



A general framework for evaluating real-time bioaerosol classification algorithms

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Abstract. Advances in automatic bioaerosol monitoring require updated approaches to evaluate particle classification algorithms. We present a training and evaluation framework based on three metrics: (1) Kendall's Tau correlation between predicted and manual concentrations, (2) scaling factor, to assess identification efficiency, and (3) off-season noise ratio, quantifying off-season false predictions. Metrics are computed per class across confidence thresholds and five stations stations, and visualised in graphs revealing overfitting, station-specific biases, and sensitivity–specificity trade-offs. We provide optimal ranges for each metric respectively calculated from correlations on co-located manual measurements, worst-case scenario off-season noise ratio, and physical sampling limits constraining acceptable scaling factor. The evaluation framework was applied to seven deep-learning classifiers trained on holography and fluorescence data from SwisensPoleno devices, and compared with the 2022 holography-only classifier. Classifier performances are compared through visualisation methods, helping identifying over-training, misclassification between morphologically similar taxa or between pollen and non-pollen particles. This methodology allows a transparent and reproducible comparison of classification algorithms, independent of classifier architecture and device. Its adoption could help standardise performance reporting across the research community, even more so when evaluation datasets are standardised across different regions.

1 Introduction

Bioaerosol, and pollen in particular, have been routinely monitored in many countries using Hirst-type manual traps (Hirst, 1952) since the mid 1950s (Hyde, 1959; Emberlin et al., 1993; Buters et al., 2018; Gehrig and Clot, 2021). This method relies on manual identification and counting of pollen grains under a microscope. It is a time-consuming process and results in a delay between data collection and delivery (Galán et al., 2014). The last decade has seen the development of numerous automatic instruments that can detect and identify bioaerosol in real- or near-real time (Tummon et al., 2021; Huffman et al., 2019), often using machine learning algorithms (Brdar et al., 2023; Erb et al., 2024).



Since 2020, SwissPollen, the Swiss national bioaerosol monitoring network operated by the Federal Office of Meteorology and Climatology MeteoSwiss (denoted MeteoSwiss hereafter), has equipped 16 sites with SwisensPoleno automatic bioaerosol monitoring systems (Sauvageat et al., 2020), which were fully operational in 2023. The SwisensPoleno uses flow cytometry to isolate single particles and measure their characteristics, which are used as input features for a classification algorithm for the identification of particles. The SwisensPoleno instrument has evolved over the last 3–4 years, from just taking holographic images to also recording standardised fluorescence spectra of each particle. As a result, classification algorithms have needed to be adapted to take into account these new data, aiming to improve classification resolution and accuracy.

The classification algorithm used for the SwissPollen network since January 2023 was based only on holographic images and hourly information about seven pollen taxa have been made available to the public since then. While considered good enough for operational use (Maya-Manzano et al., 2023), the algorithm is known to suffer from several problems. Firstly, water droplets, present in high humidity conditions (i.e. fog or rain), can be misclassified as grass pollen due to similarities in shape and size. Additionally, some genera, for example within the Betulaceae family, have been shown to be difficult to classify reliably using holographic images only (Sauvageat et al., 2020). More generally, training bioaerosol classification algorithms remains challenging, since large training datasets are required to cover the entire spectrum of diversity of the bioaerosol present in a given country or region. Additionally, the development of new deep learning classifiers is a bottleneck in the community, as it requires substantial computational, technical and human resources.

Once a new classification algorithm has been trained, it is important to evaluate whether it improves performance compared to previous algorithms, using dedicated metrics and associated visualisations, that help identify over-training and misclassification. For the SwissPollen network, this entailed assessing the performance of the algorithm for the seven pollen taxa for which accurate pollen information is of particular relevance to Swiss allergy sufferers (Wüthrich et al., 2009), i.e., Poaceae (grass), *Corylus* (hazel), *Alnus* (alder), *Betula* (birch), *Quercus* (oak), *Fagus* (beech) and *Fraxinus* (ash). Evaluation of classification algorithms is commonly carried out against manual measurements from Hirst-type method, despite the limitations related to such measurements. These include the fact that only a fraction of the sample is counted (European Committee for Standardization (CEN), 2019), the air flow can be unsteady and is not continuously measured (Triviño et al., 2023), sampling is prone to human error (Sikoparija et al., 2016), and at low particle concentrations, the measurement uncertainty is very high (Adamov et al., 2021). It is therefore important, when evaluating classification algorithms with operational manual observations, to use data from several locations and over the longest possible period. Additionally, water droplets are not identified by Hirst-type traps, meaning there is no reference available for this class of particles.

Here, we present the development and evaluation of a deep learning classification pipeline for the SwissPollen operational network, designed to work on holographic and fluorescence data from SwisensPoleno Jupiter, i.e. the SwisensPoleno instrument that produces standardised fluorescence data. We aim at providing a full pipeline and protocol to help the community 1) train and fine-tune deep-learning classifiers and 2) evaluate them with understandable metrics and associated visualisation means, that highlight over-training and misclassification. The evaluation strategy involves applying different classifiers, to operational data from five SwissPollen stations. To support systematic algorithm comparison, we develop evaluation metrics that identify



the best-performing classifiers for real-time bioaerosol identification. These metrics provide a reproducible framework for evaluating bioaerosol classification algorithms developed across the globe for any automatic bioaerosol monitoring system.

2 Methods

We present a framework for comparing several bioaerosol classifiers developed for SwisensPoleno instruments, regardless of the number of taxa of interest. The example of the operational SwissPollen network is shown to illustrate this pipeline.

2.1 Instruments and raw data

The SwisensPoleno Jupiter measures airborne particles ranging in size from 0.5 to 300 μm as they pass through the instrument in-flight. When a particle triggers the detector, two orthogonally-arranged cameras capture in-flight holograms (200×200 pixels, greyscale). Fluorescence is then induced sequentially using three excitation sources (280, 365, and 405 nm), and the emitted light is recorded within five spectral windows (333–381, 411–459, 465–501, 539–585, and 658–694 nm), each named according to its central wavelength (357, 435, 483, 562, and 676 nm). This yields 15 raw fluorescence intensity values. However, the first channel becomes saturated due to scattered light when the 365 nm excitation is used, and for single-photon excitation at 405 nm, no signal is expected in the same channel as this would violate energy conservation. This effectively reduces the number of usable fluorescence values to 13. The fluorescence data are further pre-processed to ensure consistency and robustness across instruments. The full set of measurements for an individual particle, including each pair of holographic images and fluorescence intensities, is referred to as an “event” (Sauvageat et al., 2020; Erb et al., 2024; Berg and Videen, 2011).

2.2 Training datasets

The classification algorithms were trained on three different datasets, composed of single-taxa datasets. Each dataset covers the same 15 classes labelled *Alnus*, *Betula*, *Carpinus*, *Corylus*, *Cupressus*, *Fagus*, *Fraxinus*, *Pinaceae*, *Platanus*, *Poaceae*, *Populus*, *Quercus*, *Taxus*, *Ulmus* and Raindrops. Each event includes two 200×200 pixel holographic images, with or without fluorescence data depending on the single-taxa dataset it is part of.

Classes in training datasets are built from single-taxa datasets that combine events from species of the same genus, apart for the *Pinaceae* class, which contains events of *Picea*, *Cedrus*, *Pinus*, and *Larix* genera, as well as the *Poaceae* family, which groups events from the *Alopecurus*, *Arrhenatherum*, *Bromus*, *Lolium*, *Dactylis*, *Trisetum* and *Cynosurus* genera. Further detailed information about the training datasets used can be found in the supplementary material SI_1_training datasets.csv and on the Github repository (MeteoSwiss, 2025).

The training single-taxa datasets were cleaned manually using the Open Source Swisens Data Explorer tool. Particles that were clearly not pollen were removed based on the holographic images, as were events for which the images included more than one particle or none at all.

Holographic dataset:



Class	Holography only	Holography + fluorescence	Combined training
Alnus	P-2, P-4, P-5	P-27	P-2, P-4, P-5, P-27
Betula	P-2, P-4	P-27	P-2, P-4, P-27
Corylus	P-2, P-5	P-27	P-2, P-5, P-27
Fagus	P-2, P-4, P-5	P-15, P-22	P-2, P-4, P-5, P-15, P-22
Fraxinus	P-2, P-4, P-5	P-28, P-29	P-2, P-4, P-5, P-28, P-29
Poaceae	P-2, P-4, P-5, P-19	P-2	P-2, P-4, P-5, P-19
Quercus	P-2, P-4, P-5	P-19	P-2, P-4, P-5, P-19
Raindrops	P-5, P-16	P-5, P-11, P-33	P-5, P-11, P-16, P-33

Table 1. Summary of the instruments used for generating training datasets for eight classes of interest (seven pollen taxa and the water droplets). The Holography and fluorescence dataset displays the least diversity in instrumentation.

