studies of gases, aerosols, clouds and precipitation... The journal scope is focused on studies with general implications for atmospheric science rather than investigations that are primarily of local or technical interest."

PBAP vs. biological phosphorus

- While in the abstract and most of the manuscript the impression is given primary biological aerosol particles (PBAP) could be clearly distinguished from other particles, only in the last section of the discussion (4.3) it becomes apparent that this is not the case at all: in fact, what can be distinguished with the algorithm is whether the particles contain "biological" or "inorganic" phosphorus. (Reviewer #1)
- [T]he title and introduction imply that bioaerosols will be distinguished from dust and fly ash using this method. However, it is stated in several areas of the results and discussion that what is really distinguished are biological or organic phosphorus and inorganic phosphorus. The

abstract and title should be updated to reflect what is actually being measured in this paper. (Reviewer #2)

• I also agree with the others in that the essence is not PBAP....rather a neat method of potentially distinguishing between them in the atmosphere. (Reviewer #4)

In order to address these concerns, a clear definition of "bioaerosol" as used in this paper is now in the introduction: "In this paper, "bioaerosol" is defined as primary biological aerosol particles (PBAP) (i.e. airborne whole and fragmentary bacteria, pollen and spores) and particles that contain fragments of PBAP as a part of an internal mixture." The following wording was also added in the introduction, "In this work, the presence of phosphorus in a mass spectrum is used as proxy for bioaerosol. All biological cells contain phosphorus because it is a component of nucleic acids and cell membranes. Distinguishing the specific phosphate signature of biological cells from other non-biological phosphorus is the topic of the analysis in this paper."

Uncertainties present in soil dust particles are now discussed, see the responses under "More focused discussion of uncertainties and limitations" heading in this document.

Introduction length and detail

• [T]he introduction is very long, and in parts reads more like a review than like a general introduction to a research paper, esp. P2, L22 to P4, L28. This also leads to an excessively long list of references. (…) On the other hand, the discussion on previous efforts in bioaerosol detection using single particle mass spectrometry, which is the most important aspect for the discussions within the manuscript, is very brief and could be expanded (P5 L9-16). (Reviewer #1)

• The introduction needs to be substantially revised. In its current form, the introduction first details ice nucleation, which was not explored in this work, then discusses other methods used to identify bioaerosols, then provides a very short introduction to single particle methods of bioaerosol detection. The introduction should be more focused on methods used to distinguish bioaerosols and dust, and focus more heavily on single particle methods. (Reviewer #2)

• [T]he introduction is dealing with very specific atmospheric questions, where biological particles have a crucial impact as their function as nutrient source or ice nuclei. But there are nor results/ discussion to these fields in the manuscript. (Reviewer #3)

• I thought the somewhat extensive discussion on WIBS as a bioaerosol detection technique was not necessary (although due mention should be made). The same criticism applies to the ice nucleation material. (Reviewer #4)

The introduction was revised and shortened per these comments. In particular, ice nucleation material was shortened (a short overview was retained for context), the paragraph about bioaerosols in cloud water has been cut, and material about previous measurements of bioaerosol (including WIBS) is now only a short overview.

The discussion of previous efforts to detect bioaerosol with SPMS was expanded. Per the last point a clarification about bio/dust mixtures has been added: "Particles that contain

phosphates, organic nitrates and silicates have been historically always classified as mixtures of bioaerosol and dust (Creamean et al., 2013).” This section is difficult to expand further, as those methods are very poorly characterized in previous literature (this paper aims to improve on that).

• The last paragraph of the introduction should be cut. (Reviewer #2)

We decided to keep a shortened version of the paragraph in order to maintain motivation for using phosphorus in this analysis (this could not be eliminated): “Phosphorus was chosen as the focus of this paper because of its abundance in spectra of bioaerosol, but also because it does not undergo gas-phase partitioning in the atmosphere (Mahowald et al., 2008). Therefore, the presence of phosphorus on a particle can often constrain its source and only the classes of particles that are most likely to contain phosphorus are examined here. Emission estimates qualitatively agree that mineral dust, combustion products, and biological particles constitute the principal phosphate emission sources.”

• What is the distinction between goals 1 and 2 listed in the introduction? These two goals seem quite similar to me. (Reviewer #2)

The paper has been reworded to avoid the distinction: “This work examines the prevalence of these ions in the context spectra collected with PALMS” and “The goal of this paper is to develop a method that can differentiate PALMS bioaerosol spectra from spectra of dust and combustion by-products.”

• The first paragraph of section 4.2 and a condensed version of the first paragraph of section 4.3 both belong in your introduction. (Reviewer #2)

Change made: The following has been added to the introduction: “In atmospheric particles, the composition can be mixed, containing some phosphate from inorganic sources, such as calcium phosphate, and some phosphate from microbes. For instance, soils can contain minerals, live microbes, and biogenic matter at all stages of decomposition. Therefore, classifying soil-derived particles with a binary biological/non-biological classifier has uncertainties. These uncertainties are quantified here for soils using soil samples collected in various locations.” And “Biological aerosols have been studied with SPMS, in particular the Aerosol Time of Flight Mass Spectrometer (ATOFMS; Cahill et al., 2015; Creamean et al., 2013; Fergenson et al., 2004; Pratt et al., 2009). A property of SPMS bioaerosol spectra that has been exploited for their detection is the presence of phosphate (PO^-, PO_2^-, PO_3^-) and organic nitrogen ions (CN^-, CNO^-) (Cahill et al., 2015; Fergenson et al., 2004). Those ions have also been shown to be present in non-biological particles with the same instrument, however, such as vehicular exhaust (Sodeman et al., 2005) and soil dust (Silva et al., 2000). Particles that contain phosphates, organic nitrates and silicates have been historically always classified as mixtures of bioaerosol and dust (Creamean et al., 2013). This work examines the prevalence of these ions in the context spectra collected with PALMS.”

More streamlined discussion of samples used/table of particle numbers

- In order to get an idea of the underlying statistics, the general information on how many mass spectra (positive/negative) were available for the different samples needs to be included somewhere (in the experimental or the results section), also, how many mass spectra were acquired in the field campaign. (Reviewer #1)
- Could Section 2.2 (Test samples) be streamlined a bit to be more concise? It would be good to have a table with an overview of the sampled materials; maybe some of the detailed information could go into such a table as well, to make the section easier to read and to provide a better overview for the reader. (Reviewer #1)
- P9, L3: "a portion of the data" – how many spectra? Give at least the order of magnitude. The same for the "remaining data" (P9 L5). Also specify which sample types were included for the training: on P11 L5 it is mentioned that soil data were not used. Were all other lab samples used? Please be more specific. (Reviewer #1)
- P10 L19 to L27: which bioaerosol materials were used for this? In Fig. 4 only 5 of the 8 tested materials are shown. Were the others used as well, showing similar behavior, and only were omitted for clarity? Please state clearly which materials were used for the development and if some were left out, why. – On P11 L4 it is stated that soil dust is left out from the training set because some biological material might be contained in the particles, however, in P10 L24 it is used within the class of inorganic phosphorus. If biological material is indeed present within soil dust, this does not make sense. This seems like a contradiction. (Reviewer #1)
- The paper would greatly benefit from the addition of a table with statistics of how many particles were analyzed, how many positive spectra, negative spectra, and what ion peak thresholds were used. (Reviewer #2)

Several steps were taken to address the confusion regarding which samples formed the training dataset and which did not:

1. Section 2.2 was clarified and re-structured. The section is now split into two sub-sections, "2.2.1 Training dataset" and "2.2.2 Test dataset" to make sure that confusion does not occur. All detail about sample processing was retained, as the new organization should make it easier to follow.

2. Added clarifying remarks in section 3 and Figure 4 caption, "Soil dusts are shown in Figure 4, even though they are not used as training aerosol; their histogram shows a broad distribution with a tail extending into $PO_3^-/PO_2^- > 2$ region, indicating a mixed inorganic/biological composition. In comparison, fertilized soil dusts show a similar distribution to apatite ($PO_3^-/PO_2^- < 4$) due to presence of inorganic fertilizer, which is calcium phosphate" and "Note that soil dusts were not used as part of the training dataset and that not all training aerosols are shown here for clarity."

3. Added Table 2 detailing all aerosol samples used and their statistics. The table should also make it clear which aerosol made it into the training set.

More focused discussion of uncertainties and limitations

• Section 4.2: This is an important discussion, and I would have hoped to find a similarly critical discussion of the newly developed algorithm in this manuscript, as well. There are some basic approaches to such a discussion scattered throughout the manuscript, but this should be addressed much more clearly and explicitly. By the title of Section 4.1 it is suggested that this discussion is provided in that section, but in fact the presented discussion on uncertainties and limitations of the newly developed algorithm in that section is very limited and should be much more thorough. (Reviewer #1)

• In my opinion focusing on a critical discussion of the new procedure and all linked uncertanties would be essential to strengthen the significance and impact of this paper. (Reviewer #3)

The Discussion section has been restructured. Material noted as irrelevant to the discussion of uncertainties has been taken out of section 4.1 and shortened and placed at the beginning of the Discussion. Moreover, section 4.1 was shortened and moved to section 4.3.

Additional quantification of uncertainty has been added to the SVM classification approach: "For every observation, a distance from the SVM boundary can be calculated (otherwise known as score). Those distances can then be converted to probability of correct identification. An optimized function to convert scores to probabilities was found by 10-fold cross-validation (Platt, 1999). Because in this experiment the classes are not perfectly separable, the conversion function is a sigmoid. Posterior probabilities near 0 and 1 indicate high-confidence identification. An uncertainty boundary was defined between 0.2 and 0.8. This boundary is shown in Figure 5. Points that lie in this boundary are marked as low confidence assignments. Those correspond to shaded areas in Figures 6 and 7." Changes have been made to figures 5 and 6 accordingly.

Further discussion of the uncertainty bound was included in section 4.3 : "The basic classifier presented in this paper is binary: all phosphate- and organic nitrogen-containing particles are classified either as biological or inorganic. However, spectra whose PO_3^-/PO_2^- and CN^-/CNO^- ratios are very close to the SVM boundary have more uncertain assignments than those whose PO_3^-/PO_2^- and CN^-/CNO^- ratios fall far away from the boundary. In order to provide an additional measure of classification uncertainty, a probability bound was defined as shown in Figure 5. According to this definition, 96% of particles in the training dataset were classified with high-confidence (Figure 5). In the FIN03 and CARES field datasets, 79% of phosphate-containing particles were classified with high confidence. The low-confidence assignments are shown on Figures 6A and 7A with shaded areas."

Section 4.3 (previously section 4.1) now includes a discussion of uncertainties related to dust/biological mixtures and soil dusts: "Because soil dusts are a special category, where lines between biological and inorganic phosphorus sources can be blurry because of ongoing chemical transformations, they have higher classification uncertainties than other types of

phosphate-containing aerosols. In the field data, dust/biological mixtures (defined as particles classified as biological with silicate features) are overrepresented in the low-confidence assignments. Dust/biological mixtures constitute 26% (CARES) - 46% (FIN03) of high-confidence assignments and 64% (CARES) - 68% (FIN03) of low-confidence assignments. Moreover, only 75% of phosphate-containing soil dust particles were classified with high confidence. However, in simple two-component internal mixtures of dust and biological fragments (Figure 10) phosphate features can be identified as biological with high confidence (98%)."

Discussion of misclassification rate

- 1.) What kind of misidentification exactly is contained within this value? Both false positives (mineral dust wrongly assigned to "biological phosphorus") and false negatives (biological material not assigned to "biological phosphorus") cause an uncertainty in the determined fraction of particles containing biological phosphorus. (Reviewer #1)
- 2.) The method used to calculate the "misidentification rate" should be clearly stated. Depending on the method, the number of particles within the data sets of the different test aerosols might bias the determined uncertainty, so this should be made clear to the reader. (Reviewer #1)
- 3.) It is stated that removing ragweed pollen from the training set leads to a smaller misidentification rate. Was ragweed pollen only removed from the training set, or also from the "testing" set? I guess the former, since the latter would give a wrong impression, but this needs to be stated clearly. (Reviewer #1)
- 4.) Which particles were tested for determining this misidentification rate? The pure biological / pure mineral dust / fly ash particles? What about the processed mineral dust? (Reviewer #1)
- How were misidentifications determined? (Reviewer #2)
- Page 10, line 29: how are misclassifications identified and quantified? (Reviewer #2)

In order to avoid confusion the term "accuracy" was used throughout the paper defined as: "Accuracy here is defined as percentage of correctly classified particles in the training set once the optimized boundary is found."

Context for the SVM method was provided: "The SVM algorithm was used here to optimize boundaries between clusters. To do this, the algorithm needs a training dataset, where the classes are known *a priori*. In this paper, the training dataset is defined in Table 2. Once an optimized boundary is drawn, some of the training data can still fall on the incorrect side of the boundary, when the clusters are not perfectly separable." Note that a table detailing the training set contents was added as Table 2.

We believe that the clarification above, together with Table 2 answers the questions about which particles were used to calculate the accuracy and removing the ragweed pollen from the training set.

Discussion of dust/biological mixtures

• - It should be discussed in Sect. 3 / Sect. 4.1 what effect has (…)
2) mixing mineral dust / biological material. This is discussed only in Sect. 4.3, but is an important consideration when assessing the uncertainty and limitations of the method. The discussion in Sect. 4.3 reveals several limitations which need to be discussed within this context: "At this time, we are not able to delineate between primary biological and biogenic or simply complex organic (such as humic acids) material." (P15 L30-31) This means that with the presented method, not PBAP can be determined, but whether phosphorus present in any particle is of "biological" or "inorganic" nature. The former hints at the presence of biological material, but, as also evident from the discussion on P16 L1-14, it is not possible to determine whether this biological material is part of PBAP or from an internal mixture of e.g. mineral dust and biological material, so the information retrieved remains limited, which needs to be clearly stated and discussed. (Reviewer #1)

The definition of "bioaerosol" is stated in the introduction: "In this paper, "bioaerosol" is defined as primary biological aerosol particles (PBAP) (i.e. airborne whole and fragmentary bacteria, pollen and spores) and particles that contain fragments of PBAP as a part of an internal mixture." Under this definition, internal mixtures of biological material and dust also qualify as bioaerosol.

Uncertainties related to soil dusts are now discussed in more detail, described in section 4.3. See also our comments under "More focused discussion of uncertainties and limitations" heading in this document.

• - If I understand correctly, only an internal mixture of biological material with a type of mineral dust not showing any signatures of inorganic phosphorus (illite) was tested. But what happens if mineral dust showing mass spectral signatures of inorganic phosphorus (like apatite) is internally mixed with biological material? If indeed in an ambient dataset up to 56% of all particles identified as containing biological phosphorus also contained silicate markers (P16 L1), this does not seem to be an unlikely case and needs to be addressed. (Reviewer #1)

We agree with the reviewer that an internally mixed inorganic and biological phosphorous particle is a possible atmospheric state. This is now stated in section 4.3: "The low-confidence assignments in field datasets can be related to chemical processing of particles (either at the source like in soils or during transport) or to internal mixing of biological and inorganic phosphate." To be clear, we did not test this type in the laboratory.

• [G]iven the prevalence of mixed biological/dust particles observed in ambient observations, a more detailed discussion of experiments used to characterize these mixtures is needed. (Reviewer #2)
• Section 4.3 belongs in the results section and should be discussed in greater detail since this particle type appeared to be the most atmospherically relevant. (Reviewer #2)

• Page 12, lines 13-15: why is the discussion of your experiments with mixed biological and dust particles not mentioned in this section? Clearly your ambient data shows that these particle mixtures are atmospherically relevant. (Reviewer #2)

More detail has now been included in section 4.3 (see responses above and comments under "More focused discussion of uncertainties and limitations" heading in this document). We tried to confine this discussion to one section to keep it focused because of its importance.

