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Atmospheric Measurement Techniques Discussions

Interactive comment on "Autofluorescence of atmospheric bioaerosols – spectral fingerprints and taxonomic trends of native pollen" *by* C. Pöhlker et al.

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We thank the referee #1 for her/his positive review and that she/he "strongly recommends publication in AMT after minor revisions". The minor revisions suggested by the referee have been useful to improve the manuscript quality and have been processed, as outlined in detail below. The referees' comments are listed first, followed by our responses:

[1.1] Referee comment: Concerning the presentation of the results I would like to call your attention to the paragraph between L381 and L390 (in AMTD: p. 5708, l. 11-21)

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where you summarize the findings from fluorescence microscopy analysis. The entire paragraph provides a nice conclusion of results from fluorescence microscopy part of the study. However in the following paragraph the same results are discussed in detail and it is difficult to see that the same numbers refer to the same topic of discussion. Therefore I would suggest rearranging this part to make it easy for reader.

Author response: We agree with the referee that the use of the labels (i) to (iv) in this paragraph needs clarification. Therefore the paragraph in question has been changed and the use of the labels (i) to (iv) is explained explicitly. The first sentence in the paragraph:

"In the course of our microscopy analysis, we made the following general observations: (i) (. . .)"

has been changed to:

"In the course of our microscopy analysis, we made four main observations, which are listed here as (i) to (iv) and discussed separately and in detail in the following four paragraphs: (i) (...)."

[1.2] Referee comment: One of the most important observations in this study is that the relative fluorescence emission intensities of the same species show significant variation. Do you have any evidence that this behavior of pollen may be related to the metabolic state of pollen? Did you apply any viability test to the pollen species you presented?

Author response: The referee points at the important question, how pollen metabolic state and aging influences the autofluorescence properties. Comment [2.5] of referee #2 addresses a very similar question. We decided to rework several sections in the manuscript to discuss the aspects of pollen viability and aging in more detail. In addition, several further experiments have been conducted and implemented into the manuscript, which focus on the effects of pollen aging on autofluorescence. The

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following text sections have been incorporated into the text. Added to p. 5703, l. 6:

"The metabolic status of pollen is a potentially important aspect in the analysis of pollen autofluorescence properties. It is usually described by means of pollen viability (via staining protocols with specific fluorescent dyes) and/or germinability (via in vitro tube growth experiments) (Sato et al., 1998; Ferri et al. 2008). Both, viability and germinability decay with time as a function of storage length, temperature, and relative humidity (Van Der Walt and Littlejohn, 1996). An experimental assessment of pollen viability and germinability is rather complex, and therefore, a systematic examination of pollen metabolic state and quantitative comparison with autofluorescence properties is beyond the scope of the current study. However, we performed a number of simple experiments and comparisons, which indicate that freshly collected and aged pollen samples show overall similar autofluorescence properties: (i) In general, fresh and purchased pollen showed similar appearance in fluorescence microscopy analysis (compare Fig. 2). (ii) Relatively fresh and aged pollen samples of P. alba (collected in March 2013 versus March 2011) and F. sylvatica (collected in May 2013 versus April 2009) were analyzed by fluorescence microscopy and spectroscopy. Figure S2 reveals no clear qualitative differences in fluorescence microstructure between the younger and older pollen. However, an increase in fluorescence intensity of all grains is observed. Figure S3 shows that the spectral fingerprint in the corresponding EEMs of younger versus older pollen is nearly identical, however, it also confirms the trend of increasing fluorescence intensity with aging. (iii) The EEM of very fresh (1 day after collection) S. nigra pollen (Fig. S4Q) resembles the general fluorescence signatures of commercial (aged) pollen samples. Based on these crosschecks we assume in following that the fluorescence properties of commercially obtained and freshly harvested pollen samples are overall similar, except of increasing intensity with age, and that all samples are generally comparable."

Added to p. 5709, l. 15:

"The preliminary aging experiments in the current study support this idea because a

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trend of increasing fluorescence intensity with pollen age was found (compare Sect. 2.1, Fig. S2, and Fig. S3)."

