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Methods in Ecology and Evolution

PRACTICAL TOOLS

E-PoSa: A novel and effective tool for sampling pollen directly from flowers

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Abstract

1. Pollinator insects are declining worldwide, also due to the alteration of their diet with severe implications on their health status. Pollinators diet relies mainly on flower rewards (i.e. pollen and nectar), and a precise characterization of their chemical composition is crucial in defining pollinators' nutritional ecology. In this context, the pollen represents a challenging source to investigate, especially due to operative challenges during collection operations and to the small amounts produced per flower.
2. Here, we designed and tested a novel, easy-to-assemble tool for pollen sampling: E-PoSa (Electronic Pollen Sampler), based on the use of a portable vacuum cleaner. We compared it with some of the most used sampling methods for pollen (i.e. anthers sieving and sampling of the whole anthers) by looking at the differences in their quantitative recovery and nutritional profile. Its applicability in ecological studies was also corroborated by an assessment of its recovery rate obtained from a panel of wildflower species in an operational environment.
3. The data obtained showed a significantly higher pollen recovery capacity of E-PoSa compared with the conventional sieving approach and the success in retrieving enough pollen to conduct phytochemical analyses from a broad range of flower morphologies in the field. Our results also demonstrated that high purity pollen can be collected with E-PoSa and that the device does not introduce any significant variation in the nutritional analysis compared with the conventional sieving.
4. This new sampling approach represents a cheap and easy-to-assemble tool encouraging its future use not only in the field of pollen nutrition but also in a wide variety of other contexts related to pollination ecology. Acknowledging the potential influences of the sampling techniques and moving towards shared standardized field protocols will advance the comprehension of species interactions and foraging patterns of pollinators and their nutritional needs.

KEYWORDS
E-PoSa, nutritional ecology, plant-pollinator interactions, pollen, pollinators diet

1 | INTRODUCTION

Insect pollinators are declining worldwide due to multiple global issues such as climate change (Vasiliev & Greenwood, 2021), exposure to pesticides (Goulson et al., 2015) and habitat loss (Potts et al., 2016). The depletion of dietary resources (i.e. pollen and nectar) due to the loss of flower-rich habitats and/or their contamination due to the use of agrochemicals is one of the main risk factors for pollinators (Hülsmann et al., 2015; Vaudo et al., 2015).

Considerable evidence about the importance of adequate nutrition for pollinators conservation have fostered a growing interest in the investigation of the nutritional landscape for a better understanding of the relationships existing between pollinating insects and floral resources (Leonhardt et al., 2022; Vaudo et al., 2016, 2018; Venjakob et al., 2022). Many pollinators feed on pollen, which represents the main protein source (Nicolson et al., 2018), and in this context, nutritional analyses of pollen are of paramount importance. However, these studies are often challenging at the analytical level, due to the scarcity of collected material or its frequent contamination by other floral parts (e.g. anthers and petals). Indeed, many flowers produce a low amount of pollen (<1 mg; Jeannerod et al., 2022), and most of the research dealing with pollen nutrition focuses on the generally heavier pollen pellets collected by bees (Donkersley et al., 2017; Vaudo et al., 2018).

Besides studying pollen sampled from insect corbiculae, the evaluation of the nutritional composition of pollen directly collected from plants is relevant for the characterization of specific chemical features. Furthermore, as many environmental factors can shape plant metabolism (Ahmad et al., 2018), the isolation of pollen directly from different species may be an added value in investigating plant responses to environmental pressures and consequent effects on pollinators diet. On the application side, collecting and studying pollen can also elucidate which species are the most relevant to sustain the trophic demand of pollinators both in terms of quantity of pollen produced and in relation to its nutritional quality. However, to adequately carry out the wide array of chemical analyses required to quantify pollen nutritional composition (e.g. proteins, lipids, sugars, secondary compounds and amino acids), it is required to gain at least some quantity of pollen from each plant species, since each chemical component requires specific extraction methods and the analytical sensitivity of the chemometric approaches requires a significant amount of starting material as well (Stabler et al., 2018).