• The authors should mention the prevalence of mixed biological and dust particles [in the abstract]. (Reviewer #2)

The following was added, "In addition, 36% - 56% of particles identified as biological also contained spectral features consistent with mineral dust, suggesting internal dust/biological mixtures."

• Can it be confirmed that your experiments with illite and spores did indeed contain internally mixed particles? (Reviewer #2)

Revised wording: "Internal mixtures of biological and mineral components were generated in the laboratory in order to investigate this; an exemplary spectrum of such particle is shown in Figure 10. The spectrum contains alumino-silicate markers consistent with mineral dust together with phosphate markers that, in this case, come from the biological material. In spectra of pure illite, no phosphate markers are present."

Discussion of processing of mineral dust

• - It should be discussed in Sect. 3 / Sect. 4.1 what effect has
1) processing of the mineral dust (which, as stated on P10 L17, causes CN- and CNO- to "appear and/or intensify", so might have an influence on the classification) (Reviewer #1)

A clarification was added to section 3, "Processed mineral dust had a smaller impact on the accuracy: removing it from the training dataset increased the accuracy to 97.5%."

Discussion of thresholds

• If only some of the spectra were used for the analysis, their number (and criteria for their selection) needs to be stated. (Reviewer #1)
• [I]n Sect. 3, it is stated that for the ambient data, a threshold was used to determine mass spectra containing phosphorus in a first step. Was something similar performed for the lab data (also for the training), or were all mass spectra used? (Reviewer #1)
• P13 L26 and following: What thresholds were used in order to determine whether the different marker ion signals were present or absent? Was it tried to improve the performance of the algorithm by adjusting these thresholds? (Reviewer #1)

We have added in section 3, “The only requirement for this analysis was that each spectrum used in the training set contains both phosphate and organic nitrogen (otherwise the ratios used here become undefined). This was ensured by selecting spectra, where PO_2^- > 0.001 and CNO^- > 0.001. Nearly all biological spectra in the training set satisfied this criterion (Table 2).”

Also added the following in section 4.1 (formerly section 4.2), “Note that previous literature does not provide information on the thresholds used to determine presence or absence of ions in analysis of ATOFMS spectra. Furthermore, because of hardware differences, detection limits of PALMS and ATOFMS are very different. This analysis focuses on PALMS and the threshold for “presence” was chosen as 0.001, which was observed to be the detection limit for CN^-, CNO^- and PO_3^- in the laboratory aerosol database used here.”

Pollen

• One question to the tested reference materials. A variety of pollen samples are tested. These pollen will have a size of > 10 µm, while the upper limit for the SPMS is given with 2-3µm. Is debris of the original pollen measured? In this way a discussion about the kind of biological particles, which should be captured with the new classification method (size, mixing-state = only external mixed biological particles or also external mixed biological layers) would also be a great advantage. (Reviewer #3)

• The one major scientific matter of concern to me is the laboratory work on pollen (too large for detection) unless sub-pollen. I was not at all sure what could be characterized here as the atmospheric process associated with this is complicated involving both humidity and, when appropriate, lightning. (Reviewer #4)

Yes, we are measuring pollen fragments. This was covered in the experimental section, “Pollen grains were too large (18.9 – 37.9 µm according to manufacturer’s specification) to sample with PALMS. They were suspended in ultrapure water (18.2 MΩ cm, Millipore, Bedford, MA) and the suspensions were sonicated in ultrasonic bath for ~30 minutes to break up the grains.”

All other concerns raised

• How were peak intensities determined for the various ratios (CN-/CNO- etc)? Integrated peak area? This should be stated in the methods section. (Reviewer #1)

Added to the end of section 2.1: “Raw PALMS spectra are processed using custom IDL software. Mass peak intensities used in this paper refer to integrated peak areas normalized by the total ion current.”

• P6, L3-9 (first paragraph): this paragraph is not related to the section (2.1: PALMS), but a general introduction. It should go either as a general remark in the experimental section before Sect. 2.1, or be reworked as a last paragraph into the introduction. (Reviewer #1)

This paragraph has now been revised and moved before section 2.1.

• P6, L18: "...a unipolar reflectron time of flight mass spectrometer was used..." – It should be clearly stated here that the PALMS acquires for each single particle either a negative or a positive mass spectrum, but not both simultaneously. How long were the sampling times in positive / negative mass spectra mode (e.g., switching every minute, every 15 min, every hour)? (Reviewer #1)

The following was added to 2.1: "PALMS acquires spectra in either positive or negative polarity, but not simultaneously. For field datasets presented in this paper, sampling polarity was switched every 5 minutes for FIN03 and every 30 minutes for CARES."

• P7 L25: ". . .further dissolved in ~5ml of Milli-Q water. . ." – this information is not necessary since the concentration of the original solution is not known, anyway. (Reviewer #1)

The estimated volume was removed: "…further dissolved in ultrapure water,"

• P7, L30-31: "No processing-related changes to chemistry were found." – This sentence should be clarified, e.g., ". . .were found in the mass spectra sampled with the PALMS". (Reviewer #1)

Clarified as follows: "Examination of PALMS spectra revealed no changes in chemistry resulting from different processing methods."

• P8 L4 "to aerosolize a solution of illite NX and F. solani spores" – I guess this should read "suspension" instead of "solution"? Was this suspension sonicated as well? (Reviewer #1)

Changed to "suspension" instead of "solution" and clarified: "A second disposable medical nebulizer was then used to aerosolize a suspension of illite NX and *F. solani* spore fragments."

This suspension was not sonicated, but the spores mixed with illite NX were actually fragments prepared by sonication, as described above.

• P8 L21: "for 0.1 mL experiments" is unclear. Rather something like "For experiments using 0.1 mL of nitric acid"? (Reviewer #1)

Changed to "…for experiments using 0.1 mL of nitric acid, the entire volume of HNO_3 evaporated…"

• P9, L18: Please give the start / end dates of the measurement period. (Reviewer #1)

Added the following to the end of section 2.4: "The measurements were carried out between September 14, 2015 and September 27, 2015."

• It is not clear to me why parts of the results can be found in the results section, and other parts (like the soil and internal mixtures, Sect. 4.3) in the discussion section. Maybe a single

"results and discussion" section would be more appropriate, and could also avoid some repetitions. (Reviewer #1)

Both sections were screened for repetitions, which were edited out when applicable (see track changes version).

• P10 L26 "Processing of apatite with nitric acid tends to shift the PO3-/PO2- ratio to larger values, increasing the disparity from the bioaerosols." – This is not clear to me. If inorganic material usually shows lower PO3-/PO2- ratios than bioaerosol, shouldn't this read "decreasing the disparity"? (Reviewer #1)

The Reviewer is correct, it should be "decreasing": "Processing of apatite with nitric acid tends to shift the PO_3^-/PO_2^- ratio to larger values, decreasing the disparity from the bioaerosols."

• P11 L6: "(classification with the SVM algorithm is discussed later)": maybe this could be reworded for clarity, as at first reading it seems to mean that the SVM algorithm itself will be discussed later, not the results of applying it on the soil samples. (Reviewer #1)

Clarified as follows: "(classification of soil dusts with the SVM algorithm is discussed later)"

• P13 L10-12: "Particles with positive spectra showing the characteristics of monazite. . .provides evidence of the origin of the inorganic phosphate particles." (and a similar statement on P12, L9): Since PALMS does not simultaneously provide the positive and negative ion mass spectrum of a single particle, this is not "evidence", but rather "suggests" this type of mineral dust particles as a likely origin. Please reword. (Reviewer #1)

The Reviewer is correct. Clarified as follows: "Although negative spectra of apatite and monazite cannot be definitively differentiated from fly ash or soil dust spectra, positive spectra acquired during FIN03 additionally suggest that monazite-type material was present."

And later, "Particles with positive spectra showing the characteristics of monazite coupled to back trajectories over source areas suggests the origin of the inorganic phosphate particles."

• P15 L26/27: "the numbers of biological particles fall within these estimates". The cited estimates refer to inorganic / biological phosphorus mass ratio, while the information provided by PALMS is the number ratio. Such a comparison would only be valid if it can be assumed that all types of particles contain the same amount of phosphorus, which does not seem very likely. This needs to be discussed more carefully. A similar comment applies to P16, L28-31. (Reviewer #1)

This wording was removed to avoid confusion, "The biological PALMS filter was applied to several soil dust samples (Table 2). As would be expected, soils collected in areas with less vegetation exhibit smaller biological contributions."

• Figs 1-3, Fig. 9: The mass spectra might be easier to grasp if integrated stick spectra were shown instead of the raw mass spectra. (Reviewer #1)

We disagree in this case; spectra are shown in a manner consistent with previous PALMS publications. We believe a change in this paper would have made future comparisons to prior or future by readers difficult.

• Fig. 7: "In all other aerosol classes the green bar denotes a level of misidentification." – This only applies to "Apatite + Monazite" and "Fly ash". Both "agricultural soil dust" and "Soil dusts" are expected to contain some (unknown) amount of biological material, so the performance of the algorithm cannot be validated on these samples. (Reviewer #1)

This was reworded, "In all other aerosol classes the green bar denotes a typical level of misidentification."

• Various locations, e.g., P6 L6, P12 L23, P12 L28: "phosphorous" should read "phosphorus" (Reviewer #1)

Corrected.

• P7 L8: "Snowmax" should read "Snomax" (Reviewer #1)

Corrected.

• P7 L22 and various other locations: "Milli-Q water" is laboratory slang, use "ultrapure water" instead (Reviewer #1)

Corrected.

• P8, L19: "flow" should read "flow rate" (Reviewer #1)

Corrected.

• P8, L19: for the flow rate reported in slpm, reference temperature and pressure need to be given (Reviewer #1)

Corrected, "…flow rate of 0.44 slpm (STP: 0°C, 1 atm)…"

• P8, L21: remove "." from "conducted.:" P11 L6 "latter" should read "later" P12 L5 "carbonatitie" should read "carbonatite" (Reviewer #1)

Corrected.

• P12 L6 introduce abbreviation “REE” (e.g., in the previous sentence) (Reviewer #1)

Changed to “As examples, on 09/27 the back trajectory intersects the vicinity of an active rare earth element (REE) mine…”

• P13 L23 “If a silicate components were. . .” – remove superfluous “a” (Reviewer #1)

Corrected.

• P16 L2 “This represents and upper limit” should read “an upper limit” (Reviewer #1)

Corrected.

• List of references: for several references, page numbers are given as “n/a-n/a”, e.g. P23 L16, L24, L27; P25 L1. On P23, L30: “>” should read “>”.(Reviewer #1)

Corrected.

• Table 2: In the table caption, the wording “biological filter” is unclear, please be more precise. Also not “negative particles” are “sampled”, but negative ion mass spectra are acquired. For Argentina and China, “approximate” could be omitted from column 3 (as this is already clear from the column header). (Reviewer #1)

Table 2 caption changed to read, “Soil dust samples used in this work. The last column shows the results of analysis with the SVM classifier developed here as a percentage of negative spectra acquired.”

“Approximate” left off for Argentina and China.

• Was the same laser fluence used for all experiments including the ambient work? This could affect ion peak ratios. (Reviewer #2)

The following statement was added to section 4.3, “Because the field studies were performed during very different time periods, it was difficult to control for a constant excimer laser fluence. However, laser fluence was similar for all laboratory samples acquired (3-5 mJ pulse energy). This is a possible source of uncertainty, as fragmentation patterns can differ depending on pulse energy.”

• Was a sensitivity analysis performed to confirm that your algorithm was indeed optimized for distinguishing particle types? (Reviewer #2)

The following wording was added to the results section, “Those spectral peaks were used for several reasons: (1) they are clearly visible in all biological spectra that were acquired as a part of this study (Figure 1), (2) they were used to distinguish bioaerosol from other species in previous studies (Creamean et al., 2013; Pratt et al., 2009) and (3) sources of phosphorus on aerosol particles are well-defined and documented in the literature (Mahowald et al., 2008).” For these reasons we did not perform a sensitivity analysis.

• Add lines 19-20 on page 10; lines 13-15 on page 11; lines 19-22 on page 11 here. These are details of your methods. (Reviewer #2)

In the revised manuscript we believe details of thresholds and SVM development in the results/discussion section was found to streamline the discussion.

• Page 10, lines 9-13. It seems that positive ions can also be used to filter by particle type, as was done using other single-particle methods. The author should comment on this. (Reviewer #2)

In this work, we use negative markers to compare to previous methods of distinguishing bioaerosol from other particle classes (the Boolean phosphate and organic nitrogen markers). Those markers are strongly visible only in negative spectra (see Figure 1). See the following added explanation, “Those spectral peaks were used for several reasons: (1) they are clearly visible in all biological spectra that were acquired as a part of this study (Figure 1), (2) they were used to distinguish bioaerosol from other species in previous studies (Creamean et al., 2013; Pratt et al., 2009) and (3) sources of phosphorus on aerosol particles are well-defined and documented in the literature (Mahowald et al., 2008).”

• Page 10, lines 19-20, why were only the organic nitrogen and phosphate peaks used to distinguish these classes of aerosols. From your mass spectra, it seems that the addition of other markers could help improve the separation between different classes of aerosols. (Reviewer #2)

The following wording was added : “Those spectral peaks were used for several reasons: (1) they are clearly visible in all biological spectra that were acquired as a part of this study (Figure 1), (2) they were used to distinguish bioaerosol from other species in previous studies (Creamean et al., 2013; Pratt et al., 2009) and (3) sources of phosphorus on aerosol particles are well-defined and documented in the literature (Mahowald et al., 2008).” Also note that markers in positive spectra cannot be used concurrently with markers in negative spectra because PALMS does not acquire positive and negative spectra simultaneously (now clarified, per Reviewer #1 comment above).

• Page 10, lines 23-27: do you have an explanation for your observed changes in the phosphate ion ratios for inorganic and biological phosphorus? (Reviewer #2)

Different chemical forms of phosphorus in those different particle classes are a possible explanation, now stated in section 4.1: "In apatite and monazite, phosphorus occurs as calcium phosphate. In biological particles, phosphorus occurs mostly in phospholipid bilayers and nucleic acids. In these experiments, the PO_3^-/PO_2^- ratio of those two forms is different (Figure 4A). The agricultural soils considered here cluster with the minerals and fly ash and we assume the phosphorus is due to the use of inorganic fertilizer, which is derived from calcium phosphate (Koppelaar and Weikard, 2013). Fly ash aerosol clusters similarly to apatite and monazite but with a wider distribution; this is likely because the chemical from of phosphorus in fly ash is different than in the minerals. Phosphorus present in coal is volatilized and then condenses into different forms during the combustion process (Wang et al., 2014)."

• Page 12, lines 7-10: it seems that this method also relies on a Boolean type of classification and not just ion peak ratios in order to distinguish aerosol types similar to the ATOFMS methods. The authors should mention that both methods are helpful for distinguishing particle types with similar ion peaks (e.g., fly ash and soil dust in this case). (Reviewer #2)

Added the following to section 4.1, "Such "Boolean" criteria for particle identification, can be helpful in distinguishing aerosol types when the signatures are unique to one particle type."

• Page 14, lines 23-26: would you be better able to distinguish bioaerosols if you applied a similar filter (e.g., if you looked for spectra containing Ca, Na, organic carbon, organic nitrogen, and P then applied your ion peak ratio determinations?) (Reviewer #2)

As clarified above, only negative spectra were used here for consistency with previous data analyses.

• Replace "species" with "compounds". Species denotes something biological. (Reviewer #2)

"Species" replaced with "types of particles" in Figure 4 caption, "Delineation between the clusters at a PO_3^-/PO_2^- ratio of 3 results in a 70-80% classification accuracy depending on the types of particles considered."