The influence of age is also discussed in the responses to comments [2.5] and [2.12] of referee #2.

[1.3] Referee comment: In the fluorescence microscopy section you suggest that the single particle fluorescence may substantially differ from bulk fluorescence of same material. What kind of uncertainties would this difference introduce in the case of use of light induced fluorescence (LIF) technique for online pollen detection?

Author response: The heterogeneity of single particles fluorescence properties has indeed important implications for online pollen detection strategies with LIF techniques. We reworked related sections throughout the text and clarified our statements according to the referees' comment as outlined below.

The section in p. 5710, l. 1-6:

"This aspect is important for ambient PBAP detection as highlighted by Pinnick et al. (2013) because single particle fluorescence may substantially differ from bulk fluorescence of the same material. Accordingly, bulk fluorescence spectra, such as the EEMs presented in this study, provide an average characterization of fluorescent materials, however, differences on the level of individual cells (e.g., metabolic differences) are smeared."

has been replaced by:

"This aspect is important for ambient PBAP detection as highlighted by Pinnick et al. (2013) because single particle fluorescence may substantially differ from bulk fluorescence of the same material. Bulk fluorescence spectra, such as the EEMs presented in this study, provide an average characterization of fluorescent materials, however, differences on the level of individual cells are smeared. This introduces uncertainties and therefore complicates online pollen detection with LIF techniques because pollen

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fluorescence does not appear as a constant property across all grains. Instead, it reveals a certain scattering around its average fluorescence parameters. This aspect is further discussed in Sect. 3.4"

[1.4] Referee comment: L78 (in AMTD: p. 5696, l. 8-10): According to the study which authors reference here pollen can swell and burst after taking up water. This causes a release of significant amounts of micrometer size fragments of cytoplasmic debris. Nevertheless, it is not entirely clear to me why the number concentration of small PBAP could be underestimated in such case? Since authors suggest that the pollen wall provides the key information for detection and differentiation from other bioaerosol candidates it should be still possible to detect the pollen; regardless of the swelling process.

Author response: Our statement in p. 5696, l. 4-10 refers to the fact that pollen is often regarded as a PBAP type of minor importance because of its low number concentration. However, pollen bursting can increase the number concentration of pollen fragments, which can act vehicles for allergenic proteins, drastically. At this point, the "underestimation of PBAP" is not relating to autofluorescence based detection and cell wall fluorescence. Instead, it highlights in a more general way the potential importance of pollen and its fragments in the atmosphere.

[1.5] Referee comment: Can you describe briefly why you normalized the mode intensities to the total intensity and how you chose input data for PCA?

Author response: For the PCA the following input data has been used: (i) intensities of the main fluorescence modes A, B, and C. (ii) Total fluorescence intensity, as average of fluorescence from modes A-C. (iii) Pollen grain size. (iv) Pollen grain shape. The intensity of the fluorescence modes A-C has been normalized to total fluorescence intensity because this input data provides a good distribution of data points in the PCA plot. In contrast, non-normalized mode intensities spread the data points in the PCA plot mainly according to their total intensity level, which results in a poor separation.

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The PCA section has been reworked to clarify the comment [1.5] of referee #1 and also to address the comment [2.12] by referee #2. The paragraph in p. 5705, l. 18-25:

"Principal component analysis (PCA) was used as a statistical tool to visualize taxonomic trends in pollen autofluorescence. PCA was performed using Origin 8.6 (OriginLab Corp., Northampton, Ma, USA) based on fluorescence spectroscopy data from 25 pollen species (Table 1). The following pollen features were used as PCA input data: (i) Intensities of the main fluorescence modes A (λex = 280/ $\Delta\lambda$em = 440-460), B (355/440-460), and C (460/510-530). Mode intensities are normalized to total fluorescence intensity. (ii) Total fluorescence intensity, as average of fluorescence from modes A - C. (iii) Pollen grains size as given in Table 1."

has been replaced by:

"Principal component analysis (PCA) was used as a statistical tool to visualize taxonomic trends in pollen autofluorescence. PCA was performed using Origin 8.6 (OriginLab Corp., Northampton, Ma, USA) based on fluorescence spectroscopy data from 29 pollen species (Table 1). The following pollen features were used as PCA input data: (i) Intensities of the main fluorescence modes A (λex = 280/ $\Delta\lambda$em = 440-460), B (355/440-460), and C (460/510-530). These mode intensities are normalized to total fluorescence intensity, which results in a better scattering of data points in the PCA plot, compared to the non-normalized input data. (ii) Total fluorescence intensity, as average of fluorescence from modes A - C. (iii) Pollen grain size (Table 1). (iv) Pollen grain shape, as aspect ratio of major versus minor axis (Table 1)."

References:

Ferri, A., E. Giordani, G. Padula, and E. Bellini (2008), Viability and in vitro germinability of pollen grains of olive cultivars and advanced selections obtained in Italy, Advances in Horticultural Science, 22(2), 116-122.

Sato, S., N. Katoh, S. Iwai, and M. Hagimori (1998), Establishment of reliable meth-

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ods of in vitro pollen germination and pollen preservation of Brassica rapa (syn. B-campestris), Euphytica, 103(1), 29-33.

Van Der Walt, I. D., and G. M. Littlejohn (1996), Storage and viability testing of Protea pollen, Journal of the American Society for Horticultural Science, 121(5), 804-809.

Figure captions:

Fig1. (Figure number in revised paper: FigS2.) Microscopy images in bright field (left) and fluorescence mode (right) showing influence of age on fluorescence properties of two pollen species: (A) Populus alba harvested in March 2011 versus (B) Populus alba from March 2013; (C) Fagus sylvatica from April 2009 versus (D) Fagus sylvatica from May 2013. Regions with representative group of pollen grains are shown. White numbers show excitation exposure times for red (R), green (G), and blue (B) fluorescence channels. Shorter exposure times indicate higher fluorescence intensity. Analyzed samples suggest that fluorescence intensity of all grains increases with age - same effect is observed in corresponding EEMs (Fig. S3). No obvious differences in fluorescence microstructure between younger and older grains are observed. Scale bar = 30 μm.

Fig2. (Figure number in revised paper: FigS3.) EEMs showing influence of age on fluorescence properties by means of two pollen species: (A) Populus alba harvested in March 2011 versus (B) Populus alba from March 2013; (C) Fagus sylvatica from April 2009 versus (D) Fagus sylvatica from May 2013. Spectra show that fluorescence intensity of pollen bulk samples tends to increase with age, however, spectral signature in EEMs is conserved. P. alba shows slight intensity increase with age (Fig. S3A versus Fig. S3B), corresponding with comparably slight intensity increase in fluorescence microscopy images (Fig. S2A versus Fig. S2B). In contrast, F. sylvatica shows strong intensity increase with age (Fig. S3C versus Fig. S3D), corresponding with similarly strong intensity increase in fluorescence microscopy images (Fig. S2C versus Fig. S2D).