To collect pollen, researchers have developed many sampling approaches to improve sampling efficiency and to face the critical issue of retrieving an adequate quantity of pollen to achieve reliable nutritional analyses (Jeannerod et al., 2022; Kenđel & Zimmermann, 2020; Knäbe et al., 2015; Roulston, 2005). However, many of these methods are time-consuming, as they require multiple steps of processing (e.g. 24-h drying, sieving) before obtaining the pollen samples that will be analysed (see also Table 1 for a comparison of advantages and drawbacks of the most common methods for pollen sampling). Furthermore, this wide panel of pollen sampling techniques results in a great heterogeneity among the different studies, hampering the comparison between the results obtained. With such a variety of available methodologies for the sampling of floral resources, the need to standardize the collection effort is becoming even more urgent.

Here, we propose a novel non-invasive tool for pollen sampling. The device, E-PoSa (Electronic Pollen Sampler), is based on the use of a commercially available portable vacuum specifically adapted to this purpose and allows the collection of highly pure pollen grains directly from the flower in a nondestructive way. To validate this new approach, we provide a comparison with some of the most common methodologies adopted for pollen sampling to evaluate the magnitude of the differences that occur in the recovery efficiency and in the subsequent analysis of the nutrient composition.

2 | MATERIALS AND METHODS

2.1 | Assemblage and use of E-PoSa

To conduct a study on the pollen nutritional composition of wildflowers in northern Italy, we developed an effective and easy-to-assemble device that allows the collection of pollen with a yield suitable for chemical characterization. E-PoSa is based on the use of a portable vacuum cleaner with a removable plastic mask. The tool is made up of the following parts: a portable vacuum, a 5-mL tube with the head cut and the lid drilled; an inox mesh sheet, a paper filter and laboratory film (Figure 1). The first step for assembling the device is preparing the 5-mL tube. The head of the tube needs to be cut approximately 0.5–1 cm apart from the tip using a sharp knife (Video S1). Second, the tube lid must be separated and drilled by using a conical drill bit (Video S1). The next step is cutting the paper filter with a diameter slightly larger than that of the test tube cap. Then, close the tube by paying attention that the paper filter remains in the correct position by completely covering the hole previously made on the cap (Video S1). After cutting a small square from the inox mesh sheet, heat it quickly using a lighter, place it at the top of the tube and hold it in place for a few seconds to ensure it does not detach (Video S1). The last step is to connect the tube to the head of the vacuum and secure it by using the laboratory film (Video S1). We suggest securing the tube to vacuum with multiple layers of laboratory film to avoid loosening during field sampling. The time required for the assembly is estimated to be less than 10 min. A few seconds are required for replacing the pre-assembled collection tubes. The battery life of the portable vacuum used in this study is about 30 min, but it can be easily extended by integrating it with a portable power bank that allows its use also in remote sites. Details regarding the materials utilized and their costs are reported in Table S1.

The use of E-PoSa does not require expertise. The pollen can be collected by simply turning on the vacuum and moving it on the flowers to be sampled. Pollen is aspirated and accumulates on the paper filter. Due to the transparency of the tube, the amount of pollen collected can be easily estimated by eye, and once enough pollen is collected, it can be directly transferred to another tube by removing the inox mesh from the tip of the E-PoSa and gently tapping on the bottom of the adapted tube. The system is useful with flowers

TABLE 1 The table reports a brief description and the characteristics of the three methods used in this study for pollen sampling.