"Species" replaced by "compounds" and "ions" in the Results section: "These particles contain both organic and inorganic compounds. Because they are easy to ionize, the inorganic ions sodium and potassium stand out in the positive spectra despite their minor fraction by mass."

• Page 9, line 28, change "contamination" to "contaminant". (Reviewer #2)

Corrected.

• Page 16, line 2, change "and" to "an" (Reviewer #2)

Corrected.

• As the peak intensity in SPMS is not following the mass abundance only I am not convinced that only the three discussed groups (soil dust, fly ash, biological) will show phosphorus signatures in SPMS. What is with mixed particles e.g. biological layers on soil dust or sea-salt particles? Do they show phosphorus signals? (Reviewer #3)

We revised the introduction: "Emission estimates qualitatively agree that mineral dust, combustion products, and biological particles constitute the principal phosphate emission sources. (…) In this work, calcium phosphate-rich minerals (apatite and monazite) and fly ash are chosen to represent dust and industrial combustion particle classes, respectively."

Note that the selection of those classes is based on the work of Mahowald, et al. (2008), which does not show sea salt as an important source of atmospheric phosphorus.

Mixed particles (mineral dust with biological fragments) were investigated in this study using both laboratory particle mixtures and natural soil samples. Those tend to be frequent in field datasets (as a possible soil dust influence). We added some uncertainty analysis regarding those particles, as described in the responses above.

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Improved identification of primary biological aerosol particles using single particle mass spectrometry

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Abstract

Measurements of primary biological aerosol particles, especially at altitudes relevant to cloud formation, are scarce. Single particle mass spectrometry (SPMS) has been used to probe aerosol chemical composition from ground and aircraft for over 20 years. Here we develop a method for identifying bioaerosols using SPMS. We show that identification of bioaerosol using SPMS is complicated because phosphorus-bearing mineral dust and phosphorus-rich combustion by-products such as fly ash produce mass spectra with peaks similar to those typically used as markers for bioaerosol. We have developed a methodology to differentiate and identify bioaerosol using machine learning statistical techniques applied to mass spectra of known particle types. This improved method provides far fewer false positives compared to approaches reported in the literature. The new method was then applied to two sets of ambient data collected at Storm Peak Laboratory and a forested site in Central Valley, California to show that 0.04-~~0.32~~% of particles in the 200 – 3000 nm aerodynamic diameter range were identified as bioaerosol. In addition, 36% - 56% of particles identified as biological also

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contained spectral features consistent with mineral dust, suggesting internal dust/biological mixtures.

1 Introduction

~~Primary biological aerosol, hereafter "bioaerosol", include intact and fragmentary microbes, fungal spores and vegetation. One particularly important role of bioaerosol in the atmosphere is that~~Biological atmospheric aerosol (or bioaerosol) has recently garnered interest because certain species of bacteria and plant material might impact climate via the nucleation of ice in clouds (Hiranuma et al., 2015; Möhler et al., 2008). However, many field-based measurements of ice nuclei and ice residuals do not indicate that bioaerosol ~~are~~is a major class of ice active particles (Cziczo et al., 2013; DeMott et al., 2003; Ebert et al., 2011). ~~Uncertainties~~While modeling efforts suggest that biological material is not significant in ice cloud formation on a global scale, uncertainties continue to exist because field measurements of ice nucleating particles are currently sparse~~. Modeling efforts also suggest that biological material is not significant in ice cloud formation on a global scale. Hoose et al. (2010) has shown that global average contribution of bioaerosol to heterogeneous ice nucleation in mixed phase clouds is small: with higher than realistic freezing efficiencies, the total contribution of biological aerosol remained less than 1%. Later studies by Burrows et al. (2013), Sesartic et al. (2012, 2013) and Spracklen and Heald (2014) support this result. These studies do identify circumstances where bioaerosol can have an influence on clouds. For example, at low altitudes bacteria can dominate immersion freezing rates, where the conditions are too warm for mineral dust to activate (>15°C) (Spracklen and Heald, 2014). Additionally, bioaerosol can dominate the aerosol coarse modes in certain regions. For example bioaerosol can be 50% of the coarse mode over tropical forests compared to a 5-8% global average (Spracklen and Heald, 2014). There are measurements of this enhancement in the Amazon basin, supporting possible regional effects of bioaerosol (Artaxo et al., 1990; Prenni et al., 2009)~~(Hoose et al., 2010; Sesartic et al., 2012).

In this paper, "bioaerosol" is defined as primary biological aerosol particles (PBAP) (i.e. airborne whole and fragmentary bacteria, pollen and spores) and particles that contain fragments of PBAP as a part of an internal mixture. Measurement techniques specific to bioaerosol include collection of aerosol on filters followed by analysis with microscopy techniques, either electron microscopy (EM) or optical microscopy coupled with fluorescent

staining of the samples ~~(Amato et al., 2005; Bauer et al., 2002, 2008; Bowers et al., 2009, 2011, 2012; Griffin et al., 2001; Matthias-Maser and Jaenicke, 1994; Pósfai et al., 2003a; Sattler et al., 2001; Wiedinmyer et al., 2009; Xia et al., 2013)~~(Amato et al., 2005; Bauer et al., 2002, 2008; Bowers et al., 2009, 2011; Griffin et al., 2001; Matthias-Maser and Jaenicke, 1994; Pósfai et al., 2003; Sattler et al., 2001; Wiedinmyer et al., 2009; Xia et al., 2013). Aerosol samples collected in the atmosphere have been cultured for identification of the microbial strains present (Amato et al., 2005, 2007; Fahlgren et al., 2010; Fang et al., 2007; Griffin et al., 2001, 2006; Prospero et al., 2005). ~~However, culturing techniques can underestimate microbial diversity, as not all organisms present in the atmosphere are viable or cultivable using standard media. It has been suggested that <10% of bacteria found in atmospheric aerosol are cultivable (Amato et al., 2005; Georgakopoulos et al., 2009).~~

In-situ techniques specific to biological samples are typically based on fluorescence of biological material following UV excitation. Examples include the wide-band integrated bioaerosol sensor (WIBS) which is available commercially (Kaye et al., 2000, 2005). WIBS has been successfully deployed in several locations (Gabey et al., 2010; O'Connor et al., 2014; Toprak and Schnaiter, 2013). Using fluorescence to detect biological aerosol can have interferences, however. For example, polycyclic aromatic compounds or humic acids can have similar fluorescent properties (Gabey et al., 2010; Pan et al., 1999). Cigarette smoke has similar fluorescent properties to bacteria (Hill et al., 1999). In an attempt to address interferences, WIBS collects fluorescence information using several channels with different wavelengths while also measuring the size and shape of the particles. Table 1 summarizes some recent measurements of bioaerosol. More information can be found in recent reviews focused on bioaerosols in the atmosphere, such as Després et al., (2012).

~~Table 1 summarizes recent measurements of bioaerosol in the atmosphere. Apart from WIBS, the other recent measurements are collection of the aerosol on filters followed by off line microscopy. Biological particles have been measured at variety of ground sites, including urban (Bauer et al., 2008; Fang et al., 2007; Toprak and Schnaiter, 2013), rural (Bowers et al., 2011; Harrison et al., 2005), forest (Gabey et al., 2010), marine (Griffin et al., 2001; Pósfai et al., 2003a) and remote (Xia et al., 2013). High-altitude mountain sites, such as Jungfraujoch, Storm Peak Laboratory, Mt. Rax and Mt. Bachelor Observatory are often used to gain access to free tropospheric air less impacted by local sources (Bauer et al., 2002; Bowers et al., 2012; Smith et al., 2012, 2013; Wiedinmyer et al., 2009; Xia et al., 2013). Measured concentrations~~

~~range from 2.9×10^{3} to 1.5×10^{6} particles m^{-3}, and bioaerosol can make up from 0.5% to 22% of atmospheric aerosol by number greater than 500 nm. There is a strong seasonal cycle to biological material (Bowers et al., 2012; Harrison et al., 2005; Toprak and Schnaiter, 2013). Bioaerosol tend to be primarily bacteria and some fungal spores, although pollen (O'Connor et al., 2014) and possibly viruses (Griffin et al., 2001) have been reported. Some studies have performed DNA analysis of bioaerosol, reporting a wide diversity (Smith et al., 2012, 2013; Xia et al., 2015).~~

~~Bioaerosol has also been reported in cloud water (Amato et al., 2005; Bauer et al., 2002; Sattler et al., 2001) and precipitation samples (Bauer et al., 2002; Christner et al., 2008a, 2008b; Sattler et al., 2001). This does not necessarily mean the bioaerosol play a role in droplet nucleation processes, however, as scavenging of interstitial aerosol happens frequently in and below clouds (Pruppacher and Klett, 2003). It does illustrate that microorganisms, sometimes viable ones, can be transported by the atmosphere and deposited by precipitation (Amato et al., 2005).~~

Measurements of bioaerosol in the free and upper troposphere, where they could be relevant to cloud formation, remain scarce. Four of the recent studies reported in Table 1 used an aircraft to access altitudes higher than 4,000 m ~~(DeLeon-Rodriguez et al., 2013; Pósfai et al., 2003a; Twohy et al., 2016; Ziemba et al., 2016)~~(DeLeon-Rodriguez et al., 2013; Pósfai et al., 2003; Twohy et al., 2016; Ziemba et al., 2016). Two of these used the WIBS sensor to report vertical profiles of fluorescent particles (Twohy et al., 2016; Ziemba et al., 2016). In the remaining two cases, aerosols were collected on filters and analyzed off-line. ~~Pósfai (2003a) reported results of Transmission EM (TEM) measurements of samples collected around Cape Grim that included bacteria with rod-like morphology. It should be noted that numerous other studies of samples collected on aircraft missions with TEM microscopy did not reveal the presence of any aerosols that matched morphology of biological material (Buseck and Posfai, 1999; Li et al., 2003a, 2003b; Pósfai et al., 1994, 1995, 2003b).~~ There can exist significant uncertainty in these measurements. A recent aircraft-based study by DeLeon-Rodriguez et al. (2013) reports analysis of high altitude (8-15 km) samples taken before, after and during two major tropical hurricanes. The abundances of microbes, mostly bacteria, were reported between 3.6×10^{4} and 3.0×10^{5} particles m^{-3} in the 0.25 – 1 μm size range. The methods and conclusions of this study were re-evaluated by Smith and Griffin (2013), who argued that in some instances the reported concentration of bioaerosol were not possible because they

exceeded the total aerosol by several factors. The samples were also taken over periods of hours, possibly including sampling in clouds when the high-speed impaction of droplets and ice can dislodge particles from the inlet (Cziczo and Froyd, 2014; Froyd et al., 2010; Murphy et al., 2004).

Although difficult, measurements of bioaerosol in the upper troposphere are necessary in order to constrain their influence on atmospheric properties and cloud formation processes. All of the techniques discussed above, except for WIBS, are off-line and require expertise in sample processing and decontamination. WIBS is a possible in situ detection technique for bioaerosols, but it is relatively new and, as a result, has a short deployment history. There has been considerable interest in using aerosol mass spectrometry techniques to measure bioaerosol. Single particle mass spectrometry (SPMS) has been successfully used since the mid-1990s to characterize chemical composition of atmospheric aerosol particles in situ and in real time (Murphy, 2007). The ability of SPMS to simultaneously characterize volatile and refractory aerosol components makes it an attractive tool for investigating the mechanisms of cloud formation (Cziczo et al., 2013; Friedman et al., 2013). The general principle behind SPMS, and in particular the instrument discussed in this paper, the Particle Analysis by Laser Mass Spectrometry (PALMS), is the use of a pulsed UV laser for the ablation and ionization of single aerosol particles. Ions are then accelerated into a time-of-flight mass spectrometer. Laser ablation/ionization used with SPMS produces ion fragments and clusters and is susceptible to matrix effects such that quantitative results are possible only with careful calibration and consistent composition (Cziczo et al., 2001).

Biological aerosols have been studied with SPMS, in particular the Aerosol Time of Flight Mass Spectrometer (ATOFMS; Cahill et al., 2015; Creamean et al., 2013; Fergenson et al., 2004; Pratt et al., 2009). A property of SPMS bioaerosol spectra that has been exploited for their detection is the presence of phosphate (PO^-, PO_2^-, PO_3^-) and organic nitrogen ions (CN^-, CNO^-) (Cahill et al., 2015; Fergenson et al., 2004). Those ions have also previously been shown to be present in non-biological particles with the same instrument, however, such as vehicular exhaust (Sodeman et al., 2005)~~. One goal of this work is to examine the prevalence of these ions in the context spectra collected with other SPMSs.~~ and soil dust (Silva et al., 2000). Particles that contain phosphates, organic nitrates and silicates have historically been classified as mixtures of bioaerosol and dust (Creamean et al., 2013). This work examines the prevalence of these ions in the context of spectra collected with PALMS.

~~Phosphorus is a limiting nutrient in terrestrial ecosystems (Brahney et al., 2015). On the global scale, phosphorus-containing dust aerosols are primarily responsible for delivering this nutrient to oceans and other ecosystems (Mahowald et al., 2008, 2005). Bioaerosols can be an important source of atmospheric phosphorus on local scales, especially in heavily forested areas, like the Amazon (Mahowald et al., 2005).~~Phosphorus was chosen as the focus of this paper because of its abundance in spectra of bioaerosol, but also because it does not undergo gas-phase partitioning in the atmosphere (Mahowald et al., 2008). Therefore, the presence of phosphorus on a particle can often constrain its source, and only the classes of particles that are most likely to contain phosphorus are examined here. Emission estimates qualitatively agree that mineral dust, combustion products, and biological particles constitute the principal phosphate emission sources. The global phosphorus budget has been modeled by Mahowald et al. ~~(2008)~~(2008), indicating that 82% of the total burden is emitted in the form of mineral dust. Bioaerosol accounts for 12% and anthropogenic combustion sources, including fossil fuels, biofuels and biomass burning, account for 5% ~~(Mahowald et al., 2008)~~(Mahowald et al., 2008). Recently, Wang et al. (2014) provided a higher estimate of phosphorus emissions from anthropogenic combustion sources, 31%. In this estimate, mineral dust was responsible for 27%, bioaerosol 17% and natural combustion sources 20% of total phosphorus emissions (Wang et al., 2014). ~~These examples illustrate the major factors in the global phosphorous budget but also that significant uncertainties exist in the emission inventories. A second goal of this work is to determine if the non-biological phosphate aerosols, such as those from minerals and combustion, can be detected and differentiated from bioaerosol.~~

In this work, calcium phosphate-rich minerals (apatite and monazite) and fly ash are chosen to represent dust and industrial combustion particle classes, respectively. In atmospheric particles, the composition can be mixed, containing some phosphate from inorganic sources, such as calcium phosphate, and some phosphate from microbes. For instance, soils can contain minerals, live microbes, and biogenic matter at all stages of decomposition. Therefore, classifying soil-derived particles with a binary biological/non-biological classifier has uncertainties. These uncertainties are quantified here for soils using soil samples collected in various locations.

In this work, the presence of phosphorus in a mass spectrum is evaluated as proxy for bioaerosol. All biological cells contain phosphorus because it is a component of nucleic acids and cell membranes. Distinguishing the specific mass spectral phosphate signature of

biological cells from other non-biological phosphorus is the topic of the analysis in this paper. The goal of this paper is to develop a method that can differentiate PALMS bioaerosol spectra from spectra of dust and combustion by-products.

2 Experimental

~~2.1 PALMS~~

The objective of this work is to describe and validate a new SPMS-based data analysis technique that allows for the selective measurement of bioaerosol. A dataset of bioaerosol, phosphate-rich mineral and coal fly ash single particle spectra – the three largest sources of ~~phosphorous~~phosphorus in atmospheric aerosols - was used to derive a classification algorithm for biological and non-biological phosphate-containing material. This classifier was then applied to an ambient data set collected at the Storm Peak Laboratory during the Fifth Ice Nucleation workshop—phase 3 (FIN03).