85 The **2022-Operational** algorithm was trained using only holographic images (i.e., no fluorescence). A total of 166'262 events were used, with the Carpinus class having the lowest number of events (2'247) and the Poaceae class the maximum number of events (22'666). All holographic-only single-taxa datasets that make the training dataset were produced in Switzerland between 2020 and 2021.

Holographic & fluorescence dataset:

90 The **2025-Beta-1**, **2025-Gamma-1**, and **2025-Omega-1** algorithms were trained on a dataset that includes events with both holographic images and fluorescence measurements. The training dataset includes a total of 563'063 events, with a minimum of 9'461 for the Ulmus class and a maximum of 75'197 for the Poaceae class. The single-taxa datasets that make this training dataset are part of those produced and described in Erb et al, 2025 (Erb et al., 2025).

Combined dataset:

95 The **2025-Beta-2**, **2025-Gamma-2**, **2025-Gamma-3** and **2025-Omega-2** algorithms were trained on a mix of the two aforementioned datasets as well as a few additional single-taxa datasets containing both holography and fluorescence data chosen in a targeted way to compensate for the lack of diversity for certain classes. This training dataset is composed of 104 single-taxa datasets, the classes containing a minimum of 14'391 (class Populus), a maximum of 216'741 (class Corylus), and a total of 893'361 events.

100 More instruments were used for producing the Combined training dataset compared to the Holography & Fluorescence dataset 1. This integrates more instrument variability, especially for eight classes of interest (Alnus, Betula, Corylus, Fagus, Fraxinus, Poaceae, Quercus and Raindrops), which cover the most allergenic taxa in Switzerland, and Raindrops, which are often misclassified as Poaceae pollen. Due to the differences in the amounts of events in each class, they were weighted accordingly.



105 2.3 Classifier architecture and parameters

The 2022-operational algorithm is based on the VGG16 architecture (Simonyan and Zisserman, 2014), applied separately to the two orthogonal holographic images of each particle, i.e. two single matrices of grey levels. Each image is processed through convolutional layers to extract features, which are then combined in fully-connected layers. The final classification is obtained via a Softmax layer that outputs a probability distribution, from which the most likely pollen class is selected. The architecture
 110 of the 2022-operational algorithm is described in more detail in Sauvageat et. al, 2022 (Sauvageat et al., 2020). During the training process, the dataset was split into two subsets: 80 % where used to train the classifiers, while the remaining 20 % were reserved for validation on unseen data.

The 2025-Beta-1, 2025-Gamma-1, 2025-Omega-1, 2025-Beta-2, 2025-Gamma-2, 2025-Omega-2 and 2025-Gamma-3 algorithms follow a triple-branch convolutional neural network architecture designed to process holographic and fluorescence
 115 input. The classifier works on events that have both kinds of data, or holography only by setting up one input to zero. The two holography input branches independently process an holographic image as a single matrix of grey levels through a sequence of convolutional layers, followed by ReLU activation, and max pooling, ending in a size of 1024 for each branch. The fluorescence branch contains a fully-connected dense layer followed by a drop out. The extracted feature maps from both branches are flattened and concatenated before entering a fully-connected classification head composed of dense layers with ReLU activations
 120 and dropout for regularization. A final Softmax layer produces the class pseudo-probabilities for particle identification.

While the global structure remains consistent, each classifier presents various architectural differences. All classifiers share the same convolutional blocks (two convolutional layers followed by max pooling, and three convolutional layers followed by max pooling, respectively repeated tow and three times) and a flatten layer. The Beta classifiers then have three fully connected dense layers, while the Omega classifiers have two, and the Gamma classifiers only one. The output from the fluorescence
 125 branch has a size of 26, apart from the 2025-Gamma-3 classifier which has a size of 13. Further details about the classifiers and a ready-to-use ONNX (Bai et al., 2019) version can be found in the following Github repository. Additionally, Crouzy et al. (2025) (Crouzy et al., 2025) is a companion short note describing the new operational SwissPollen classifier.

2.4 SwissPollen operational evaluation datasets

The algorithms are evaluated using operational data from the 2024 pollen season at five stations (Table 2) where manual
 130 Hirst-type instruments were run in parallel. These stations are located on the Swiss plateau at low altitude (Peel et al., 2007), where the climate is between oceanic and continental, and where a majority of the Swiss population lives(Federal Statistical Office (Switzerland), 2017). The start and end dates were selected to maximise overlaps between the manual and operational automatic measurements (avoiding manual instrument downtime). Note that prior to 2024, the software used to pre-process the fluorescence data was not the same, therefore the results from (Erb et al., 2024) were not implemented operationally and data
 135 from earlier years cannot be used.

The predictions from the eight classifiers are aggregated into daily concentration averages. This allows easy comparison with the manual observations that cannot provide reliable data at a subdaily resolution due to limited sampling and spreading of



Location	Coordinates	Start date	End date	Poleno ID
Buchs	47.17327, 9.47261	16.01.2024	14.09.2024	P-13
Basel	47.56181, 7.58391	12.01.2024	04.05.2024	P-27
Payerne	46.81337, 6.94290	04.05.2024	31.08.2024	P-30
Luzern	47.05768, 8.29679	16.01.2024	10.06.2024	P-21
Neuchâtel	47.00029, 6.94983	11.01.2024	01.06.2024	P-18

Table 2. Location as well as start and end dates of the operational time series used to evaluate the eight classification algorithms. The pollen concentrations at each site were compared with manual measurements from the Hirst-type trap over the same period at the same location.

particles on the sampling tape. Note that when the SwisensPoleno becomes saturated due to an excessive number of particles, it estimates how many particles were missed and provides a correction factor. This factor, referred to as the multiplier, is used to adjust the event count accordingly. For all event types, except for raindrops, this multiplier was applied to obtain an event count that is as accurate as possible.

The pollen concentrations for these five stations are available on the Github repository (MeteoSwiss, 2025). These data are used as a reference, even though not providing a perfect picture of pollen concentrations, as 1) only a fraction of the sample is counted (European Committee for Standardization (CEN), 2019), 2) the air flow of the device can vary and is not monitored continuously (Triviño et al., 2023), 3) the method can be subjected to human error (Sikoparija et al., 2016), and 4) the measurement uncertainty is high (Adamov et al., 2021). However, it is reliable to identify what pollens are in the air and therefore identify pollen seasons. For these reasons, we diversified the validation datasets by selecting operational data from five different stations, and aim, in the future, at not relying on these manually obtained concentration data to evaluate our algorithms.

2.5 Algorithm evaluation

The performance of the classification algorithms was evaluated using the following three metrics on the operational data from five SwissPollen stations:

Kendall's Tau correlations were used to capture the correlation between the reference (here, manual) and automatic measurements. The Kendall correlation was chosen rather than the Pearson correlation because it is less sensitive to outliers. The better the classifier, the higher the Kendall's Tau value. Note that for the season considered, the Kendall correlation is markedly lower than the Pearson correlation.

The off-season noise ratio was designed to measure the out-of-season false positives, considered as noise. It is calculated as the ratio between the mean predicted pollen concentration outside the pollen season divided by the sum during the season. Note that these values are sensitive to the size of the off-season, and is therefore dependant from the period and station, but comparable between two classifiers evaluated on the same period and station. The lower the ratio, the fewer false positives were detected outside of the season. Class-specific seasons for each pollen taxa were automatically identified from the manual



measurements. A sliding window of seven consecutive days was applied and days were considered as belonging to the season when at least four of the seven days had average pollen concentration greater than 20 particles per cubic meter. For some stations and taxa, the automatic detection of the start and end of the season could not be automatically detected (manual data lacking at the beginning of the season, low season, or a season season occurring in multiple phases with an early rise, a temporary decline, and a later peak). For these reasons, some seasons were manually defined (Poaceae, Corylus, Quercus and Fagus for some of the stations) and the first, respectively last day with non-null, respectively null concentration counts was selected as beginning, respectively end of the season. However, start and end of seasons can be defined differently, as soon as kept the same to compare classifiers. This makes this metric independent from manual concentrations, as not necessarily relying on them.

The scaling factor is the factor by which predicted pollen concentrations need to be multiplied to obtain values in the same range as the manual time series. Scaling factors between 1-20 were considered reasonable for SwisensPoleno, with values larger than 20 indicating the automatic measurement system would reach detection limit of the manual device. The scaling factor is computed by minimizing the mean square error between the manual and automatic data over the interval [0.001, 1000] (Virtanen et al., 2020). The scaling factor is calculated for each class, confidence threshold and station individually, and averaged over all stations so it can be applied in an operational setup.

The three metrics are also used to identify the optimal confidence threshold serving as a filter to remove events with low confidence. To compare these curves, the area under each metric curve was computed using the trapezoidal rule. We therefore compute the $\Delta AUC = AUC_{max} - AUC_{min}$, corresponding to the area of the range of each metric across all stations and thresholds, that we compare across classifiers to evaluate the evolution of the range of each metrics.

Note that the confidence threshold and scaling factors are selected individually for each taxa, but are applied uniformly to all SwisensPolenos.