Method	Description	Volume/ weight quantifiable	Operative environment	Destructive	Advantages	Constraints	Selected references
Anthers	Removal of the whole anthers from the flowers. The anthers are directly subjected to the extraction and analytical evaluation of nutrients	No	In situ	Yes	Fast. It allows the sampling of a high amount of matrices and is applicable to all flower species	Pollen grains are not isolated from the vegetative tissues of the anthers, making the chemical evaluation of pollen biased	Kenđel and Zimmermann (2020), Arathi et al. (2018)
Mesh	Anthers are removed from the flowers and dried at 30°C for 12–24 h. Pollen grains are then isolated from the anthers through sieving using mesh with the desired size	Yes	Ex situ	Yes	The mesh size can be selected based on the specific size of the studied pollen grains. It allows a complete isolation of the pollen grains from other floral parts thus reducing contamination risk	It requires a drying phase prior to the sieving process that can last up to 24 h. It is not performable directly on the field and the recovery is usually very low	Jeannerod et al. (2022), Jacquemart et al. (2019)
Electronic Pollen Sampler	Pollen is vacuumed from the anthers with the assistance of a portable vacuum cleaner adapted with filters and collection tubes	Yes	In situ	No	It allows the sampling and isolation of pollen grains directly on the field. It is a non-invasive method (i.e., it does not require to sample floral units and to bring them in the lab) which allows to collect pollen from many flowers in a short time and can be used in remote locations. Materials needed are cheap	The use of this tool is limited by the battery capacity of the vacuum cleaner. This limit can be overcome by using a powerbank	This study
Stem collection	Stems bearing flower units are cut and left in water overnight in the laboratory. The pollen is then shaken onto a flat surface and scraped into storage with a plastic ruler. The use of a tuning fork is suggested to retrieve pollen from poricidal anthers (and to a lower extent also from nonporicidal anthers). If the pollen becomes contaminated with other floral parts, a step of sifting through a sieve is required	Yes	Ex situ	Yes	The sampling procedure of the stems is fast and feasible with all plant species. If the sifting step is carried out, it allows a complete isolation of the pollen grains from the floral parts thus reducing contamination risks	The procedure is time consuming. Prior to separating the pollen from the mature anthers, the stems need to be maintained in the water overnight. A step of sifting through a sieve is also suggested to be sure that the pollen samples do not present any contamination by other floral parts, similarly to the "Mesh" technique	Roulston (2005)

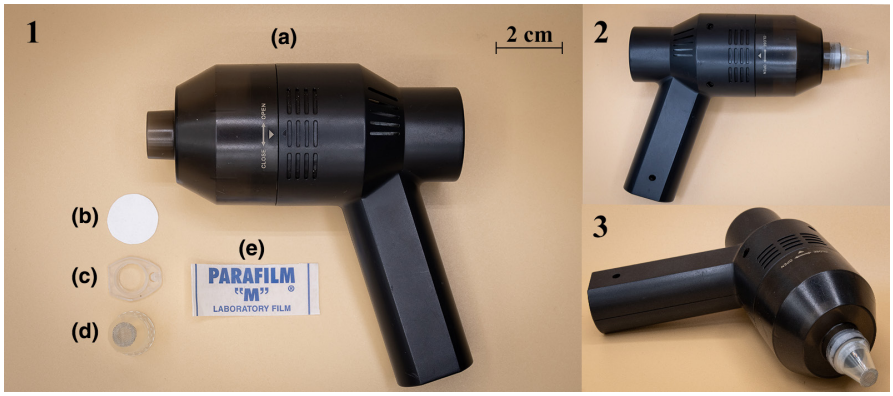

FIGURE 1 Assemblage of the Electronic Pollen Sampler (E-PoSa). (1) Overview of all the materials needed for the assembly of the E-PoSa device (a): portable vacuum; (b): paper filter with 25 μm pore size; (c): 5-mL Eppendorf tube's cap drilled; (d): 5-mL Eppendorf tube with stainless steel mesh with 75 μm mesh size at the tip; (e): strip of laboratory film); (2) top view of the fully assembled E-PoSa; (3) frontal view of the fully assembled E-PoSa.

FIGURE 2 Images of the flowers of all species studied. (a) *Tropaeolum majus* L. (Fam.: Tropaeolaceae); (b) *Hippeastrum vittatum* Herb. (Fam.: Amaryllidaceae); (c) *Alstroemeria aurea* Graham (Fam.: Alstroemeriaceae).

of different morphology. In the case of small flowers or flowers that have anthers within the corolla (e.g. Lamiaceae or Fabaceae), a 20 μL tip can be attached to the tube and fixed with laboratory film to allow a more accurate and effective pollen sampling.

2.2 | Study species

The target flower species were selected based on their taxonomy to account for a wider set of families characterized by different floral morphologies and different amounts of pollen produced. For the collection of anthers and pollen grains, a panel of three species was selected: *Tropaeolum majus* L. (Fam.: Tropaeolaceae), *Hippeastrum vittatum* Herb. (Fam.: Amaryllidaceae), *Alstroemeria aurea* Graham (Fam.: Alstroemeriaceae; Figure 2a–c). The flowers were covered with a nylon mesh 24 h before sampling to avoid possible depletion of resources by pollinator visits. The study took place at the C.R.E.A Institute (Council for Agricultural Research and Economics) of Sanremo, Italy, where the studied species were cultivated in greenhouses.