2.1 PALMS

The NOAA PALMS instrument has been discussed in detail elsewhere (Cziczo et al., 2006; Thomson et al., 2000). Currently, there are two copies of the PALMS instrument, both of which were used in this work. The laboratory PALMS is a prototype for the flight PALMS, which is more compact and can be deployed unattended at field sites and on aircraft (Thomson et al., 2000). Briefly, PALMS uses an aerodynamic lens to sample aerosols and impart them with a size-dependent velocity (Zhang et al., 2002, 2004). Aerodynamic particle diameter is measured by timing the particles between two continuous-wave laser beams (532 nm Nd:YAG in laboratory PALMS and 405 nm diode in flight PALMS). The particles are ablated and ionized in one step by a 193 nm excimer laser. A unipolar reflectron time of flight mass spectrometer is then used to acquire mass spectra. PALMS acquires spectra in either positive or negative polarity, but not simultaneously. For field datasets presented in this paper, sampling polarity was switched every 5 minutes for FIN03 and every 30 minutes for CARES.

Due to the high laser fluence used for desorption and ionization (~10^9 W/cm^2), PALMS spectra show both atomic ions and ion clusters, which complicate spectral interpretation. SPMS is considered a semi-quantitative technique because the ion signal depends on the abundance and ionization potential of the substance, rather than solely its abundance (Murphy, 2007). Additionally, the ion signals can depend on the overall chemical composition of the particle, known as matrix effects (Murphy, 2007). The lower particle size

threshold for PALMS is ~200 nm diameter and is set by the amount of detectable scattered light. The upper size threshold is set by transmission in the aerodynamic lens at ~3 µm diameter (Cziczo et al., 2006). In PALMS, Particles toward the larger end of this size range are transmitted into the laser beam more efficiently than smaller particles. The 193 nm excimer laser can ionize all atmospherically-relevant particles within this size range with little detection bias (Murphy, 2007). The ionization region is identical in the laboratory and flight PALMS instruments. Raw PALMS spectra are processed using a custom IDL software. Mass peak intensities used in this paper refer to integrated peak areas normalized by the total ion current.

2.2 ~~Test~~Aerosol standards

Table 2 shows numbers of negative spectra for all analyses in this paper. A portion of the data from each of the bioaerosol and non-biological phosphate samples was used as "training data" to build the classification algorithm. The remaining test data were classified using the trained algorithm.

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2.2.1 Training dataset

A collection of phosphorus-containing samples of biological and inorganic origin were used ~~for~~to train the classification algorithm used in this work. Some of the samples were analyzed with the laboratory PALMS at the Aerosol Interaction and Dynamics in the Atmosphere (AIDA) facility at Karlsruhe Institute of Technology (KIT) during the Fifth International Ice Nucleation Workshop—phase 1 (FIN01) with the remainder sampled at MIT.

Biological aerosol sampled at AIDA included two aerosolized cultures of *Pseudomonas syringae* bacteria, Snomax (Snomax International, Denver, CO) (irradiated, desiccated and ground *Pseudomonas syringae*) and hazelnut pollen wash water. The ~~Snowmax~~Snomax and *P. syringae* cultures were suspended in water and aerosolized with a Collison-type atomizer. The growth medium for *P. syringae* cultures was Pseudomonas Agar Base (CM0559, Oxoid Microbiology Products, Hampshire, UK).

Biological aerosol sampled at MIT included giant ragweed (*Ambrosia trifida*) pollen, oak (*Quercus rubra*) pollen, European white birch (*Betula pendula*) pollen, *Fusarium solani* spores and yeast. Samples of dried pollens and *F. solani* spores were purchased from Greer (Lenoir, NC). Information supplied by the manufacturer indicates that *F. solani* fungus was grown on enriched trypticase growth medium and killed with acetone prior to harvesting the

spores. Ragweed and oak pollen originated from wild plants, while the birch pollen originated from a cultivated plant. Pollen was collected, mechanically sieved and dried. The yeast used in this experiment was commercial active dry yeast (Star Market brand). The yeast powder was sampled by PALMS from a vial subjected to slight manual agitation. Pollen grains were too large (18.9 – 37.9 µm according to manufacturer's specification) to sample with PALMS. They were suspended in ~~Milli-Q~~ultrapure water (18.2 MΩ cm, Millipore, Bedford, MA) and the suspensions were sonicated in ultrasonic bath for ~30 minutes to break up the grains. Large material was allowed to settle to the bottom and a few drops of the clear solution from the top of the suspensions were further dissolved in ~~~5 mL of Milli-Q~~ultrapure water, and the resulting solutions were aerosolized with a disposable medical nebulizer (Briggs Healthcare, Waukegan, IL). A diffusion dryer was used to remove condensed phase water prior to sampling with PALMS. *F. solani* spores were sampled in two different ways: (1) dry and unprocessed, in the same way as the yeast and (2) fragmented in ultrasonic bath and wet-generated, in the same way as pollen samples. ~~No processing related~~Examination of PALMS spectra revealed no changes ~~to~~in chemistry ~~were found~~resulting from different processing methods.

Samples of fly ash from four coal-fired U.S. power plants were used as proxy for combustion aerosol: J. Robert Welsh Power Plant (Mount Pleasant, TX), Joppa Power Station (Joppa, IL), Clifty Creek Power Plant (Madison, IN) and Miami Fort Generating Station (Miami Fort, OH). The samples were obtained from a commercial fly ash supplier, Fly Ash Direct (Cincinnati, OH). Fly ash was dry-generated with the shaker.

Apatite and Monazite-Ce mineral samples were generated from ~3" pieces of rock. The rocks were ground and the samples aerosolized with the shaker. Both apatite and monazite were sampled and processed at MIT. The apatite rock was contributed by Adam Sarafian (Woods Hole Oceanographic Institution, Woods Hole, MA).

Two samples of German soil were used as an example of agricultural soil that was known to be fertilized with inorganic phosphate. These were also sampled at the AIDA facility during FIN01. Note that while all other soil samples are used as test aerosols for a completed classifier, those two in particular are used in the training set to account for the presence of inorganic fertilizer.

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Samples of apatite and J. Robert Welsh Power Plant fly ash were also subjected to processing with nitric acid to approximate atmospheric aging. Powdered sample was aerosolized from

the shaker to fill a 9 L glass mixing volume. A hot plate below the volume was used to heat the air inside to 31°C measured in the center of the volume with a thermocouple. PALMS sampled at a flow rate of 0.44 slpm (STP: 0°C, 1 atm) from the 9 L volume. This constituted unprocessed aerosol. 80% HNO_3 was then placed with a Pasteur pipette at the heated bottom of the mixing volume. Two experiments were conducted: for experiments using 0.1 mL of nitric acid, the entire volume of HNO_3 evaporated, producing an estimated partial pressure of about 0.005 atm in a static situation. In 1 mL experiments some liquid HNO_3 remained at the bottom of the volume with an estimated partial pressure of HNO_3 of 0.04 atm. The aerosol and gas-phase HNO_3 were allowed to interact for 2 minutes at which point PALMS began sampling from the volume.

2.2.2. Test dataset

Samples of natural soil dust were collected from various locations listed in Table 3. Five sampled were investigated at the AIDA facility during FIN01 (Bächli soil, Argentina soil, Ethiopian soil, Moroccan soil and Chinese soil) with the remaining analysis at MIT (Storm Peak and Saudi Arabian soil).

Internally mixed biological/mineral particles were also analyzed at MIT. Illite NX (Clay Mineral Society) without bioaerosol was sampled dry, using a shaker (Garimella et al., 2014), and wet-generated, using a medical nebulizer containing ~~Milli-Q~~ultrapure water. A second disposable medical nebulizer was then used to aerosolize a ~~solution~~suspension of illite NX and *F. solani* ~~spores~~spore fragments. This wet generated aerosol was also dried with a diffusion dryer prior to PALMS sampling.

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~~Samples of fly ash from four coal-fired U.S. power plants were used as proxy for combustion aerosol: J. Robert Welsh Power Plant (Mount Pleasant, TX), Joppa Power Station (Joppa, IL), Clifty Creek Power Plant (Madison, IN) and Miami Fort Generating Station (Miami Fort, OH). The samples were obtained from a commercial fly ash supplier, Fly Ash Direct (Cincinnati, OH). Fly ash was dry generated with the shaker.~~

~~Apatite and Monazite-Ce mineral samples were generated from ~3" pieces of rock. The rocks were ground and the samples aerosolized with the shaker. Both apatite and monazite were sampled and processed at MIT. The apatite rock was contributed by Adam Sarafian (Woods Hole Oceanographic Institution, Woods Hole, MA).~~

~~Samples of apatite and J. Robert Welsh Power Plant fly ash were also subjected to processing with nitric acid to approximate atmospheric aging. Powdered sample was aerosolized from the shaker to fill a 9 L glass mixing volume. A hot plate below the volume was used to heat the air inside to 31°C measured in the center of the volume with a thermocouple.~~ ~~PALMS sampled at a flow of 0.44 slpm from the 9 L volume.~~ ~~This constituted unprocessed aerosol. 80% HNO_3 was then placed with a Pasteur pipette at the heated bottom of the mixing volume. Two experiments were conducted.: for 0.1 mL experiments the entire volume of HNO_3 evaporated, producing an estimated partial pressure of about 0.005 atm in a static situation. In 1 mL experiments some liquid HNO_3 remained at the bottom of the volume with an estimated partial pressure of HNO_3 of 0.04 atm. The aerosol and gas phase HNO_3 were allowed to interact for 2 minutes at which point PALMS began sampling from the volume.~~

~~Samples of natural soil dust were collected from various locations listed in Table 2. Five sampled were investigated at the AIDA facility during FIN01 (Bächli soil, Argentina soil, Ethiopian soil, Moroccan soil and Chinese soil) with the remaining analysis at MIT (Storm Peak and Saudi Arabian soil). Two samples of German soil were used as an example of agricultural soil that was known to be fertilized with inorganic phosphate. These were also sampled at the AIDA facility during FIN01.~~

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2.3 Statistical analysis

A support vector machine (SVM), a supervised machine learning algorithm (Cortes and Vapnik, 1995), was used as the statistical analysis method for analysis of these data. ~~A portion of the data from each of the bioaerosol and non-biological phosphate samples was used as "training data" to build the algorithm. The remaining data were differentiated by the trained algorithm and the correctness judged based on their source.~~ In this case a non-linear binary classifier was constructed, using non-linear kernel functions (Ben-Hur et al., 2001; Cortes and Vapnik, 1995). A Gaussian radial basis function kernel was empirically determined to provide the best performance in this case. For this work, the SVM algorithm was implemented in MATLAB 2016a (MathWorks, Natick, MA) using the Statistics and Machine Learning toolbox.

2.4 Field data

The method was employed on ~~an~~two ambient data ~~set~~sets, one acquired at the Desert Research Institute's (DRI's) Storm Peak Laboratory located in Steamboat Springs, CO~~.~~ and the other acquired in Cool, CA site during Carbonaceous Aerosol and Radiative Effects Study (CARES). Storm Peak Laboratory is located on Mt. Werner at 3220 m elevation at 106.74 W, 40.45 N. This high altitude site is often in free tropospheric air, mainly during overnight hours, with minimal local sources (Borys and Wetzel, 1997). Ambient air was sampled using the Storm Peak facility inlet with the flight PALMS instrument in September, 2015. Measurements were made during Fifth International Ice Nucleation Workshop—phase 3 (FIN03). The measurements were carried out between September 14, 2015 and September 27, 2015.

The CARES study was carried out in the Summer, 2010 and included deployment of instruments at two different ground sites, one urban (Sacramento, CA) and another in the Sierra Nevada foothills area rich in biogenic emissions (Cool, CA site) (Zaveri et al., 2012). Thermally-driven winds tend to transport the urban plume into the Sierra Nevada foothills and sometimes back again into the Sacramento area (Zaveri et al., 2012). The laboratory PALMS instrument was deployed at the Cool, CA site at 450 m elevation at 121.02 W, 38.87 N in a trailer throughout the campaign. It sampled ambient air between June 4, 2010 and June 24, 2010.

3 Results

Figure 1 shows the spectra of biological species: *P. syringae* bacteria, Snomax and hazelnut pollen wash water particles. These particles contain both organic and inorganic ~~species~~compounds. Because they are easy to ionize, the inorganic ~~species~~ions sodium and potassium stand out in the positive spectra despite their minor fraction by mass. Sulfates, phosphates and nitrates are present, and visible in their associations with potassium. Negative spectra are dominated by CN^-, CNO^-, phosphate (PO_2^- and PO_3^-) and sulfate (HSO_4^-). Higher mass associations of potassium and sulfates, phosphates and nitrates occur ($K_3H_2SO_3^-$, $K_2H_3NO_4^-$, $K_3H_2PO_2^-$ and $K_3H_3SO_3^-$). Chlorine is present on some particles. Chlorine is a known ~~contamination~~contaminant from the Agar growth medium since spectra of aerosolized Agar devoid of bacteria contain large amounts of chlorine (not shown here).

Figure 2 shows spectra of apatite. In positive polarity, apatite spectra are dominated by calcium, its oxides, and in associations with phosphate ($CaPO^+$, $CaPO_2^+$, $CaPO_3^+$, $Ca_2PO_3^+$ and $Ca_2PO_4^+$) and fluorine (CaF^+, Ca_2OF^+ and Ca_3OF^+). Negative spectra are dominated by

phosphates (PO^-, PO_2^- and PO_3^-) and fluorine is often present. Lab-generated apatite spectra analyzed in this study contain little organic. This may be a result of post-processing of the apatite sample, in particular the use of ethanol as a grinding lubricant. In contrast, ethanol was not used in grinding the monazite sample here and its spectra exhibit peaks associated with organic matter (C_2H^-).

Figure 3 shows spectra of coal fly ash from the J. Robert Welsh Power Plant. The positive spectra contain sodium, aluminum, calcium, iron, strontium, barium and lead. As in apatite, calcium/oxygen, calcium/phosphate and calcium/fluorine fragments are present. Fly ash particles also contain sulfate ($H_3SO_3^+$). The negative spectra contain phosphates (PO_2^-, PO_3^-), sulfates (HSO_4^-) and silicate fragments, such as $(SiO_2)_2^-$, $(SiO_2)_2O^-$, $(SiO_2)_2Si^-$ and $(SiO_2)_3^-$.

The results of HNO_3 processing experiments are also shown in Figures 2 and 3. Processing with nitric acid had an effect on both apatite and fly ash: the calcium/fluorine positive markers (CaF^+, Ca_2OF^+ and Ca_3OF^+) and the negative fluorine marker (F^-) are either reduced in intensity or completely absent after processing. Additionally, CN^- and CNO^- appear and/or intensify after processing.