3 Results and Discussion

We trained seven classification algorithms on holography and fluorescence data and compared them with the 2022 classifier, trained on holography data only. We expect to observe improvements, particularly in terms of the identification of water droplets as well as for the Betulaceae family—*Alnus sp.*, *Betula sp.* and *Corylus sp.*. Note that a 2025-Alpha-1 classifier using EfficientNet B0 pre-trained on ImageNet was trained (Tan and Le, 2020; Erb et al., 2024), but did not provide classifications that were good enough to be discussed in this paper. We decided to not use pretrained classifiers so that the classifier can be tailored to bioaerosol holographic (grayscale) images and fluorescence, but also to keep our models relatively small and light, therefore fast to train, apply and adapt.



3.1 Selection of the classifier

The three metrics (Kendall's Tau, Off-season noise ratio and Scaling factor) are calculated for several confidence thresholds (i.e., the output of the Softmax function of the classifier). The lower the confidence threshold, the more particles are used to calculate a metric, thus including particles that are classified with lower confidence.

195 The comparison of the three metrics for *Alnus*, *Betula* and *Corylus* is shown in Fig 1. Results for the four other classes of interest can be found in Appendix A1. Overall, there is an improvement for all three metrics at all five sites compared to the 2022 classifier (Fig 1). The off-season noise ratio typically tends to reduce with increasing confidence thresholds, highlighting that by excluding the low confidence classifications fewer off-season false positives are observed. The Kendall's Tau values are also higher, indicating the correlation between the automatic and manual time series is improved. Finally, the averaged scaling
 200 factor values are lower than for the 2022 classifier. However, for all classification algorithms, the scaling factors increase as the confidence threshold increases. Since this results in the exclusion of more unsure classifications, it logically reduces the number of particles in the class and therefore the difference between the predictions and manual counts widens, thus increasing the scaling factor.

Interestingly, the range of each metric is larger for this first version of 2025 classifiers than for the 2022 classifier. The area
 205 difference (ΔAUC) between the maximum and minimum curves, which represents the spread in values for the five sites, is marked for the scaling factor. It indicates that the scaling factors for the 2022 classifier for *Alnus*, *Betula* and *Corylus* (ΔAUC respectively 3.5, 8.0 and 26.4) display a smaller range than those of the 2025-Beta-1 (ΔAUC respectively 995.3, 584.6 and 400.8), the 2025-Gamma-1 (ΔAUC respectively 269.9, 105.8 and 98.0) and the 2025-Omega-1 (ΔAUC respectively 994.5, 716.7 and 371.9) classifiers. Additionally, the range of the scaling factor increases as the confidence threshold increases. This
 210 trend is not as obvious for the two other metrics (Supplementary Material SI_2_metrics.csv and SI_3_metrics_area.csv). The greater spread in metrics values for the 2025 classifiers indicates larger differences between predictions across the five sites. This may be a result of 1) biological diversity in pollen morphology (Hasegawa et al., 2022) which is not fully represented in the training datasets; or 2) over-training of the 2025 classifiers for a small number of specific instruments, i.e., those that were used to produce the training datasets (Table 2). Consequently, when applied to data produced by other instruments, the algorithm
 215 does not perform as well. It is possible that each instrument has a specific signal in the holographic and/or fluorescence data, resulting in the 2025 classifiers focusing on instrument-induced artifacts instead of the particle morphology and composition.

To minimise over-training, the three 2025 classifiers were retrained with the same architecture but on training data from more instruments. The resulting combined training dataset not only includes more events (893'361 versus 563'063) but also contains datasets that have only holographic images (166'262 events or 18.61 % of the entire dataset). Fig 1 further displays
 220 the metrics for the three 2025 classifiers trained on these data. The positive impact of using training datasets from a larger number of instruments is highlighted by a reduction of the range of the scaling factor. This is especially clear when comparing the 2025-Gamma-1 and 2025-Gamma-2 classifiers for *Alnus* (ΔAUC reduced by a factor 84.3), *Betula* (ΔAUC reduced by a factor 13.6) and *Corylus* (ΔAUC reduced by a factor 28). While some discrepancies remain, especially at higher confidence thresholds, the scaling factors are coherent at lower confidence thresholds for the five sites. These results suggest training on



225 a very large number of events from a number of different instruments without fluorescence and restricting the complexity of
 fluorescence processing layers limits artifacts related to fluorescence-related over-training. Despite the overall improvement
 brought by the second generation of 2025 classifiers, they still exhibit a wider spread in metric values compared to the 2022
 classifier. This may suggest a degree of over-fitting to the fluorescence data, which can be mitigated by selecting the right
 confidence threshold: although the average performance has improved, the variability across instruments indicates a residual
 230 lack of generalisation, possibly due to differences in fluorescence data quality or standardisation between devices.

In a final step, the 2025-Gamma-3 classifier was trained on a similar architecture to that of 2025-Gamma-2, but with modifi-
 cation of the output of the fluorescence branch, reducing it from 26 to 13 features. This was to condense important information
 from the fluorescence data into fewer features so as not to dilute the fluorescence signal and limit overtraining. The predic-
 tions of this classifier display wider ranges for the scaling factors compared to the 2025-Gamma-2 algorithm for *Alnus*, *Betula*
 235 and *Corylus* ($\Delta\text{AUC } 70.4 > 3.2$, $106.5 > 7.8$ and $34.2 > 3.5$ respectively). A similar increase of the range in scaling factors is
 also observed for the other classes (see Appendix A1, Supplementary Material SI_2_metrics.csv and SI_3_metrics_area.csv),
 except for *Fraxinus* ($\Delta\text{AUC } 1.5 < 1.8$). The 2025-Gamma-3 algorithm is thus less consistent across stations, denoting an over-
 simplification of fluorescence processing layers. Furthermore, the Kendall's Tau correlations and off-season noise ratios show
 little improvement compared to those of the 2025-Gamma-2 classifier. Therefore, 2025-Gamma-2 will be selected as the next
 240 operational classifier for the SwissPollen network, with proper selection of confidence threshold to keep variations between
 monitoring sites under control.

Among the classifiers evaluated, the 2025-Gamma-2 classifier consistently outperformed all others, showing improvements
 across all three metrics compared to the 2022 baseline, and exhibiting a reduced spread in scaling factors relative to the
 other 2025 classifiers. Notably, the Gamma-2 architecture differs from the Beta-2 and Omega-2 variants in the size of the
 245 holography branches outputs prior to concatenation, which are 1024, 64, and 128 respectively. The fact that the best-performing
 classifier retains the largest holography feature space (while the fluorescence branch remains fixed at 26 features) highlights
 the continued importance of broad holographic information for accurate classification. This finding is consistent with previous
 studies highlighting the challenge of avoiding over-fitting on fluorescence data in pollen classifiers (Maya-Manzano et al.,
 2023). Our results show that fluorescence data contribute positively to overall classification performance, despite a tendency
 250 for over-fitting, provided that appropriate confidence thresholds are applied. Over-fitting is mitigated in part by expanding the
 training datasets with holography data collected from a broader range of instruments. We anticipate a similar improvement if
 such diversity is introduced for fluorescence training data as well.

3.2 Determination of optimal parameters for the SwissPollen operational network

Ideally, a threshold of 0 would be used, but in practice the confidence thresholds need to be set as low as possible to maximise
 255 the number of events kept and optimise the three metrics across classes and the five evaluation sites. To do so, we used Fig
 2, which directly compares the metrics for the 2022 and 2025-Gamma-2 classifiers. All metric values are also listed in the
 Supplementary File SI_2_metrics.csv.