2.3 | Pollen sampling

Three pollen collection approaches were adopted (Table 1; Figure 3): (i) Anthers were collected by carefully removing them from the flowers using forceps; (ii) pollen grains obtained by dehisced anthers through multiple steps of sieving starting with a mesh of 100 μm size to a final mesh of 50 μm in order to isolate pollen grains from other floral parts and used as control group (hereafter: mesh); and (iii) E-PoSa developed in this study. For each sampling approach, we collected pollen from 25 flowers per species except for *H. vittatum*, for which only 15 flowers were sampled. The pollen obtained was pooled to gain enough material for all the subsequent analyses. All the collected samples were dried in an oven at 30°C for 12 h. The three methods were compared for their recovery efficiency and the differences occurring in the macronutrient profiling. Details on the nutritional analysis are reported in Appendix S1.

2.4 | Statistical analysis

The differences in the recovery of pollen (expressed as mg per flower) per each species were evaluated by using Generalized Linear Models assuming a Gamma distribution of the response variable. The fixed effect was the sampling method. The software used was R (version 4.3.1) and the package used was 'nlme' (Pinheiro et al., 2023) and 'ggplot2' (Wickham, 2016). For details on the statistical analysis on pollen nutritional composition (see Appendix S1).

FIGURE 3 Photographs of the different sampling techniques utilized for pollen collection: (a) Removal of the anthers from *A. aurea* flower; (b) separation of pollen grains from the anthers of *H. vittatum* using a mesh; (c) pollen sampling with the Electronic Pollen Sampler device from *T. majus*.

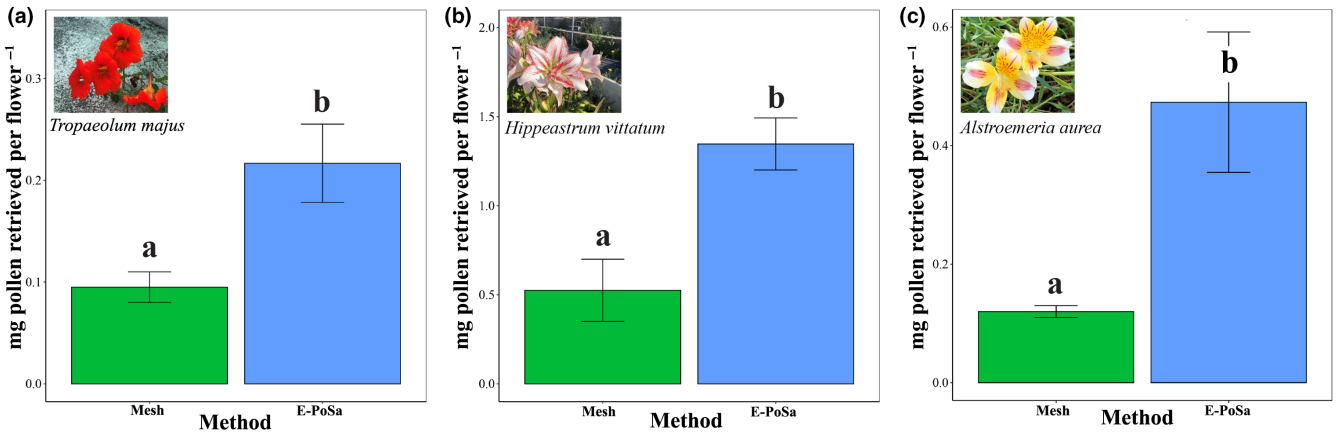

FIGURE 4 Barplots reporting the yield of pollen retrieved expressed as mg per flower depending on the sampling method (i.e., mesh and Electronic Pollen Sampler [E-PoSa]). (a) Data on *Tropaeolum majus*, (b) data on *Hippeastrum vittatum*, and (c) data on *Alstroemeria aurea*. For each of the species compared, $N = 2$ for mesh and $N = 3$ for E-PoSa. Values are the mean ± SEM. Significant differences ($p < 0.05$) are reported by different letters.