A classifier was designed to use the ratios of phosphate (PO_2^-, PO_3^-) and organic nitrogen (CN^-, CNO^-) spectral peaks. ~~This approach has previously been used with PALMS data to differentiate mineral dusts using silicate and metal peaks to reveal underlying differences in chemistry (Gallavardin et al., 2008). Figure 4A shows normalized histograms of the PO_3^-/PO_2^- ratio for the test aerosol. The aerosols that contain inorganic phosphorus, such as apatite, monazite, fly ash and soil dust, cluster at $PO_3^-/PO_2^- < 4$. The bioaerosols cluster at $PO_3^-/PO_2^- > 2$. Processing of apatite with nitric acid tends to shift the PO_3^-/PO_2^- ratio to larger values, increasing the disparity from the bioaerosols. Ragweed pollen is an exception, with a wide cluster in PO_3^-/PO_2^- from 1 to 5.~~ Those spectral peaks were used for several reasons: (1) they are clearly visible in all biological spectra that were acquired as a part of this study (Figure 1), (2) they were used to distinguish bioaerosol from other species in previous studies (Creamean et al., 2013; Pratt et al., 2009b) and (3) sources of phosphorus on aerosol particles are well-defined and documented in the literature (Mahowald et al., 2008). The only requirement for this analysis was that each spectrum used in the training set contains both phosphate and organic nitrogen (otherwise the ratios used here become undefined). This was ensured by selecting spectra, where $PO_2^- > 0.001$ and $CNO^- > 0.001$. Nearly all biological spectra in the training set satisfied this criterion (Table 2). Figure 4A shows normalized histograms of the

PO_3^-/PO_2^- ratio for the laboratory aerosol. The aerosols that contain only inorganic phosphorus, such as apatite, monazite and fly ash cluster at PO_3^-/PO_2^- less than 4 and often less than 2. The bioaerosols cluster at PO_3^-/PO_2^- greater than 2 and often greater than 4. Ragweed pollen is an exception, with a wide cluster in PO_3^-/PO_2^- from 1 to 5. Processing of apatite with nitric acid tends to shift the PO_3^-/PO_2^- ratio to larger values, decreasing the disparity from the bioaerosols. Ragweed pollen is an exception, with a wide cluster in PO_3^-/PO_2^- from 1 to 5. Soil dusts are shown in Figure 4, even though they are not used as training aerosol; their histogram shows a broad distribution with a tail extending into $PO_3^-/PO_2^- > 2$ region, indicating a mixed inorganic/biological composition. In comparison, fertilized soil dusts show a similar distribution to apatite ($PO_3^-/PO_2^- < 4$) due to presence of inorganic fertilizer, which is calcium phosphate.

The SVM algorithm was used here to optimize boundaries between clusters. To do this, the algorithm needs a training dataset, where the classes are known *a priori*. In this paper, the training dataset is defined in Table 2. Once an optimized boundary is drawn, some of the training data can still fall on the incorrect side of the boundary, when the clusters are not perfectly separable. Accuracy here is defined as percentage of correctly classified particles in the training set once the optimized boundary is found. A simple ~~delineation~~1D classifier can be made based only on the ratio of phosphate peaks ~~at~~ PO_3^-/PO_2^- ~~=~~greater or less than 3. The ~~misclassification rate~~accuracy of this simple filter is ~~20 – 30~~70 - 80% for the materials considered here, with ragweed pollen and fly ash as the greatest sources of confusion between the bioaerosol and non-biological classes. ~~A lower misclassification between~~A higher accuracy for differentiation of the bioaerosol and non-biological classes can be achieved if the ratio of organic nitrogen peaks is also taken into account. Figure 4B shows normalized histograms of CN^-/CNO^- ratios for the test aerosol. In contrast to PO_3^-/PO_2^- ratios, CN^-/CNO^- ratios do not, by themselves, exhibit a clear difference between the classes. A superior separation is achieved when data are plotted in a CN^-/CNO^- vs. PO_3^-/PO_2^- space, as shown in Figure 5. In this case, two clusters appear. The soil dust class was left out from the training set because it is not known *a priori* if and how much biological material it contains (classification of soil dusts with the SVM algorithm is discussed ~~latter~~later). The boundary between the classes in CN^-/CNO^- vs. PO_3^-/PO_2^- space is non-linear~~: the SVM algorithm "draws" this boundary~~, as shown in Figure 5. The ~~misidentification rate~~accuracy in this 2D classification is ~~~3~~97%. As before, ragweed pollen is the cause of most errors; if it is removed from training dataset, the ~~misidentification rate falls to <1~~accuracy increases to 99%. Processed mineral

dust had a smaller impact on the accuracy: removing it from the training dataset increased the accuracy to 97.5%.

For every observation, a distance from the SVM boundary can be calculated (otherwise known as score). Those distances can then be converted to probability of correct identification. An optimized function to convert scores to probabilities was found by 10-fold cross-validation (Platt, 1999). Because in this experiment the classes are not perfectly separable, the conversion function is a sigmoid. Posterior probabilities near 0 and 1 indicate high-confidence identification. An uncertainty boundary was defined between 0.2 and 0.8. This boundary is shown in Figure 5. Points that lie in this boundary are marked as low confidence assignments. Those correspond to shaded areas in Figures 6 and 7.

Once trained with the ~~laboratory data~~training set, the SVM algorithm was used to analyze the FIN03 and CARES field ~~dataset~~datasets collected at Cool, CA and Storm Peak. As a first step, "phosphorus-containing" particles were identified in ~~the dataset~~both datasets. The criterion for phosphorus-containing used for this work is the presence of both PO_2^- and PO_3^- ions at fractional peak area (area of peak of interest/total spectral signal area) greater than 0.01. This threshold was set by examination of the ambient mass spectra to determine when the phosphate peaks are ~~above the noise threshold.~~distinct. Ambient particles commonly have numerous small peaks at masses below ~200 due to a diversity of organic components. The height of this background is ~0.01 and data below this level are considered uncertain. Phosphorus-containing ambient spectra were then classified by the SVM algorithm as bioaerosol or inorganic ~~phosphorous~~phosphorus if the CNO^- ion was also present at fractional peak area greater than 0.001. If CNO^- fractional area was less than 0.001, the spectrum was also classified as inorganic phosphorus.

During the FIN03 campaign, phosphorus-containing particles represented from 0.2 to 0.5% by number of the total detected particles in negative ion mode depending on the sampling day and a 0.4% average for the entire dataset. As shown in Figure 6A when the binary classifier described in this work was applied to the phosphorus-containing particles, bioaerosol represented a 29% subset by number (i.e., 0.1% of total analyzed particles). ~~This~~During the CARES campaign, phosphorus-containing particles were 1.1% to 4.2% by number of the total particles detected in negative ion mode, with 2.4% average for the dataset (Figure 7A). Bioaerosol particles represented 63% subset by number (i.e., 1.2% of total analyzed particles). This range (0.1% – 1.2%) is within, and towards the lower end, of previous estimates with

biological-specific techniques (Table 1). This lower end estimate may, in part, be due to PALMS sampling particles in the 200-500 nm diameter range as well as larger sizes. Previous estimates tend to show increased bioaerosol in the super-micrometer range and data are often unavailable for the numerous particles smaller than 500 nm diameter.

The origin of the non-biological phosphate particles is likely phosphate-bearing mineral dust or fly ash. The CARES site experienced influences of aged marine, urban and local biogenic sources. Within the urban plumes, a likely source of inorganic phosphate is industrial combustion aerosol. At Storm Peak a likely source is mining of phosphate rock and nearby monazite deposits. Figure 6B shows HYSPLIT back trajectories for the ten days of the FIN03 campaign; the air masses sampled cross deposits of either phosphate rock (apatite) or rare earth elements (monazite or ~~carbonatitic~~carbonatite). As examples, on 09/27 the back trajectory intersects the vicinity of an active rare earth element (REE) mine in Mountain Pass, CA and on 09/18 and 09/20 the airmass intersected active phosphate mines in Idaho. Although negative spectra of apatite and monazite cannot be definitively differentiated from fly ash or soil dust spectra, positive spectra acquired during FIN03 ~~provide additional evidence~~additionally suggest that monazite-type material was present. In Figure 2, panels G and H show non-biological phosphate-rich ambient spectra from FIN03. Figure 2 panels E and F (monazite) contains similar features and matching rare earth elements.

In total, 56% and 36% of phosphate-containing particles analyzed in FIN03 and CARES respectively categorized as biological also contained silicate features. Considered in more detail in the next section, a subset of these may represent internal mixtures of biological and mineral components.

4 Discussion

~~4.1 Uncertainty in bioaerosol identification in PALMS spectra~~

The method of identification of bioaerosol described here is based on ratios of phosphate and organic nitrogen peaks. This work is specific to PALMS but can be considered a starting point from which identification and differentiation can be made with similar instruments. Previous work with PALMS shows this ratio approach can be used to identify differences in chemistry, for example among mineral dusts (Gallavardin et al., 2008). In this case the classes are bioaerosol and non-biological ~~phosphorous~~phosphorus; Figure 4A shows that phosphorus ionizes differently in these classes. In apatite and monazite, phosphorus occurs as calcium phosphate. In biological particles, phosphorus occurs mostly in phospholipid bilayers and

nucleic acids. In these experiments, the PO_3^-/PO_2^- ratio of those two forms is different (Figure 4A). The agricultural soils considered here cluster with the minerals and fly ash and we assume the ~~phosphorous~~phosphorus is due to the use of inorganic fertilizer, which is derived from calcium phosphate (Koppelaar and Weikard, 2013). Fly ash aerosol clusters similarly to apatite and monazite but with a wider distribution; this is likely because the chemical from of phosphorus in fly ash is different than in the minerals. Phosphorus present in coal is volatilized and then condenses into different forms during the combustion process (Wang et al., 2014).

~~Phosphorus peak ratios in biological particles cluster differently than in inorganic phosphorous particles with ragweed pollen an exception (Figure 4A). No satisfactory explanation for this observation has been found although contamination with phosphate fertilizer cannot be ruled out. The classification error of the biological filter using PO_3^-/PO_2^- and CN^-/CNO^- ratios is 3% with ragweed alone the source of most of the error. This unexplained behavior is a cause for concern, as the list of biological samples used as a training set is extensive, but not exhaustive and other exceptions could exist.~~

During the FIN03 campaign at Storm Peak, 0.2-0.5% of particles by number detected in negative polarity contained measureable phosphorus (Figure 6A). On most days, the majority of phosphorus-rich particles were inorganic. Particles with positive spectra showing the characteristics of monazite coupled to back trajectories over source areas ~~provides evidence of~~suggests the origin of the inorganic phosphate particles. Although apatite/monazite particles make up a small portion of ambient particles at Storm Peak they are potentially interesting not only due to their possible confusion with biological phosphate but also as a tracer for industrial mining and processing activities. Currently, such activities are taking place in Idaho and until very recently at Mountain Pass, CA (U.S. Geological Survey, 2016a, 2016b). Smaller exploration activities are also taking place at the Bear Lodge, WY and the REE-rich areas in Colorado, Idaho and Montana are of interest (U.S. Geological Survey, 2016a).

~~4.2~~During the CARES campaign more particles contained phosphorus (1.1% - 4.2%) and a higher percentage of phosphate-rich particles were identified as biological (63% vs. 29% in FIN03). Because the site contains strong local biogenic and urban influences, the sources of biological particles are probably local. As shown in Figure 7B, aged marine particles were also present on many days; however, only 4% of particles identified as biological also contained markers associated with sea salts.

4.1 Comparison with existing literature

Previous studies have attempted to identify bioaerosol with SPMS based on the presence of phosphate and organic nitrate components. Creamean et al. (2013) and Pratt et al. (2009b) suggested a "Boolean criterion" where the existence of CN^-, CNO^- and PO_3^- in a particle resulted in its classification as biological. If ~~a~~ silicate components were additionally present, the particle was classified as an internal mixture of mineral dust and biological components (Creamean et al., 2013; 2014). Such "Boolean" criteria for particle identification, can be helpful in distinguishing aerosol types when the signatures are unique to one particle type.

The selectivity of this simple three-component filter (presence or absence of CN^-, CNO^- and PO_3^-) for biological particles was investigated for PALMS using the test aerosol database with results shown in Figure ~~7. The~~8. Note that previous literature does not provide information on the thresholds used to determine presence or absence of ions in analysis of ATOFMS spectra. Furthermore, because of hardware differences, detection limits of PALMS and ATOFMS are known to be different (Murphy, 2007). This analysis focuses on PALMS and the threshold for "presence" was chosen as 0.001, which was observed to be the detection limit for CN^-, CNO^- and PO_3^- in the laboratory aerosol database used here. The simple filter successfully picks biological material. However, it also has a high rate of false positives. For the material that contains inorganic phosphorus (i.e., samples known to be devoid of biological material) the three-component filter selects 56% of fly ash, 56% of agricultural dust and 32% of apatite and monazite. Soil dust is identified as biological 78% of the time.

The effect of misidentification of inorganic phosphate as biological can be considered in the context of the atmospheric abundance of the three major phosphate bearing aerosols: mineral dust, fly ash and bioaerosol (estimates given in Table ~~3~~4). Because the emissions estimates vary, the highest fraction of bioaerosol is the case of the highest estimate of bioaerosol coupled to the lowest estimate of fly ash and mineral dust (Table ~~3~~4 and Figure ~~8A~~9A). Conversely, the lowest fraction of bioaerosol is the case of the lowest estimate of bioaerosol coupled to the highest estimate of fly ash and mineral dust (Table ~~3~~4 and Figure ~~8B~~9B).

The misidentification rates ~~shown~~noted above are then propagated onto the high and low estimates. As an example, the fraction of aerosol phosphate due to fly ash (1% in the high and 5% in the low bioaerosol estimate) is multiplied by .56 to indicate the fraction of fly ash that would be misidentified as biological phosphate with the simple three-component filter. This misidentification effect is repeated for the mineral dust emission rate and misidentification

fraction. For simplicity, we considered the mineral dust fraction to be desert soils, termed aridsols and entisols, which are predominantly present in dust-productive regions, such as the Sahara or the dust bowl (Yang et al., 2013). According to Yang and Post (2011), the organic phosphate content of those soils is 5-15% but this is a second order effect when compared to misclassification. In the high bioaerosol scenario 17% of the phosphate aerosol is biological (Figure ~~8A~~9A) but when misidentification is considered 81% of particles are identified as such (Figure ~~8C~~9C). In the low bioaerosol scenario 2% of the phosphate aerosol is biological (Figure ~~8B~~9B) but when misidentification is considered 77% of the particles are identified as such (Figure ~~8D~~9D). This illustrates that simplistic identification can lead to large misclassification errors of aerosol sources.

Misidentification can also lead to misattribution. Pratt et al. (2009b) analyzed ice residuals sampled in an orographic cloud and suggested a biological source using the simple three-component filter applied to spectra containing calcium, sodium, organic carbon, organic nitrogen and phosphate. The processed apatite spectrum in Figure 2, devoid of biological material, contains all of these markers. Similar to the Storm Peak dataset, the Pratt et al. (2009b) wave cloud occurred in west-central Wyoming which is near the Idaho phosphate rock deposits (Figure 6) and four U.S. states with active mining of phosphate rock for use as inorganic fertilizer in agriculture (U.S. Geological Survey, 2016b).

~~The~~As noted above, the Pratt et al. (2009b) and Creamean et al. (2013, 2014) studies were performed with a different SPMS, the ATOFMS (Gard et al., 1997; Pratt et al., 2009a). Because the ATOFMS uses a desorption/ionization laser of a different wavelength (266 nm) the SVM algorithm used here may not directly translate to that instrument~~.~~ (Murphy, 2007). Instead, the calculation above assumes only that the misidentification rates between the simple three-component filter and the SVM algorithm applies.

4.~~3~~2 Soil dust and internal dust/biological mixtures

Soil dust is an important but complicated category of phosphate-containing atmospheric particles. Modeling studies, such as Mahowald et al. ~~(2008), treat all phosphorus in soil dust aerosol as inorganic.~~(2008), treat all phosphorus in soil dust aerosol as inorganic. However, the phosphorus in soil investigated here took both organic and inorganic forms. Walker and Syers (1976) proposed a conceptual model of transformations of phosphorus depending on the age of the soil. At the beginning of its development, all soil phosphorus is bound in its primary mineral form, matching that of the parent material, which is primarily apatite (Walker

and Syers, 1976; Yang and Post, 2011). As the soil ages, the primary phosphorus is released. Some of it enters the organic reservoir and is utilized by vegetation, some is adsorbed onto the surface of secondary soil minerals (non-occluded phosphorus) and then gradually encapsulated by secondary minerals (Fe and Al oxides) into an occluded form. The total phosphorus content of the soil decreases as the soil ages, due to leaching. The organic fraction can encompass microorganisms, their metabolic by-products and other biological matter at various stages of decomposition. Soil microorganisms are the key players in converting organic phosphorus back into the mineral form (Brookes et al., 1984). Yang and Post (2011) estimated organic and inorganic phosphorus content of various soils based on available data. Spodosols (moist forest soils) have the highest fraction of organic phosphorus (~45%) and aridsols (sandy desert soils) have the lowest (~5%) (Yang and Post, 2011). Yang et al. (2013) compiled a global map of soil phosphorus distribution and its forms and found that 20%, on average, of total phosphorus is organic. Wang et al. (2010) arrive at 34% of soil phosphorus as organic globally.