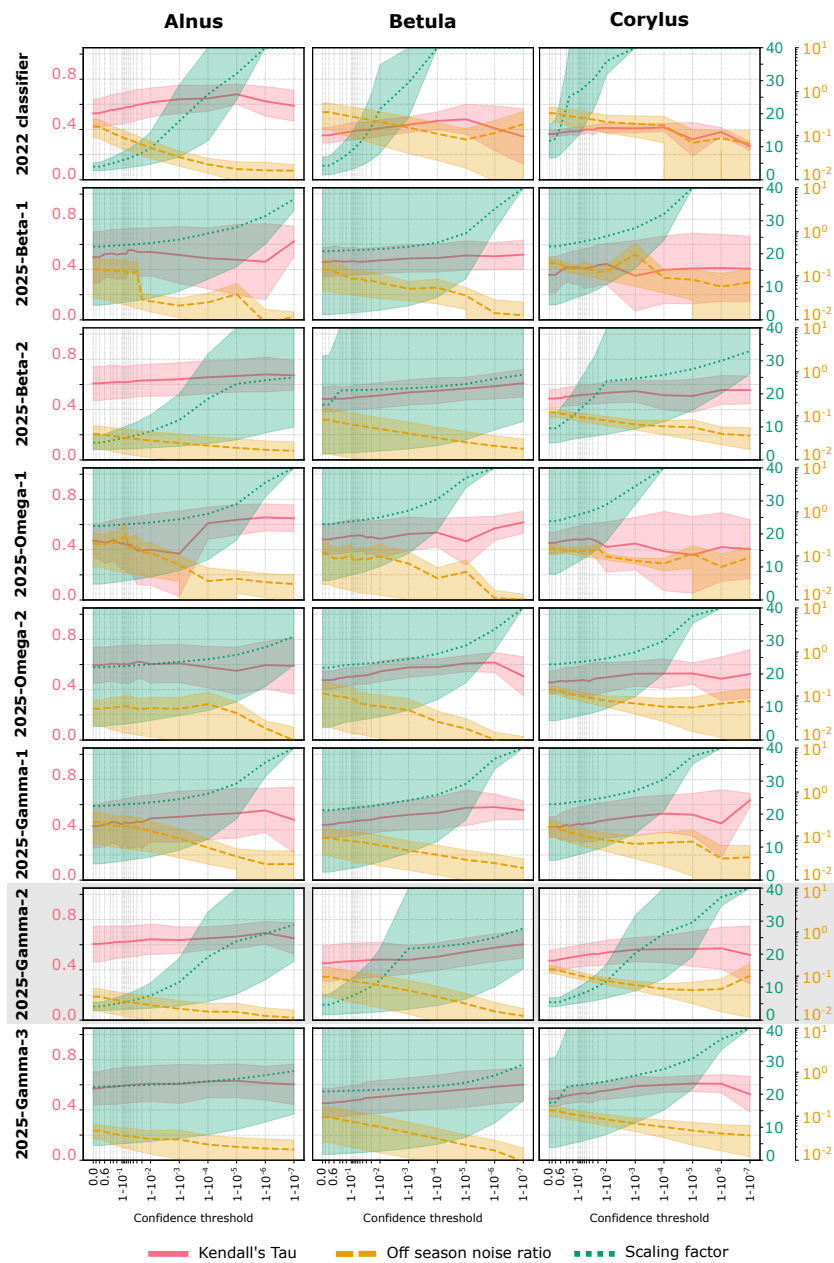


Figure 1. Kendall's Tau correlation, scaling factor and off-season noise ratio as a function of the confidence threshold for Alnus, Betula and Corylus (columns) and for each classifier (rows). The yellow, green and red areas represent the spread of values across the five sites while the thick lines show the averages. Note that the axis of the scaling factor stops at 40, while values range up to 1000. The grey frame highlights the best classifier. The 2025-Gamma-2 panel is presented in Crouzy et al. (2025) (Crouzy et al., 2025)



Optimised parameters are selected so that: 1) The scaling factor remains low, so the detection limit of the device is taken into account. To avoid overestimating concentrations in the absence of reliable physical detections, a maximum scaling factor of 24 grains/m³ per particle detected was applied for SwisensPoleno classifiers. This value ensures that a single detection over one hour, corresponding to 2.4 m³ of air sampled by the instrument (operating at 40 L/min), does not exceed the commonly accepted Hirst detection threshold of 10 grains/m³ (Triviño et al., 2023). One particle detected in this volume corresponds to approximately 0.42 grains/m³, and applying a scaling factor of 24 yields a final concentration of 10 grains/m³. This constraint helps prevent artificially inflated values in low-concentration conditions, where manual measurements are known to be less reliable.

2) The Kendall's Tau correlation between the predicted and manual pollen concentrations should be as high as possible. To provide a reference for what level of correlation can be expected between manual devices, we analysed time series from three co-located Hirst-type pollen traps operating in Payerne between 26 March and 26 July 2013 (Adamov et al., 2021). For each taxon, we computed the average of the three pairwise Kendall's Tau coefficients, which are much lower than Pearson correlations, as an estimate of attainable correlation: *Alnus* (0.48), *Betula* (0.63), *Corylus* (0.50), *Fagus* (0.66), *Fraxinus* (0.75), *Poaceae* (0.64), and *Quercus* (0.66).

3) The off-season noise ratio quantifies the mean predicted pollen concentration during the off-season relative to the in-season period. Lower values indicate better classifier specificity, as they reflect minimal pollen predictions outside the pollen season. To provide a reference, we computed this ratio using three manual time series for *Betula* taken from (Adamov et al., 2021). *Betula* is a genus with a well-defined seasonal pattern. The average ratio was 0.009. Additionally, we estimated a worst-case scenario by artificially increasing all off-season values to 35 grains/m³ (Brito, 2010), yielding an average ratio of 0.165. Based on these results, ratios exceeding 0.165 may reflect chronically high off-season over-prediction or intermittent high over prediction and reduced classifier accuracy, while ratios close to 0.01 can be considered indicative of high-quality predictions, as they reflect only rare occurrences of significant off-season false positives. It should be noted that this metric is sensitive to the definition and duration of the pollen season, which varies across taxa and geographical locations, as well as to the size of the off-season included in the calculation.

For *Alnus*, we chose a 0.9 confidence threshold. Around 0.9, the off-season noise ratio and the scaling factor reach a plateau, so raising the threshold further brings little improvement. Across the whole range of thresholds, Kendall's Tau also exceeds that of the 2022 classifier. At a confidence threshold of 0.9 for *Betula*, the curve of Kendall's Tau correlations begins to plateau, indicating a reduction in correlation improvement beyond this point. This threshold also effectively limits the range of scaling factors while maintaining a reasonable off-season noise ratio. The class *Corylus* displays a large improvement in terms of the range of scaling factors across stations with the 2025-Gamma-2 algorithm. For this class, the confidence threshold of 0.9 was selected mostly based on the off-season noise ratio, for which a plateau begins, while keeping both the scaling factor and Kendall's Tau at better levels than the 2022 classifier. Selecting a common confidence threshold for all Betulaceae also brings coherence and simplicity to our approach.

All in all, *Alnus*, *Betula*, and *Corylus* have optimised metrics at a confidence threshold of 0.9. It yields mean scaling factors of 5.00, 6.34, and 7.38, all well below the maximum threshold of 24 grains/m³ per detection. The corresponding Kendall's