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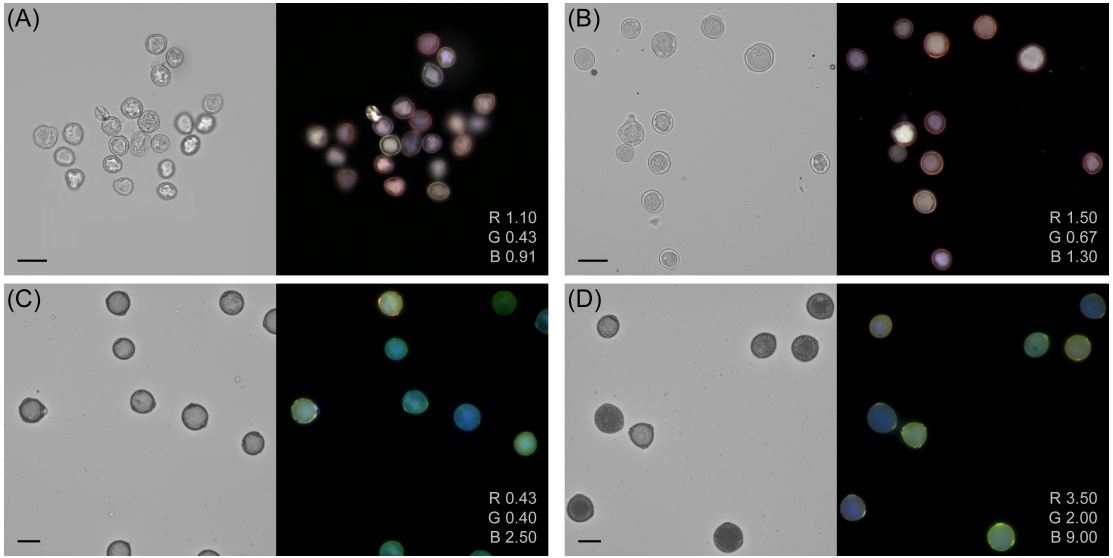

Fig. 1.

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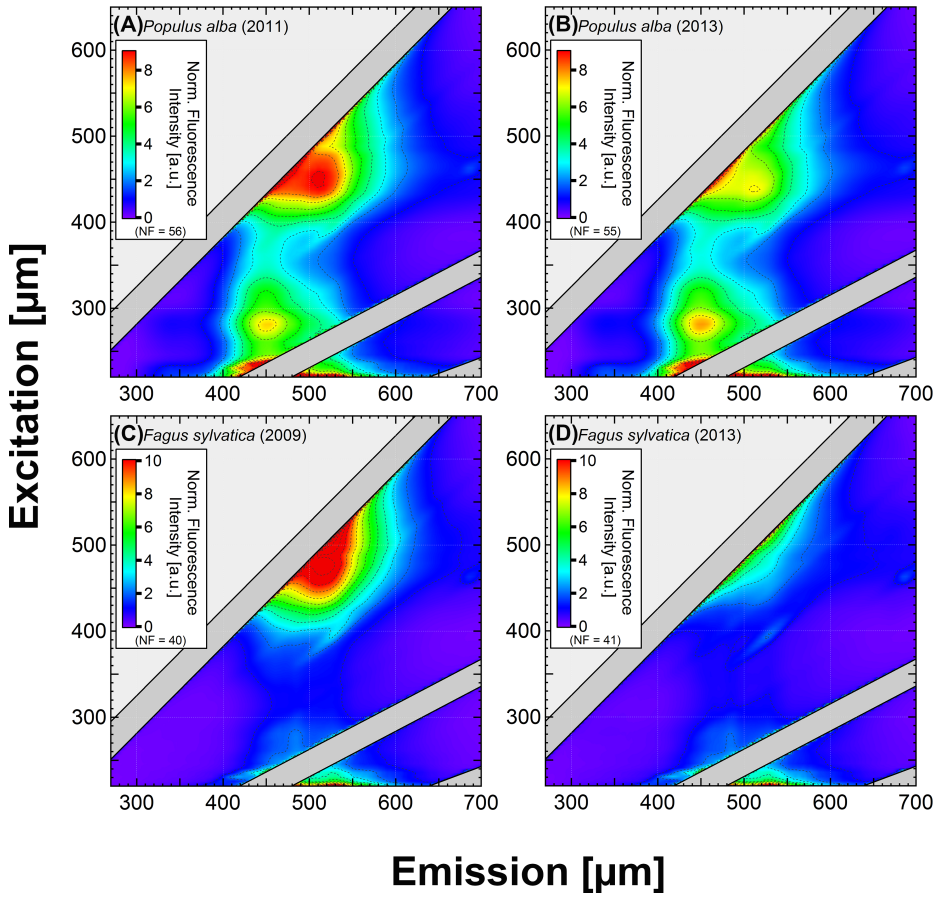

Fig. 2.

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