The biological PALMS filter was applied to several soil dust samples (Table ~~2) and the numbers of biological particles in all cases fall within these estimates.~~3). As would be expected, soils collected in areas with less vegetation exhibit smaller biological contributions. We note that organic phosphorus content is not necessarily a direct indicator of microbes since it also encompasses decomposed biogenic and organic matter. At this time, we are not able to delineate between primary biological ~~and,~~ biogenic or simply complex organic (such as humic acids) material.

In the FIN03 field dataset, 56% of particles identified as biological also contained silicate markers normally associated with mineral dust. In the CARES dataset the percentage of such particles was 36%. This represents ~~and~~an upper limit of particles that are an internal mixture of dust and biological material. As stated in the last paragraph, this biological material probably does not consist of whole cells sitting on mineral particles; such internally mixed mineral dust particle with surface whole or fragments of biological material are not supported by EM (Peter Buseck, personal communication~~, 2016~~). It currently remains unclear if such internally mixed particles would be counted as biological with an optical microscope after fluorescent staining.

Internal mixtures of biological and mineral components were generated in the laboratory in order to investigate this; an exemplary spectrum of such particle is shown in Figure ~~9~~10. The

spectrum contains alumino-silicate markers consistent with mineral dust together with phosphate markers that, in this case, come from the biological material. In spectra of pure illite, no phosphate markers are present. Using the classifier developed in this paper on the laboratory-generated internally mixed particles correctly identifies the phosphate signatures to be biological.

4.3 Uncertainty in bioaerosol identification in PALMS spectra

Phosphorus peak ratios in biological particles cluster differently than in inorganic phosphorus particles with ragweed pollen an exception (Figure 4A). No satisfactory explanation for this observation has been found although contamination with phosphate fertilizer cannot be ruled out. The accuracy of the biological filter using PO_3^-/PO_2^- and CN^-/CNO^- ratios is 97% with ragweed alone the source of most of the error. This unexplained behavior is a cause for concern, as the list of biological samples used as a training set is extensive, but not exhaustive and other exceptions could exist.

The basic classifier presented in this paper is binary: all phosphate- and organic nitrogen-containing particles are classified either as biological or inorganic. However, spectra whose PO_3^-/PO_2^- and CN^-/CNO^- ratios are very close to the SVM boundary have more uncertain assignments than those whose PO_3^-/PO_2^- and CN^-/CNO^- ratios fall far away from the boundary. In order to provide an additional measure of classification uncertainty, a probability bound was defined as shown in Figure 5. According to this definition, 96% of particles in the training dataset were classified with high-confidence (Figure 5). In the FIN03 and CARES field datasets, 79% of phosphate-containing particles were classified with high confidence. The low-confidence assignments are shown on Figures 6A and 7A with shaded areas. The low-confidence assignments in field datasets can be related to chemical processing of particles (either at the source like in soils or during transport) or to internal mixing of biological and inorganic phosphate.

Because soil dusts are a special category, where lines between biological and inorganic phosphorus sources can be blurry because of ongoing chemical transformations, they have higher classification uncertainties than other types of phosphate-containing aerosols. In the field data, dust/biological mixtures (defined as particles classified as biological with silicate features) are overrepresented in the low-confidence assignments. Dust/biological mixtures constitute 26% (CARES) - 46% (FIN03) of high-confidence assignments and 64% (CARES) - 68% (FIN03) of low-confidence assignments. Moreover, only 75% of phosphate-containing

soil dust particles were classified with high confidence. However, in simple two-component internal mixtures of dust and biological fragments (Figure 10) phosphate features can be identified as biological with high confidence (98%).

Because the field studies were performed during different time periods, it was difficult to control for a constant excimer laser fluence. However, laser fluence was similar for all laboratory samples acquired (3-5 mJ pulse energy). This is a possible source of uncertainty, as fragmentation patterns can differ depending on pulse energy.

5 Conclusion

This paper examines criteria that can be used with SPMS instruments to identify bioaerosol. We propose a new technique of bioaerosol detection and validate it using a database of phosphorus-bearing spectra. A simple binary classification scheme was optimized using a SVM algorithm, with ~~a classification error of 3%. Using the binary classifier developed in this paper, ambient~~97% accuracy. Ambient data collected ~~at Storm Peak~~ during ~~the~~ FIN03 ~~campaign was~~and CARES campaigns are then analyzed~~.~~ with this binary classifier. Particles with phosphorus were up to 0.5% for FIN03 and 4.2% for CARES by number of all ambient particles in the 200 – 3000 nm size range. On average, 29% (FIN03) and 63% (CARES) of these particles were identified as biological.

~~Our work expands on previous SPMS sampling that used a more simple Boolean three marker criterion (CN^-, CNO^- and PO_3^-) to classify particles as primary biological or not (Creamean et al., 2013; 2014).~~ Our work expands on previous SPMS sampling that used a more simple Boolean three marker criterion (CN^-, CNO^- and PO_3^-) to classify particles as primary biological or not (Creamean et al., 2013; 2014). We show that the presence of these markers is necessary but not sufficient. We show a false positive rate of the Boolean filter between 64% and 75% for a realistic atmospheric mixture of soil dust, fly ash and primary biological particles.

The trained SVM algorithm was also used to measure the biological content of soil dusts. Different soil dust samples can have different content of biological material with a range from 2 – 32% observed here. Consistent with the literature, samples taken from areas with vegetation exhibit a higher biological content.

Acknowledgements

The authors gratefully acknowledge funding from NASA grant # NNX13AO15G, NSF grant # AGS-1461347, NSF grant # AGS-1339264, and DOE grant # DE-SC0014487. M. A. Z. acknowledges the support of NASA Earth and Space Science Fellowship. The authors would like to thank Ottmar Moehler and the KIT AIDA facility staff for hosting the FIN01 workshop and Gannet Hallar, Ian McCubbin and DRI Storm Peak Laboratory for hosting the FIN03 workshop. The authors thank the entire CARES, FIN01 and FIN03 teams for support and Peter Buseck for useful discussions.

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Table 1. Measurements of biological aerosol in the atmosphere (NR – not reported, FBAP – fluorescent particles, attributed to bioaerosol). *Comment in response to DeLeon-Rodriguez et al., 2013 by Smith and Griffin (2013).

Site	Elevation (m)	Technique	Concentration of bioaerosol detected (particles m^{-3})	% of total particles (size range)	Type of bioaerosol	Reference
Ground sites						
Jungfraujoch	3,450	Fluorescent microscopy	3.4×10^4 (free troposphere) 7.5×10^4 (over surface)	NR	Bacteria	Xia et al., 2013
Storm Peak Lab	3,220	Fluorescent microscopy	$9.6\times10^5 – 6.6\times10^6$	~~0.5-5% (0.5-20 μm)~~NR	Bacteria (51%) Fungi (45%) Plant material (4%)	Wiedinmyer et al., 2009
Storm Peak Lab	3,220	~~Fluorescent microscopy~~ Flow cytometry	3.9×10^5 (spring) 4.0×10^4 (summer) 1.5×10^5 (fall) 2.7×10^4 (winter)	22% (0.5-20 μm)	Bacteria	Bowers et al., 2012
Mt. Rax (Alps)	1,644	Fluorescent microscopy	1.1×10^4 (bacteria) 3.5×10^2 (fungi)	NR	Bacteria and fungi	Bauer et al., 2002
Various locations in Colorado	1,485-2,973	Fluorescent microscopy	1.0×10^5 - 2.6×10^6	NR	Bacteria	Bowers et al., 2011
Vienna	150-550	Fluorescent microscopy	$3.6\times10^3 – 2.9\times10^4$	NR	Fungi	Bauer et al., 2008
U.S. Virgin Islands	NR	Fluorescent microscopy	$3.6\times10^4 – 5.7\times10^5$	NR	Bacteria and possible viruses	Griffin et al., 2001
Various sites in the U.K.	50-130	Fluorescent microscopy	$5.3\times10^3 – 1.7\times10^4$ (spring) $8.3\times10^3 – 1.5\times10^4$ (summer) $6.0\times10^3 – 1.4\times10^4$ (fall) $2.9\times10^3 – 1.0\times10^4$ (winter)	NR	Bacteria	Harrison et al., 2005
Danum Valley, Malaysian Borneo	150-1,000	WIBS	2.0×10^5 (above forest canopy)	NR	FBAP	Gabey et al., 2010

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			1.5×10^6 (below forest canopy)			
Karlsruhe, Germany	112	WIBS	2.9×10^4 (spring) 4.6×10^4 (summer) 2.9×10^4 (fall) 1.9×10^4 (winter)	4-11% (0.5-16 μm)	FBAP	Toprak and Schnaiter, 2013
Aircraft campaigns						
Cape Grim	30-5,400	TEM	NR	1% (>0.2 μm)	Bacteria	Pósfai et al., 2003
Flights around the Gulf of Mexico, California and Florida	3,000-10,000	Fluorescent microscopy	$3.6\times10^4 - 3.0\times10^5$	3.6-276% (0.25-1 μm)*	Mostly bacteria	DeLeon-Rodriguez et al., 2013
Flights over southeastern U.S. (SEAC^{4}RS)	Vertical profiles up to 12,000	WIBS	3.4×10^5 (average, <0.5 km) 7.0×10^4 (average, 3 km) 1.8×10^4 (average, 6 km)	5-10% (0.6-5 μm)	FBAP	Ziemba et al., 2016
Flights over Colorado, Wyoming, Nebraska and South Dakota	Vertical profiles up to 10,000	WIBS	$1.0\times10^4 - 1.0\times10^5$ (<2.5 km) $0 - 3.0\times10^3$ (>2.5 km)	NR	FBAP	Twohy et al., 2016

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Table 2. Summary of particle statistics for samples used to both train and test the classifier.

Category	**Total negative spectra**	**Used for training the classifier**
Bare apatite	338	135
Processed apatite (~0.1 mL)	994	359
Processed apatite (~1 mL)	987	203
Fertilized soil dusts	1953	1774
Fly ash	3986	3536
Processed fly ash (~0.1 mL)	824	312
Monazite	415	371
P. syringae	1429	1429
Snomax	497	497
F. solani (whole)	1053	1010
F. solani (fragmented)	1129	1127
Yeast	778	757
Birch pollen	1136	1137
Hazelnut pollen	183	183
Oak pollen	1193	1191
Ragweed pollen	1207	1187
Bächli soil dust	501	Not used
Moroccan soil dust	460	Not used
Ethiopian soil dust	502	Not used
Storm Peak Lab dust	464	Not used
Argentinian soil dust	507	Not used
Chinese soil dust	1002	Not used
Saudi Arabian soil dust	3131	Not used
Illite NX (dry-generated)	1002	Not used
Illite NX (wet-generated)	1030	Not used
Illite NX/*F. solani* mixed	1396	Not used
FIN03 ambient sampling	26019	Not used
CARES ambient sampling	19011	Not used

Table 3. Soil dust samples used in this work. The last column shows the results of analysis with the ~~biological filter~~SVM classifier developed here as a percentage of negative ~~particles sampled~~spectra acquired.

Sample	Site description	Approx. collection coordinates	% biological
Bächli	Outflow sediment of a glacier in a feldspar-rich granitic environment. No vegetation.	46.6 N, 8.3 E	6.0
Morocco	Rock desert with vegetation. Close proximity to a road.	33.2 N, 2.0 W	20.4
Ethiopia	Collected in Lake Shala National Park from a region between two lakes. Area vegetated by shrubs and acacia trees.	7.5 N, 38.7 E	32.1
Storm Peak Lab	Collected near Storm Peak Lab. Grass and shrubs present.	40.5 N, 106.7 W	31.3
Argentina	La Pampa province. Top soil collected from arable land with sandy loam (Steinke et al., 2016).	37 S, 64 W ~~(approximate)~~	21.3
China/Inner Mongolia	~~Xilingele steppe. Top soil collected from a pasture with loam (Steinke et al., 2016).~~Xilingele steppe. Top soil collected from a pasture with loam (Steinke et al., 2016).	44 N, 117 E ~~(approximate)~~	2.0

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Saudi Arabia	Various samples from several locations. Arid, sandy soils.	24.6 N – 26.3 N, 46.1 E – 49.6 E	14.5

1

2

Table ~~3~~4. Literature estimates of emission rates of primary biological particles, dust and fly ash.

Particle	Emissions (Tg yr^{-1})	
	low estimate	*high estimate*
Dust	1490 (Zender, 2003)	7800 (Jacobson and Streets, 2009)
Primary biological	~~186 (Mahowald et al., 2008)~~186 (Mahowald et al., 2008)	298 (Jacobson and Streets, 2009)
Fly ash	14.9 (Garimella et al., 2016)	390 (Garimella et al., 2016)

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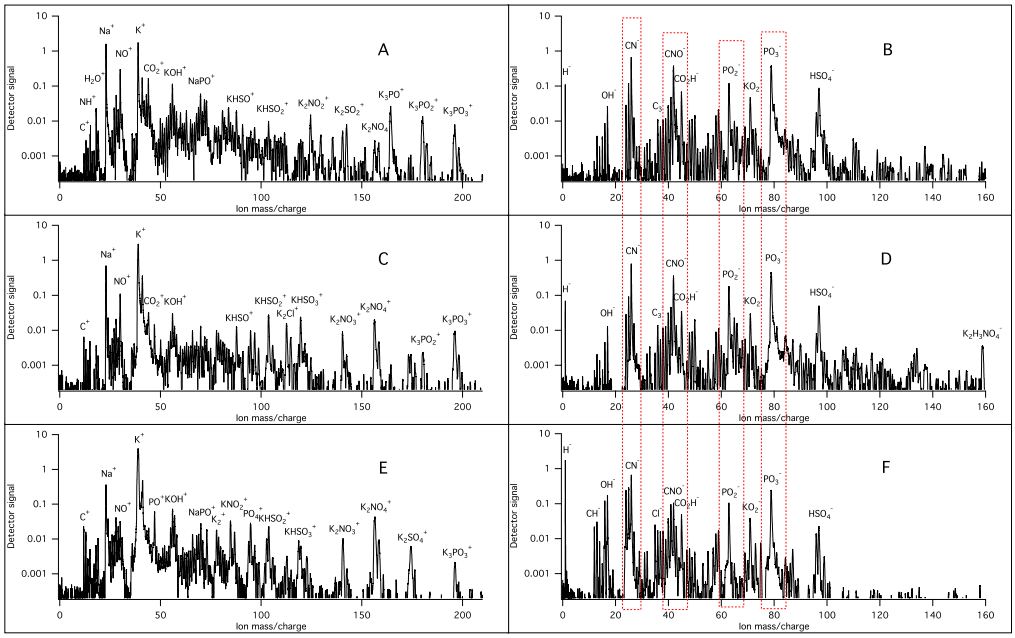


Figure 1. Representative PALMS spectra of bioaerosol. A and B: Snomax. C and D: *P. syringae*. E and F: Hazelnut wash water. Right and left columns are positive and negative polarity, respectively. Red dotted lines are features indicated in the literature as markers for biological material.