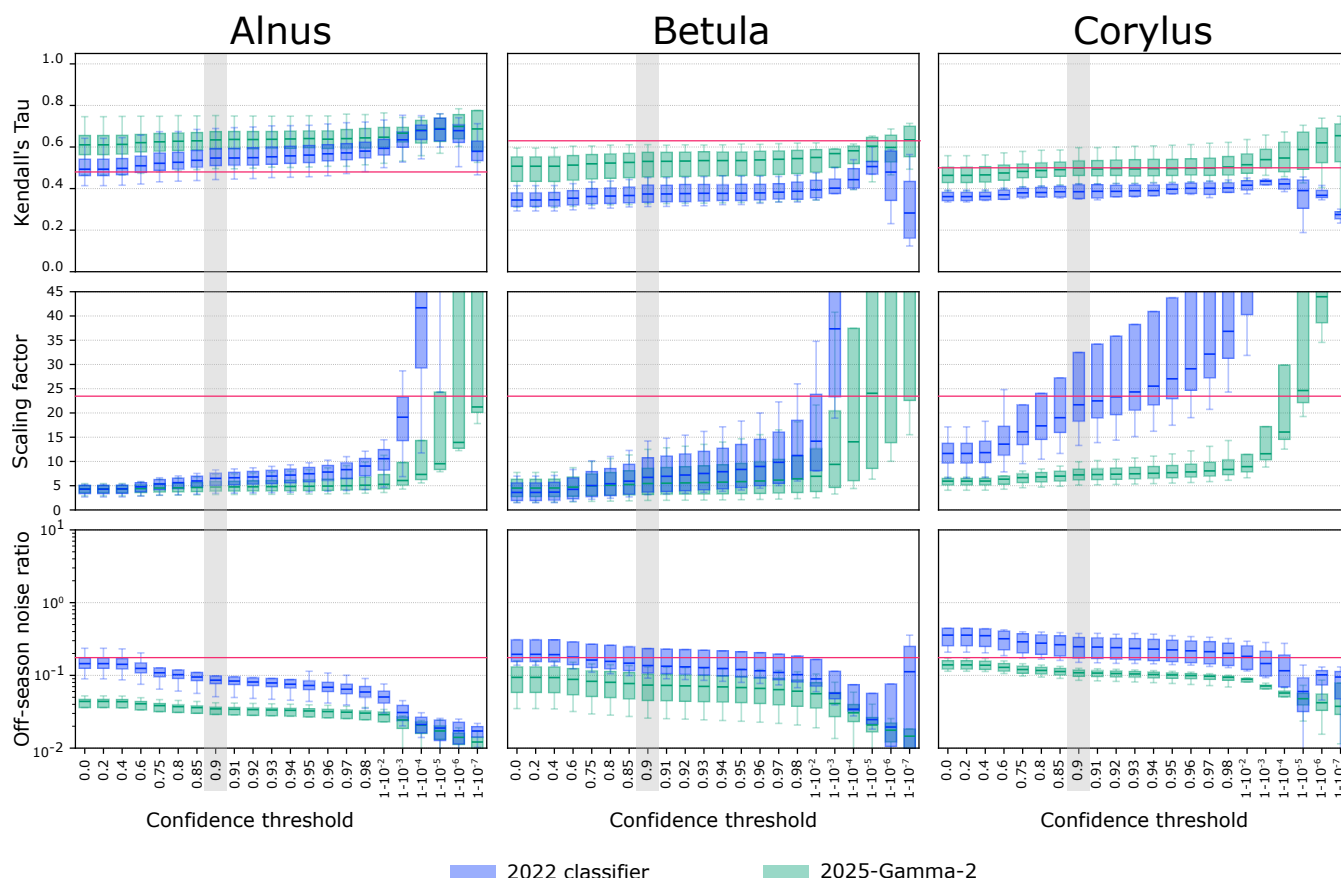


Figure 2. Kendall Tau correlation, scaling factor and off-season noise ratios as a function of confidence threshold for the five stations and for the 2022 and 2025-gamma-2 classifiers. Columns show Alnus, Betula and Corylus, from left to right. Metrics are computed at each threshold, in green for the 2025-Gamma-2 classifier, in blue for the 2022 classifier. Scaling factors higher than 45 are not shown. The boxplots illustrate the values of each metric, at each confidence threshold, for all stations. Red lines indicate the acceptability threshold values for each individual metric and taxa.

Tau values were 0.52 (above the 0.48 attainable correlation for Alnus), 0.43 (below the 0.63 for Betula), and 0.44 (below the 0.50 for Corylus). Off-season noise ratios were 0.031, 0.076, and 0.11, respectively, which are all within the acceptable range (0.01–0.165) defined by manual references and worst-case estimates (Fig 2). Interestingly, for Alnus, the automatic predictions show a stronger correlation with the reference manual time series than the averaged correlation observed between co-located Hirst traps. This suggests that the classifier is able to consistently capture the seasonal pattern of this taxon, as far as can be evaluated from manual reference measurements.

The scaling factors for other classes range from 2.195 for Fraxinus up to 8.570 for Fagus. This relatively limited range indicates that the measurement system detects and classifies pollen grains relatively well, particularly given the known uncer-



tainties of the manual observations. Values of optimal confidence thresholds and corresponding scaling factors for all classes can be found on the Github repository where we deposited the classifier (MeteoSwiss, 2025).

Fig 3 displays the difference between the scaled predictions and the manual measurements for the five evaluation sites. At Neuchâtel (*Alnus*, *Betula* and *Corylus*) and Luzern (*Betula*) the classifier predictions are too low, while for Basel and Buchs (both *Betula* and *Corylus*) they tend to be too high. Informing the Swiss public of pollen concentrations that are too high or too low is problematic, however, it is unfeasible to have individual sets of scaling factors for each instrument across a national network. More accurate scaling factors based on studies currently being carried out at the Swiss Federal Office for Metrology METAS may help resolve this issue, as for the moment it is impossible to tell which part of the variability comes from the automatic system or from the manual measurements (deviations from the flow up to 72 % were observed (Oteros et al., 2016)). We however notice clear seasonal misclassification (false-positives), for example, pollen being classified as *Corylus* when *Alnus* or *Betula* seasons kick in. Operationally, these issues can be resolved using the supervisor approach (Crouzy et al., 2022).

3.3 Improvements in classification of Betulaceae family and identification of water droplets

The 2025-Gamma-2 classifier was designed to use both holography and fluorescence data, with the expectation that this would resolve some of the shortcomings identified in the 2022 classifier (Sauvageat et al., 2020). The 2025 algorithm improves the classification of the Betulaceae pollen grains, when comparing the Kendall's Tau correlations between the predicted and manual values (Fig 4), as already illustrated in Fig 1 and Fig 2. We also observe a slight improvement of Kendall's Tau values between the prediction of one of the Betulaceae and the manual values for another class of the Betulaceae (e.g., comparing predicted values for *Betula* with the manual observations of *Alnus*) (Fig 4, all values in the Supplementary File SI_4_corr_pred_ref.csv). This suggests that the 2025-Gamma-2 classifier tends to produce fewer misclassification than the 2022 classifier. For example, the Kendall's Tau values, averaged across stations, between the 2025-Gamma-2 predictions for classes *Alnus* and *Corylus* and the manual timeseries for *Betula* dropped by -0.24 and -0.07, respectively. Therefore, fewer *Alnus* and *Corylus* pollen are classified as *Betula*. This is also the case when comparing the predictions of class *Betula* to the manual timeseries' of *Alnus* and *Corylus* (-0.11 and -0.12). When looking at the ranges of the Kendall's Tau across stations, we notice that this is not the case for all of them, suggesting again a possible overtraining of the classifier. Overall, the 2025 classifier tends to produce fewer misclassification for the Betulaceae family compared to the 2022 algorithm.

The 2022 classifier also tended to misclassify Poaceae pollen as *Corylus* (Sauvageat et al., 2020). The 2025 algorithm reduces this problem, with the averaged correlation between the predicted Poaceae and manual *Corylus* timeseries dropping from 0.05 to -0.02. Again, the Kendall's Tau ranges across stations indicate that the classifier improves on some stations but not on other, suggesting a possible overtraining. Conversely, the 2025 algorithm classifies more Poaceae as *Betula* than the 2022 classifier, increasing the correlation by 0.07, however, it remains low (0.24).

To confirm the results of the timeseries correlations and more fully understand the differences between the 2022 and 2025-Gamma-2 classifiers, we selected two periods and visualised, event by event, what class each classifier allocated it to (Fig 5). Two seasons were selected, the first from 1 January to 15 March 2024, covering the main *Alnus* and *Corylus* peaks, the second

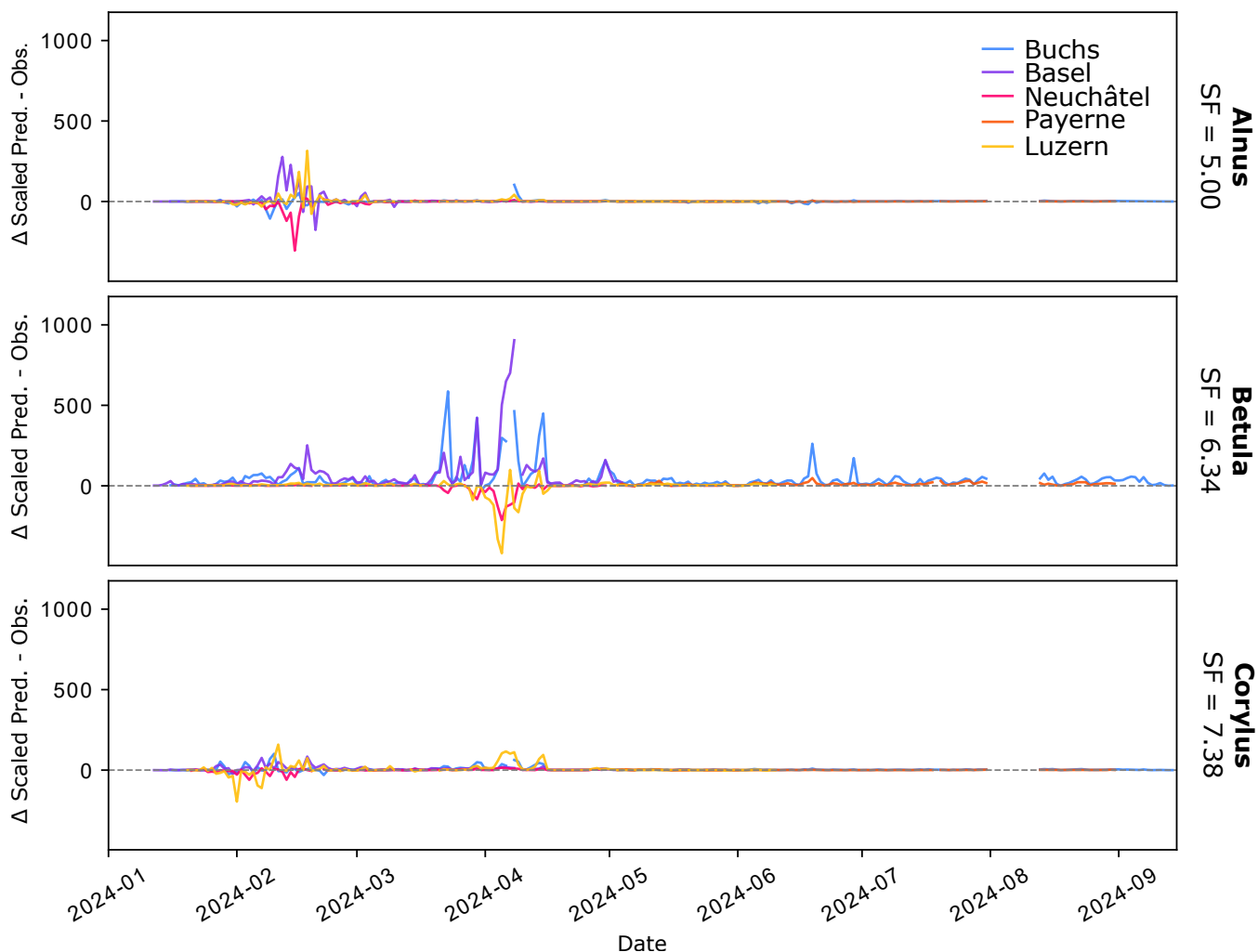


Figure 3. Difference between scaled 2025-Gamma-2 predictions and the manual observations. A scaling factor (SF) is calculated for each station by minimisation of the Mean Square Error between the predictions and the manual observation timeseries. These SF are then averaged across stations to obtain a value for each class for the whole network. Each line displays the difference between the scaled daily pollen predictions from 2025-Gamma-2 and the corresponding manual observations for one of the five stations.

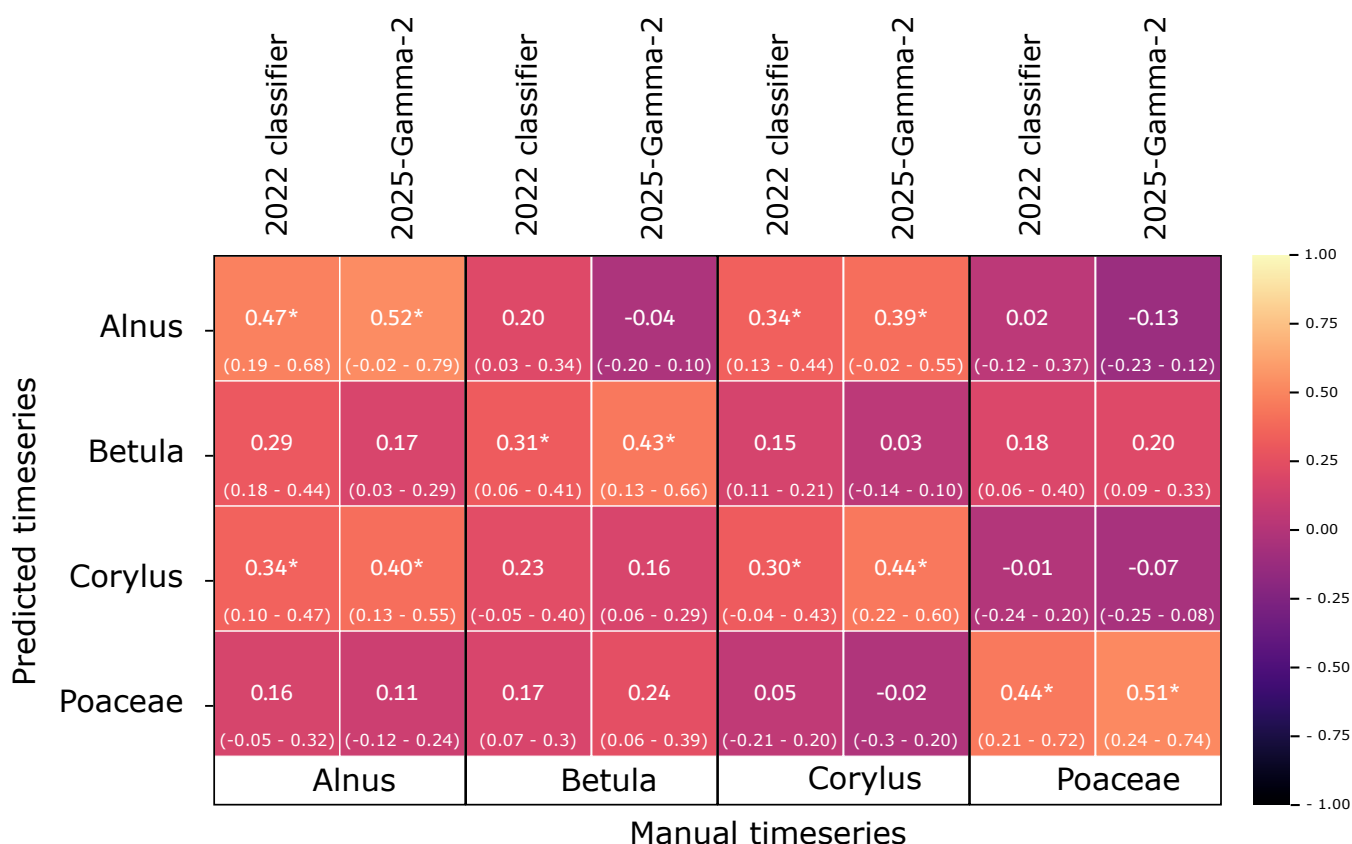


Figure 4. Heatmap of the averaged Kendall Tau correlations between the predicted and manual timeseries for the 2022 and 2025-Gamma-2 classifiers. Numbers and corresponding colours indicate the Kendall's Tau values between the predicted timeseries of the class on the y axis, and the manual observation timeseries of the class on the x axis. The minimum and maximum Kendall's Tau across stations are reported in parenthesis. * indicates that the Kendall Tau value is higher than 0.5 when calculated between the manual timeseries of the two classes, indicating an expected correlation between two classes. All predicted timeseries have been produced with the optimal confidence threshold identified for the class and algorithm.



335 from 1 May to 30 September 2024 covering the main Poaceae season. Classes of taxa which pollen the classifier was trained to recognize but that are not releasing pollen during those periods were labelled as "Other" and any events classified as such can thus be considered as false positives. All values are in the Supplementary File SI_5_flow.csv.

Fig 5 clearly highlights the improvement of the 2025 classifier when it comes to reducing the out-of-season noise. This is shown by the reduction by 63.13 % and 33.87 % of events classified as "Other" for each period respectively, between the
 340 predictions from the 2022 classifier and the 2025-Gamma-2 classifier. Importantly, 37'616 events (51.06 %) that were classified as "Other" by the 2022 algorithm are reclassified as *Taxus* by the 2025 algorithm. *Taxus* is of relatively little interest in terms of allergies in Switzerland and thus the evaluation of this class is beyond the scope of this paper. However, it is airborne during this period, so it is quite feasible that these results are accurate. Nevertheless, there is still a large proportion of particles classified as "Other": 22.2 % of all events from the first period, and 57.7 % of all events from the second period, many of which
 345 are likely false positives. This may be a result of the restricted number of classes on which the algorithm was trained (15 in total), forcing all pollen, even other taxa not included in training data, to be allocated a class. This is one of the disadvantages of the types of algorithms used in this study. One indication that this indeed may be the case is that there are considerably more "Other" classifications in the later period. This is possibly the result of the presence of many fungal spores, for which there is no class in the algorithm but that pass the first step in the identification process (i.e., the sphericity test, (Sauvageat et al., 2020)). However, the portion of false positives can be reduced using more restrictive confidence thresholds. Additionally, post-processing algorithms could be applied to the predicted data, that specifically corrects false positives outside of the pollen season of a given taxa (Crouzy et al., 2022). Fig 5 (C) focuses on the flows of classification in *Alnus*, *Betula* and *Corylus* between 2022 and 2025 classifiers. It shows that 74.6 % (169025 events) of events classified as *Betula* by the 2022 classifier are re-classified in the 2025 classifier, probably helping in the improvement of the correlation between *Betula* predictions and
 355 manual timeseries (Fig 4). We also see that 20.4 % of the events classified as *Alnus* by the 2025 classifier come from events previously classified as *Betula*, while 31.2 % events remain classified as *Betula* by both classifiers. Notably, few events are re-classified from *Alnus* (14.4 % of events classified as *Corylus* by 2025 classifier) and *Betula* (6.2 % of events classified as *Corylus* by 2025 classifier) as *Corylus*, while 48.5 % were previously classified as Other. Finally, 63.6 % of the events classified as *Alnus* by the 2025 classifier was already classified as *Alnus* by the 2022 classifier. All in all, these results highlight drastic
 360 changes in the classification of events as *Betula* and *Corylus*, while the class *Alnus* remains relatively stable. Additionally, there are few re-classifications from *Alnus* and *Betula* to *Corylus* and vice-versa, indicating that our 2025 classifier does not drastically improve discriminating *Corylus* pollen grains from *Alnus* and *Betula*. Number of events in each of these classes and timeframes can be found in Supplementary data file SI_5_flow.csv.