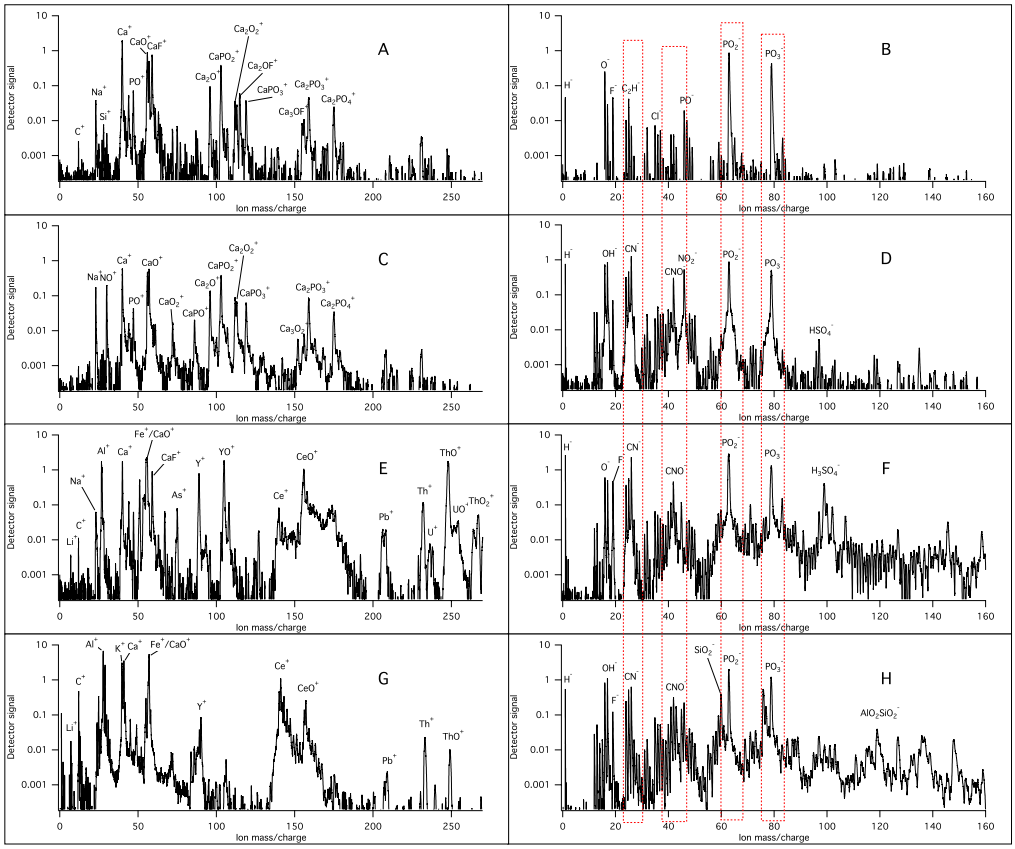


Figure 2. Representative PALMS spectra of phosphorus-rich minerals and ambient aerosol. A and B: Unprocessed apatite. C and D: Apatite processed with HNO_3 (see text for details). E and F: Monazite-Ce. G and H: Ambient particles sampled at Storm Peak matching monazite chemistry. Right and left columns are positive and negative polarity, respectively. Red dotted lines are features indicated in the literature as markers for biological material.

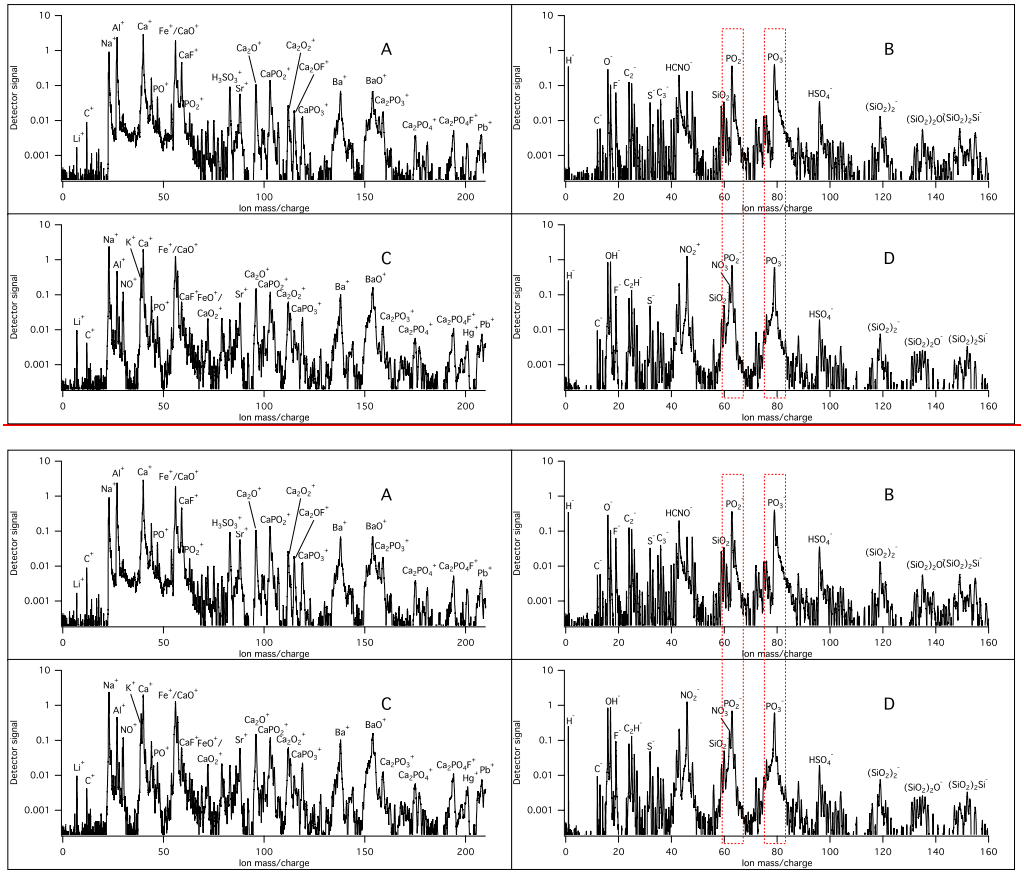


Figure 3. Representative PALMS spectra of coal fly ash from the J. Robert Welsh power plant. A and B: Unprocessed fly ash. C and D: Fly ash processed with HNO_3 (see text for details). Right and left columns are positive and negative polarity, respectively. Red dotted lines are features indicated in the literature as markers for biological material.

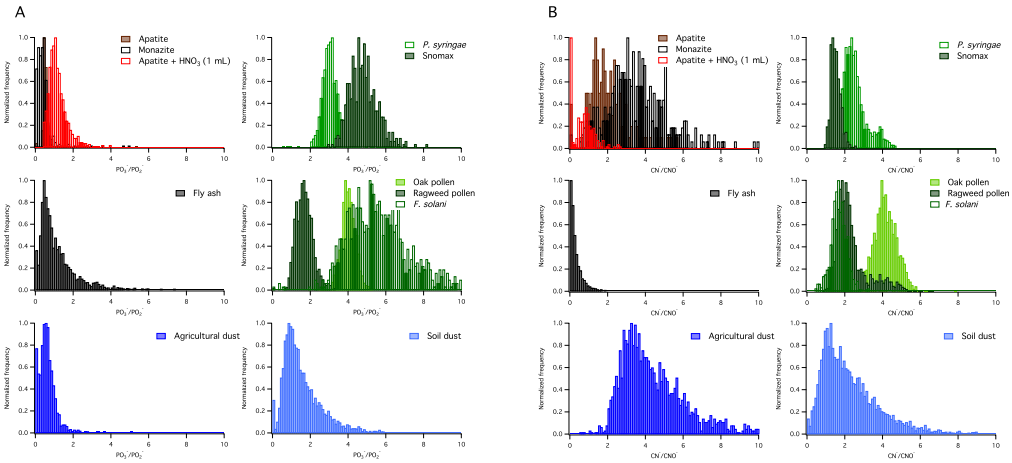
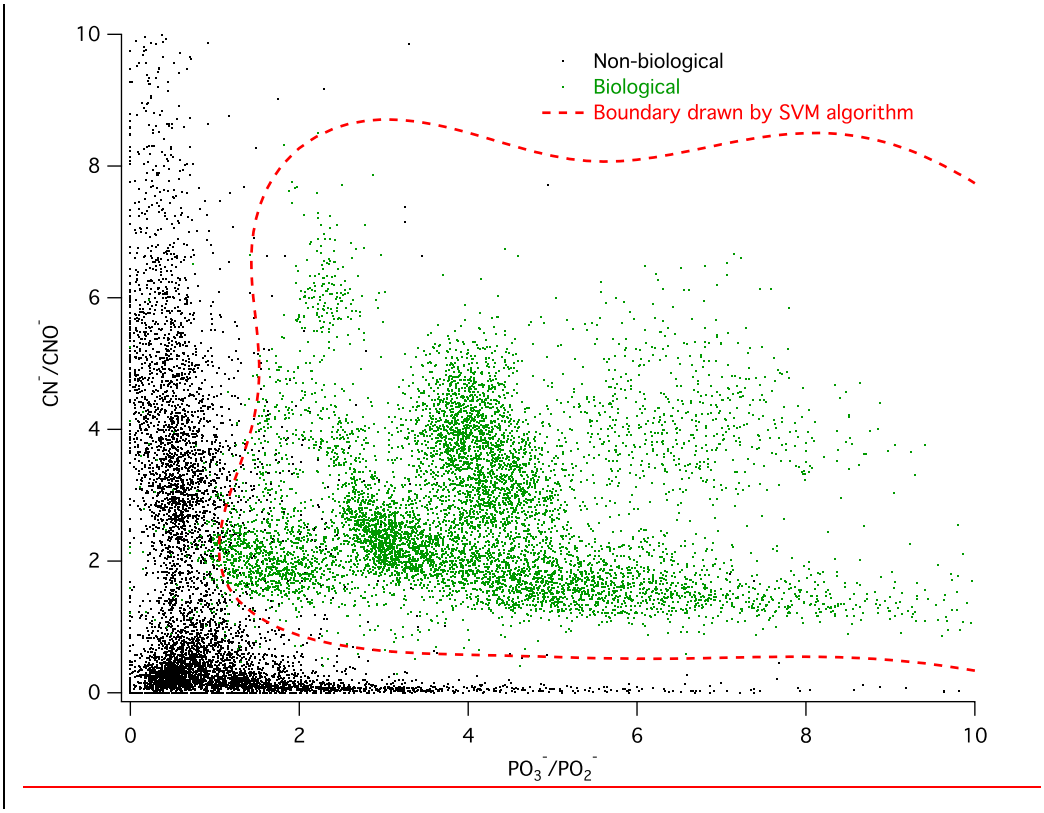


Figure 4. A: Normalized histograms of the PO_3^-/PO_2^- ratio for the ~~test~~laboratory aerosol. B: Normalized histograms of the CN^-/CNO^- ratio for the same ~~test~~laboratory aerosol as in A. Delineation between the clusters at a PO_3^-/PO_2^- ratio of 3 results in a 70-80% classification accuracy depending on the ~~species considered~~types of particles considered. Note that soil dusts were not used as part of the training dataset and that not all training aerosols are shown here for clarity.


Non-biological
Biological
Boundary drawn by SVM algorithm
CN-/CNO-
PO3-/PO2-
0
2
4
6
8
10

1

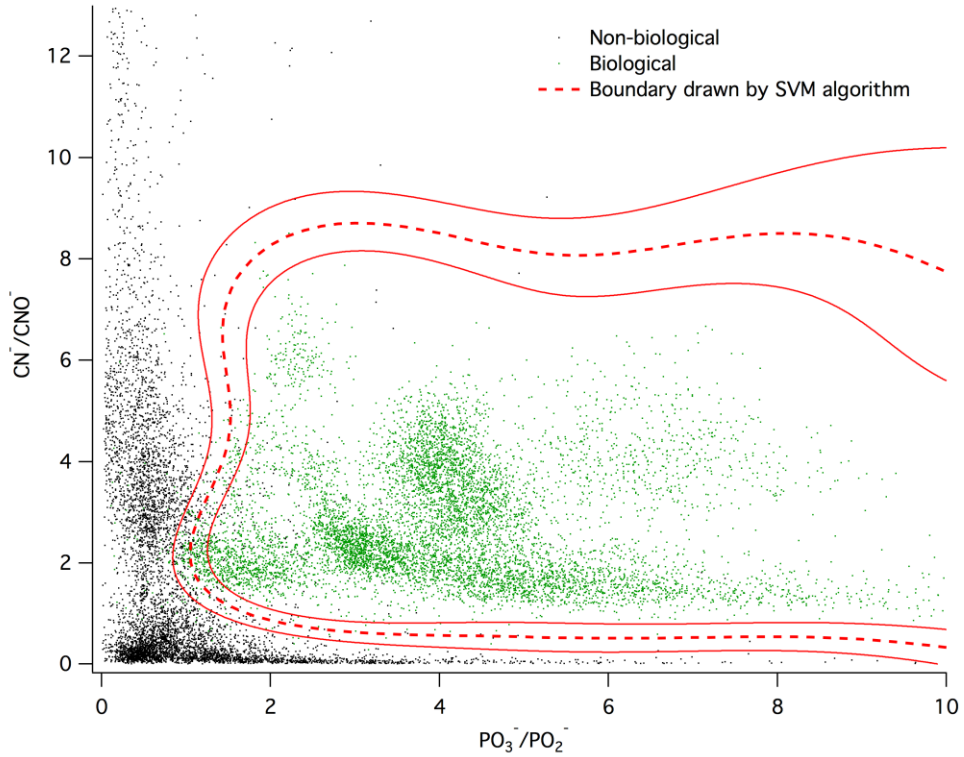
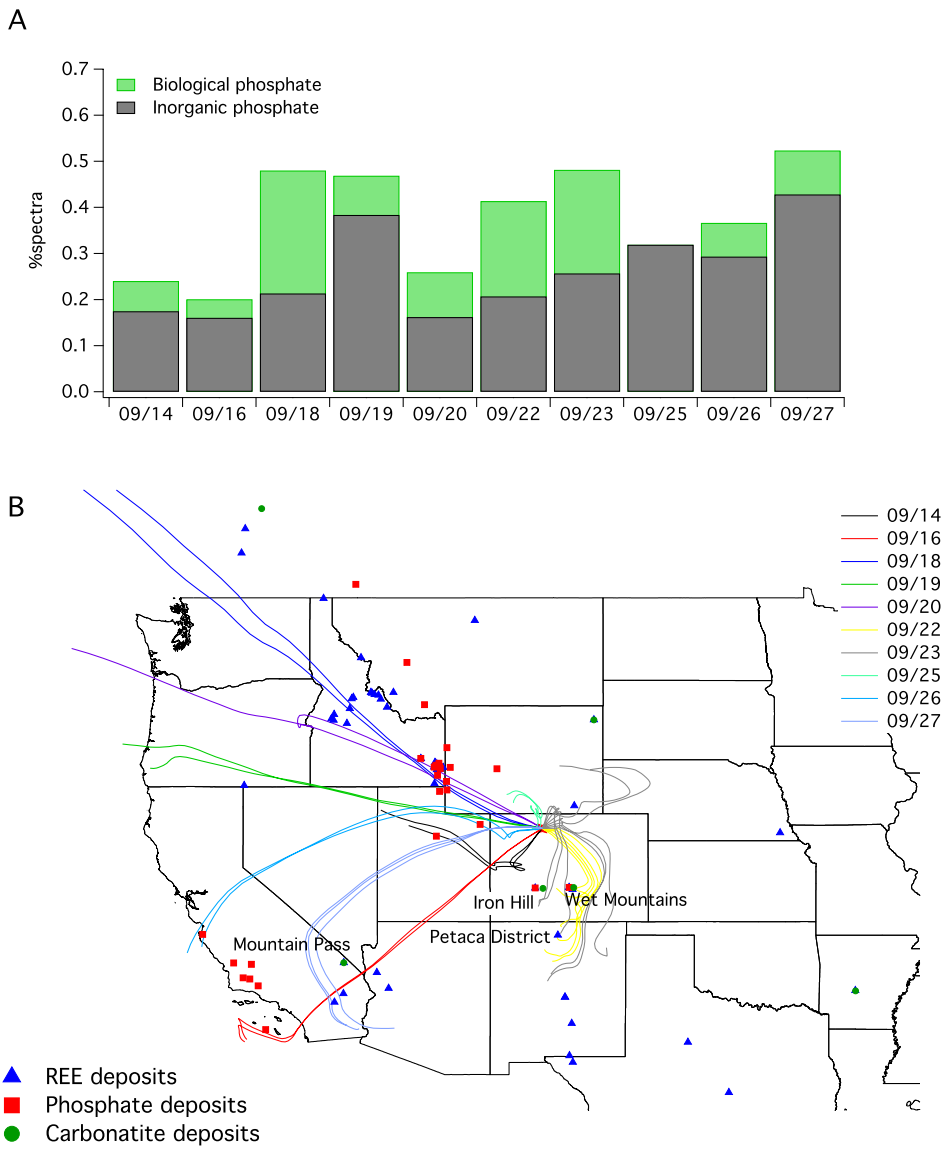


Figure 5. Inorganic and biological particle clusters in CN^-/CNO^- vs. PO_3^-/PO_2^- space. The SVM algorithm delineates between the clusters with the red dashed line with an overall 97% classification accuracy. Solid red lines indicate the uncertainty boundary (see text for further details).