Another improvement of the 2025 classifier is the reduction of water droplets classified as Poaceae pollen. This is clearly
 365 illustrated in Fig 5 A and B, where 2025-Gamma-2 classifies 194.96 % and 248.45 % more events as water droplets compared to the 2022 classifier for the two periods, respectively. The 2022 classifier allocates 12'895 events (18.07 % of the 2025 classifier's water droplet count) to Poaceae, which are re-classified as water by the 2025 algorithm (data can be found in Supplementary files SI_6_P33_2025Gamma2.csv and SI_6_P33_2022Operational.csv). Such confusion is particularly obvious at the High Altitude Research Station Jungfrauoch (3567m above sea level, often located in the clouds) where long-term, year-round



Figure 5. Changes in classification of individual events between the 2022 and 2025-Gamma-2 classifiers. Three time periods are presented: A) 1 January to 15 March 2024, and B) 1 May to 30 September 2025 and C) 1 January to 30 September 2024. Colours correspond to the different classes, which are consistent between the two algorithms. The 2022 classifier is shown on the left while the 2025-Gamma-2 classifier is on the right. The values under each class indicate the number of events assigned to each class. The flows highlighted in light blue are discussed specifically in the text. In A) and B) the class labelled "Other" contains all classes that are not in season during the two periods considered. In C) we focus on the classification of Alnus, Betula and Corylus, and all other classes are grouped under Other.



370 observations of a wide range of environmental parameters including meteorology, atmospheric composition (aerosols, trace gases), radiation, permafrost, glaciology, cosmic radiation, and human physiology are conducted (Fig 6). This is especially clear when a lot of water droplets are detected by the device, such as in the morning of the 14th of July. The improvement in the 2025-Gamma-2 classifier is likely the result of the inclusion of fluorescence, since most water droplets are not expected to fluoresce.

375 There is also an improvement in the correlation between Corylus predictions and the manual measurements (Fig 5 (A)). Notably, 29'083 events (33.64 % of the 2025 Corylus events) are reclassified from "Other" in the 2022 algorithm, as are 13'333 events (15.44 % of the 2025 Corylus event) from Ulmus, and 12'411 events (14.37 % of the 2025 Corylus events) from Alnus. These additional classifications as Corylus by the 2025 algorithm result in the improved correlation with the manual data. Interestingly, just 26'621 events (30.82 % of the 2025 Corylus events) are classified as Corylus by both the 2022 and 2025
 380 algorithms. In contrast, just 1'698 events (1.06 % of the 2025 Alnus events) are reclassified from Corylus to Alnus, indicating there are considerably more Alnus events misclassified as Corylus by the 2022 algorithm rather than the other way around. Fig 5 (B) also highlights the stability in the classification of Pinaceae (+18.55 %), which are easily identifiable because of their size and shape. This period was extended after the grass season to include times when few pollens but many water droplets are in the air.

385 **3.4 Potential for future improvements**

Despite the considerable improvements in the 2025 algorithm, the classifiers still suffers certain limitations. The correlations between the Corylus predictions and the Alnus manual timeseries, as well as the correlation between the Alnus predictions and the Corylus values slightly increase by +0.06 (0.34 and 0.40) and +0.05 (0.34 and 0.39), respectively (Fig 3). However, these correlations remain lower or equal to the averaged Kendall's Tau between co-located manual timeseries for Alnus and Corylus
 390 (0.40). The evaluation of these classes is particularly challenging given that their seasons overlap (indicated by * on Fig 4) and that the genuine correlations between the manual Alnus and Corylus timeseries are thus high (0.4). It is therefore difficult to discern using correlations alone, if there is any improvement. Nevertheless, figures such as Fig 5 can be used to help visualise how the classification of individual events change and how this may impact false positive classifications.

Another limit of the 2025 classifier is the reduction in the classification of Quercus, which, for an unknown reason, are better
 395 classified by the 2022 algorithm than the 2025 classifier (see figure B1). For this reason, in the operational SwissPollen setup, we will keep relying on the 2022 classifier for the classification of Quercus. Additionally, the 2025-Gamma-2 model could be evaluated for other classes that were not considered in this analysis. This could allow to operationalise more taxa and provide a wider service to the Swiss population.

4 Conclusion

400 We propose a framework based on a number of easily-calculated metrics that can be used to evaluate particle classification algorithms based on deep learning. It includes Kendall's Tau correlations, scaling factors, and the off-season noise ratio. The

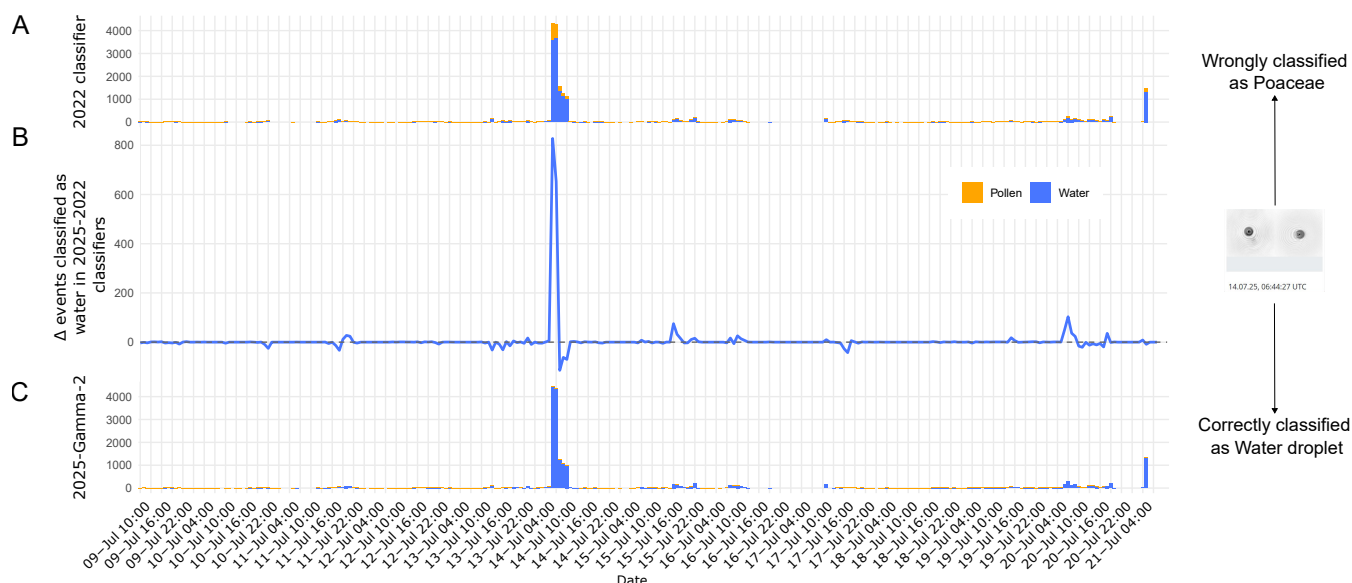


Figure 6. Pollen and water droplet counts of individual events between 9-21 July 2025 at the Jungfraujoch station. A) and C) display the hourly number of events classified as water droplets (blue) and pollen (yellow, all classes summed) for the 2022 and 2025-Gamma-2 classifiers, respectively. B) represents the difference in the number of water droplets classified by 2025-Gamma-2 minus 2022 classifier. A positive value therefore highlights an increase in the number of events classified as water by the 2025-Gamma-2 classifier. The instrument at the Jungfraujoch station typically detects mostly water droplets that are often misclassified as pollen, especially Poaceae, which is much reduced in the predictions of the 2025-Gamma-2 classifier. Note that, due to different operational pre-filtering based on size and morphology that is beyond the scope of this paper, both classifiers are not applied on exactly the same number of events, explaining negative values in subplot B.

generality of this framework makes it applicable to any automatic instrument that counts and identifies airborne particles. We encourage the community to use this framework as a standard tool to evaluate such classifiers. As an example, we apply the framework to evaluate a newly developed classifier for the SwisensPoleno instrument, comparing this algorithm using both
 405 holography and fluorescence data with the current operational classifier based only on holographic images.

Our results show that the addition of fluorescence data improves the classification of several pollen taxa, particularly Poaceae, for which there were many false positives resulting from misclassified water droplets in the 2022 algorithm. The 2025 classifier also better distinguishes between genera from the Betulaceae family (*Alnus*, *Corylus*, and *Betula*), and classifies notably better *Fagus* and *Fraxinus* pollen grains. According to the metrics we developed, the only taxa of the 15 included in the classifier for
 410 which the 2025 algorithm does not show an improvement is *Quercus*. Furthermore, while the 2025 classifier predicts notably less false positives than the 2022 algorithm, there is still a large proportion of events that are misclassified. This may be a consequence of the limited number of particle classes used (15) and future work aims to test algorithms with larger numbers of classes. One avenue to follow will be through clustering of a mix of operational and labelled training data, which could be used to produce more training datasets representative of the bioaerosol present in the air. In particular, more focus will be



415 placed on the inclusion of fungal spores, which has been shown to have considerable potential on one hand for new health and
agricultural applications but on the other hand also because they are likely generating unwanted false-positive detections (Erb
et al., 2024). Other classifier architectures should also be explored, as some have shown great results for classifying conifer
pollen grain microscopic images with genus resolution, such as ResNet (Rostami et al., 2025). These could be combined with
a branch treating fluorescence inputs to try to improve classification on holographic images, despite their reduced resolution
420 compared to microscopic images.

Although the 2025 classifier has been developed and evaluated specifically for Switzerland, the approach is readily adaptable
to other biogeographical regions, at the condition that suitable training and/or evaluation datasets are provided. Beyond this
case study, we expect that the training and evaluation protocol introduced here will serve as a useful framework for the com-
munity, enabling robust comparison of bioaerosol classifiers, even between instrument types. Ultimately, this should support
425 the selection of the most appropriate algorithm for each regional context and strengthen the reliability of automatic bioaerosol
monitoring worldwide.

5 Code availability

All the code used for this study can be found on the public GitHub repository [MeteoSwiss/swisspollen-models](#).

6 Data availability

430 All level-1 data used for this study can be found on the public GitHub repository [MeteoSwiss/swisspollen-models](#). Due to
technical limitations, level-0 can be provided upon request.

7 Author contribution

Marie-Pierre Meurville: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Classifier, Valida-
tion, Visualisation, Writing – original draft; **Bernard Clot:** Conceptualization, Data curation, Methodology, Project admin-
435 istration, Supervision, Writing – review and editing; **Sophie Erb:** Data curation; **Maria Lbadaoui:** Data curation; **Fiona**
Tummon: Conceptualization, Formal analysis, Investigation, Methodology, Writing – review and editing; **Gian Lieberherr:**
Conceptualization, Formal analysis, Investigation, Methodology, Classifier, Validation, Writing – review and editing; **Benoît**
Crouzy: Conceptualization, Formal analysis, Investigation, Methodology, Classifier, Validation, Writing – review and editing.

8 Declaration of competing interest

440 The authors declare that they have no conflict of interest.



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Appendix A

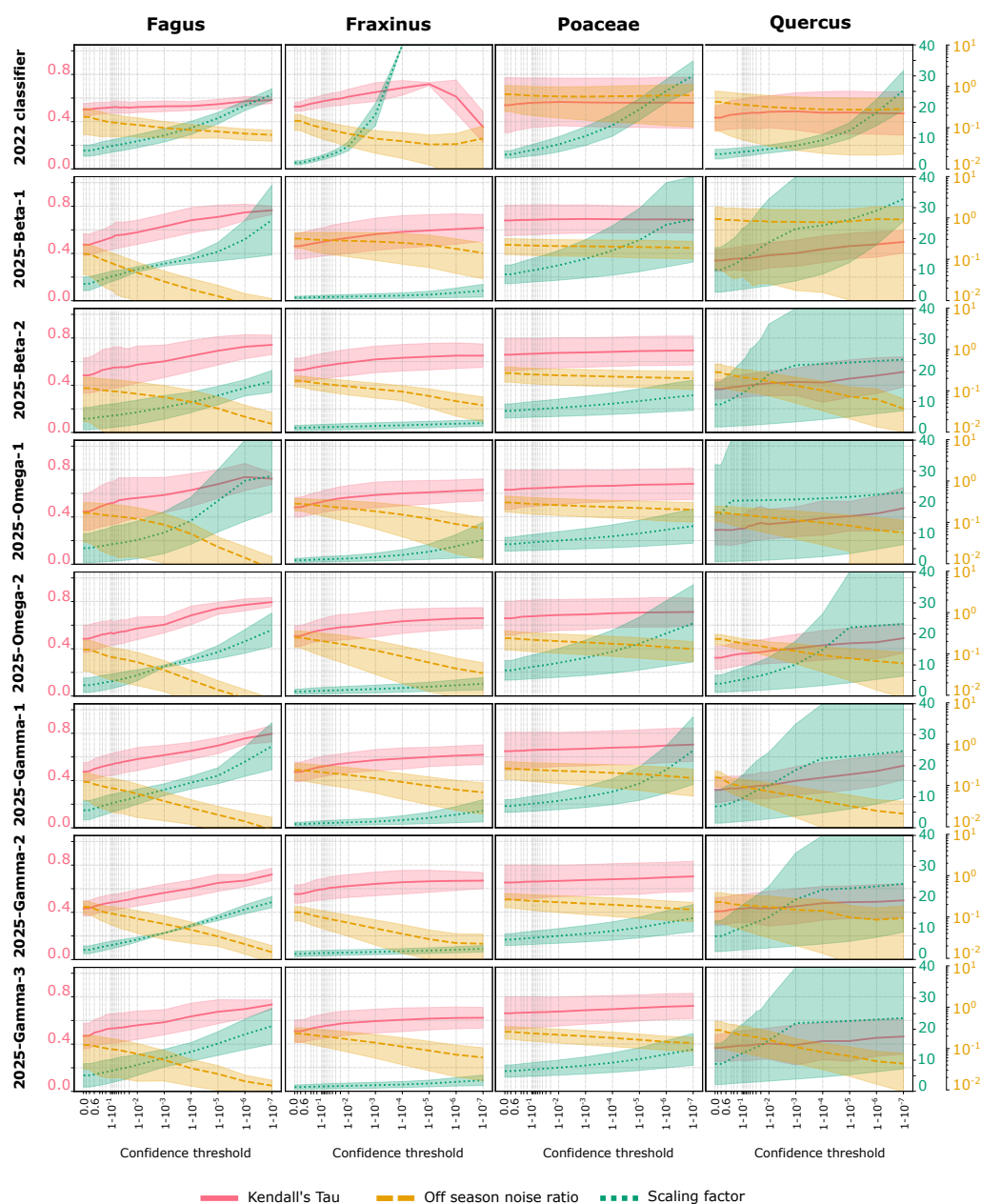


Figure A1. Kendall Tau correlation, scaling factor and Off-season noise ratio as a function of the confidence threshold for Fagus, Fraxinus, Poaceae and Quercus for the five sites and eight classifiers.

The yellow, green and red areas illustrate the spread of the Kendall Tau, scaling factor and off-season noise ratio, respectively. Lines represent the averaged metrics across the five sites. Note that the axis of the scaling factor stops at 40, while values can be as high as 1000.

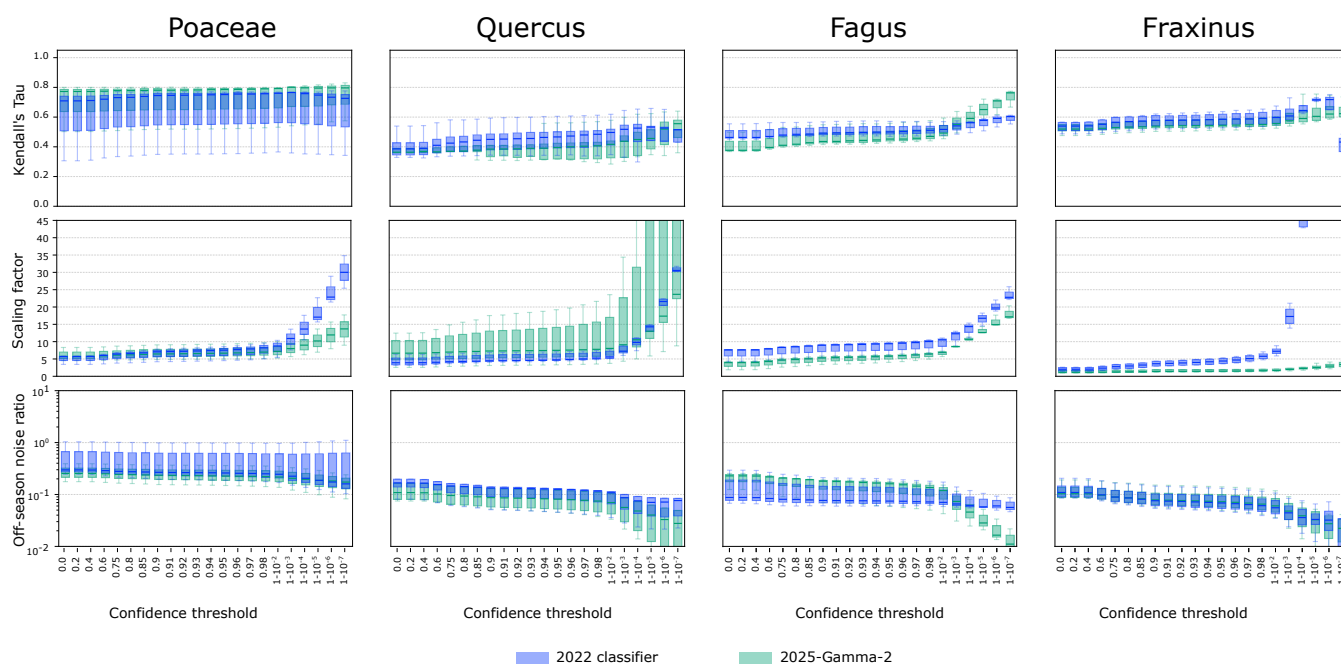


Figure B1. Kendall Tau correlation, scaling factor and off-season noise ratio as a function of the confidence threshold for Poaceae, Quercus, Fagus and Fraxinus. Values are shown for the 2022 (green) and 2025 (blue) classifiers and for the five sites. Scaling factors greater than 40 are not shown.



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