A
Biological phosphate
Inorganic phosphate
%spectra
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
09/14
09/16
09/18
09/19
09/20
09/22
09/23
09/25
09/26
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B
09/14
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09/27
Iron Hill
Wet Mountains
Petaca District
Mountain Pass
REE deposits
Phosphate deposits
Carbonatite deposits

1

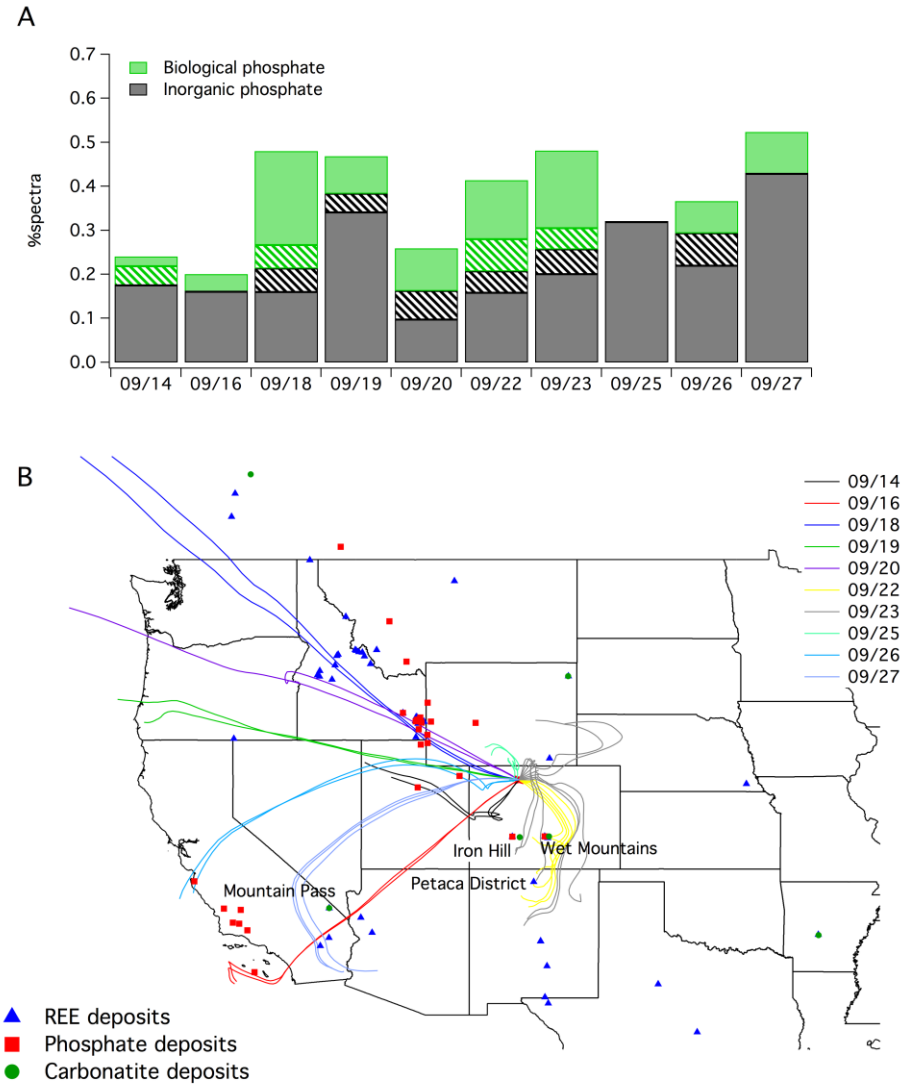

Figure 6. A: The percentage of ambient aerosol particles from ~~the~~FIN03 dataset categorized as biological and inorganic (phosphate-bearing mineral dust or fly ash) phosphate using the criteria developed in this work. Hatched regions indicate uncertain assignments per the boundaries in Figure 5. Note that at this location and time of year inorganic phosphate dominates biological. B: HYSPLIT back trajectories plotted for ten measurement days at Storm Peak Laboratory. Locations of REE, phosphate and carobonatite deposits, sourced from U.S. Geological Survey, are co-plotted (Berger et al., 2009; Chernoff and Orris, 2002; Orris and Grauch, 2002).

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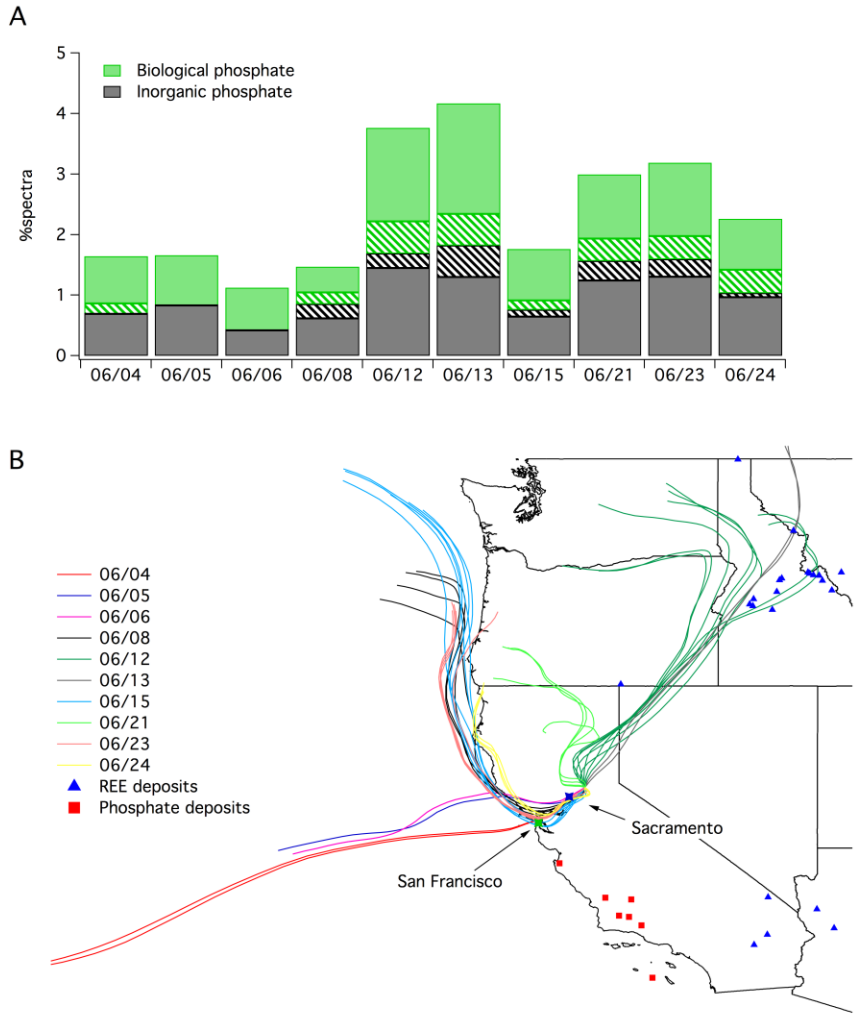

Figure 7. A: The percentage of ambient aerosol particles from CARES dataset categorized as biological and inorganic (phosphate-bearing mineral dust or fly ash) phosphate using the criteria developed in this work. Hatched regions indicate uncertain assignments per the boundaries in Figure 5. B: HYSPLIT back trajectories plotted for ten measurement days at the Cool, CA site. Locations of REE, phosphate and carobonatite deposits, sourced from U.S. Geological Survey, are co-plotted (Berger et al., 2009; Chernoff and Orris, 2002; Orris and Grauch, 2002) along with locations of major urban centers.

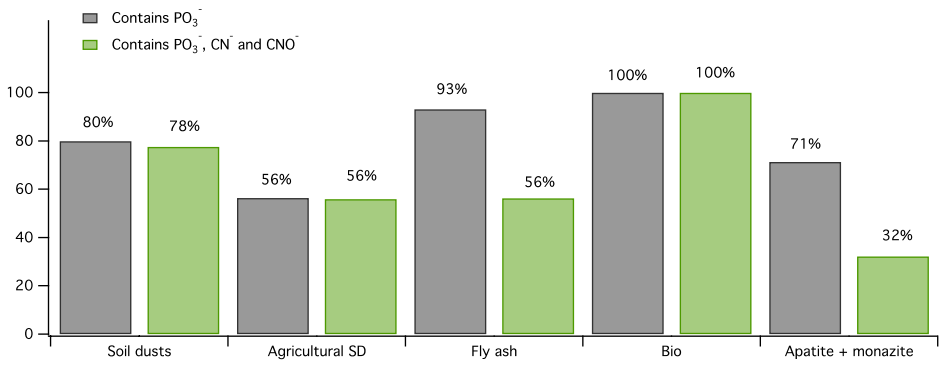

Figure ~~7~~8. Percentage of particles that include PO_3^-, CN^- and CNO^- markers in five classes of atmospherically-relevant aerosol spectra acquired with PALMS in this work. Note that the green bars indicate the percentage of particles of each type identified as biological using literature criteria. In the case of bioaerosol the identification is correct. In all other aerosol classes the green bar denotes a typical level of misidentification.

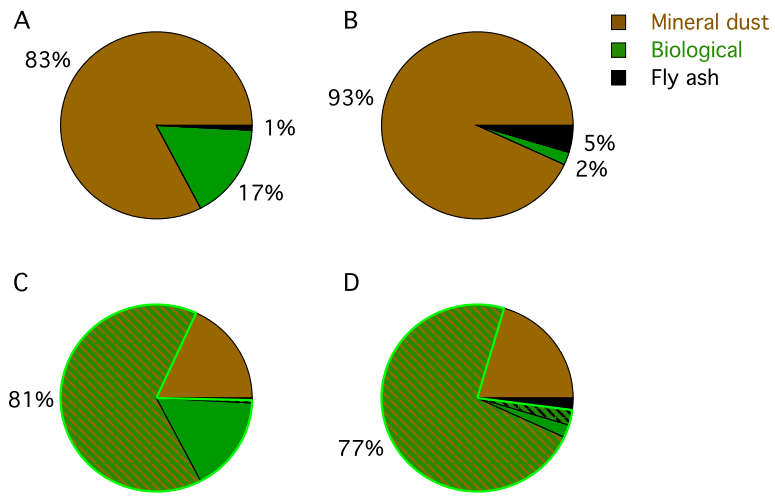

Figure ~~8~~9. Abundance of bioaerosol, mineral dust and fly ash in the atmosphere constructed using emissions estimates in Table 3 A: Highest estimate for bioaerosol coupled to lowest estimates for dust and fly ash. B: Lowest estimate of bioaerosol in the atmosphere coupled to highest estimates for dust and fly ash. C and D: Effect of misidentification of phosphate- and organic nitrogen-containing aerosol as biological using the emissions in A and B, respectively. The hatched regions correspond to the misidentified fractions of mineral dust and fly ash. In these estimates the correct emissions (solid green region) in A and B (17 and 2%, respectively) are overestimated (hatched green region of misidentified aerosol plus solid green region) in C and D (as 81 and 77%, respectively).

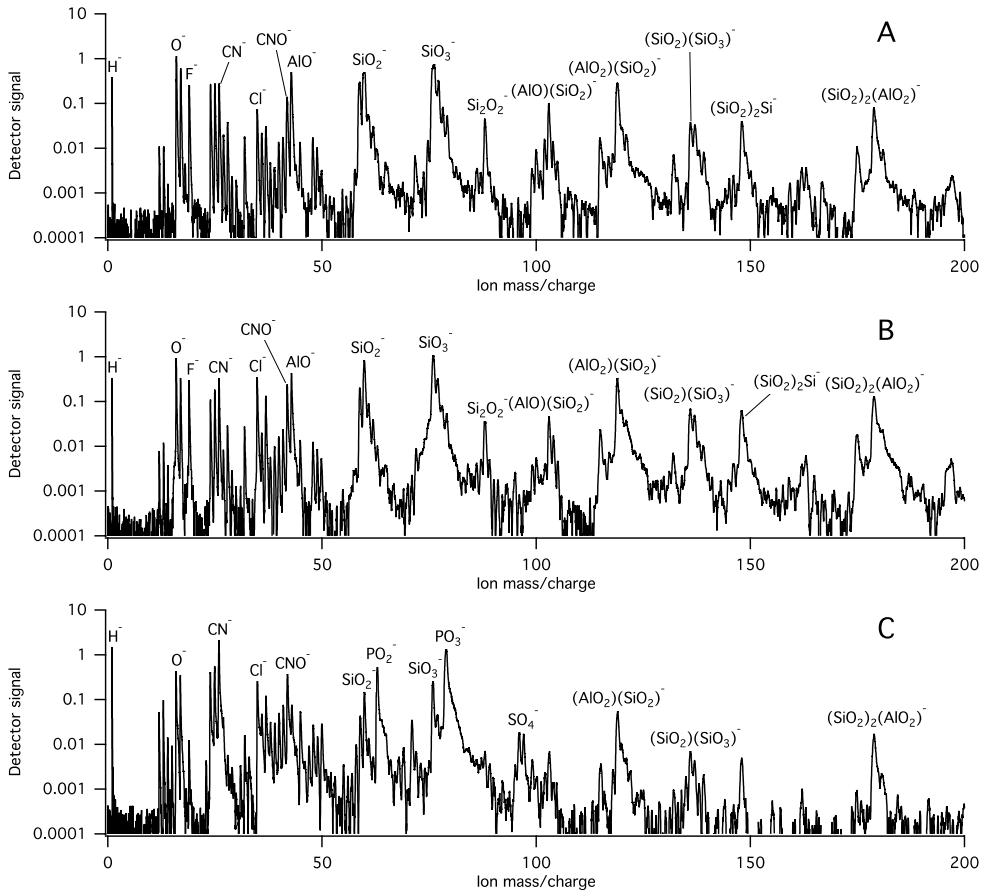

Figure ~~9~~10. Exemplary PALMS negative polarity spectra of A: dry-dispersed illite NX, B: wet-dispersed illite NX from a distilled, deionized water slurry and C: similarly wet-dispersed illite NX but from a water slurry that also contained *F. solani* spores. Note that phosphate features are absent in A and B but present in C due to addition of biological material to the mineral dust.