2.5 | Validation in operative environment

The E-PoSa was employed also in the operative environment to collect pollen from a diverse array of wildflowers in the framework of a project aiming at the characterization of the pollinators nutritional landscape of northern Italian meadows. The sampling protocol adopted was the same as in the greenhouse validation experiment. The number of flowers/inflorescences sampled per species was recorded in the field and related to the weight of the pollen retrieved to estimate both the pollen recovery per floral unit and the minimum number of floral units to be sampled to perform nutritional analysis.

3 | RESULTS

Compared with other sampling approaches, the adoption of the E-PoSa represents a cost-effective and easy-to-use tool for the sampling of pollen from a wide range of flower species with different flower morphology and pollen yields. As anthers sampling does not allow measuring the weight of the pollen retrieved directly, it was not possible to compare the mass recovered by this technique with the mesh and the E-PoSa. Pollen collection through the E-PoSa device resulted in a significantly higher amount of pollen retrieved in all the three analysed species, as shown in Figure 4. The present method let us perform a field sampling on at least 22 wildflower species belonging to eight families showing a broad range of flower morphology, which were successfully collected for the characterization of pollen nutrients (see Table 2 for details).

3.1 | Pollen macronutrient composition

The estimation of the macronutrient composition of pollen is shown in Figure 5 and Table S2. Generally, the results from all the three investigated species show that the sampling with the E-PoSa does not introduce any further bias than anthers, and in most cases, it removes them, nearing the results of the nutritional investigation to those of mesh sampled pollen (Figure 5a–c).

TABLE 2 The table provides information on pollen recovery from different wildflower species. It includes family, floral morphology, and the need for Electronic Pollen Sampler adaptation with the tip. The 'minimum floral units' column indicates the required number of floral units for obtaining 2 mg of pollen, the minimum amount for macronutritional profiling (proteins, lipids and carbohydrates) using the protocol described in Appendix S1.

Species	Family	Floral morphology	Floral shape	Need of tip adapter	Floral unit considered	µg/floral unit	Minimum floral units
Taraxacum officinale	Asteraceae		Composite flower	No	Inflorescence	196	11
Sonchus oleraceous	Asteraceae		Composite flower	No	Inflorescence	168	12
Hypochaeris radicata	Asteraceae		Composite flower	No	Inflorescence	133	16
Cyrsium vulgare	Asteraceae		Composite flower	No	Inflorescence	139	15
Crepis sp.	Asteraceae		Composite flower	No	Inflorescence	62	33
Bellis perennis	Asteraceae		Composite flower	No	Inflorescence	54	38
Erigeron annuus	Asteraceae		Composite flower	No	Inflorescence	37	55
Cichorium intybus	Asteraceae		Composite flower	No	Single flower	25	80
Lotus corniculatus	Fabaceae		Keel flower	Yes	Single flower	89	23
Trifolium repens	Fabaceae		Keel flower	Yes	Inflorescence	13	154
Trifolium pratense	Fabaceae		Keel flower	Yes	Inflorescence	10	200
Salvia pratensis	Lamiaceae		Tubular flower with biradial symmetry	No	Single flower	36	56
Lamium purpureum	Lamiaceae		Tubular flower with biradial symmetry	Yes	Single flower	29	69
Prunella vulgaris	Lamiaceae		Tubular flower with biradial symmetry	Yes	Single flower	20	100
Malva sylvestris	Malvaceae		Bowl flower	No	Single flower	695	3
Oxalis acetosella	Oxalidaceae		Bowl flower	No	Single flower	18	112
Plantago lanceolata	Plantaginaceae		Blossom incospicuos	No	Inflorescence	230	9
Veronica persica	Plantaginaceae		Disk flower	Yes	Single flower	19	106
Rubus sp.	Rosaceae		Bowl flower	No	Single flower	85	24
Prunus sp.	Rosaceae		Bowl flower	No	Single flower	72	28
Potentilla reptans	Rosaceae		Bowl flower	No	Single flower	67	30
Sambucus nigra	Viburnaceae		Brush blossom	No	Inflorescence	720	3

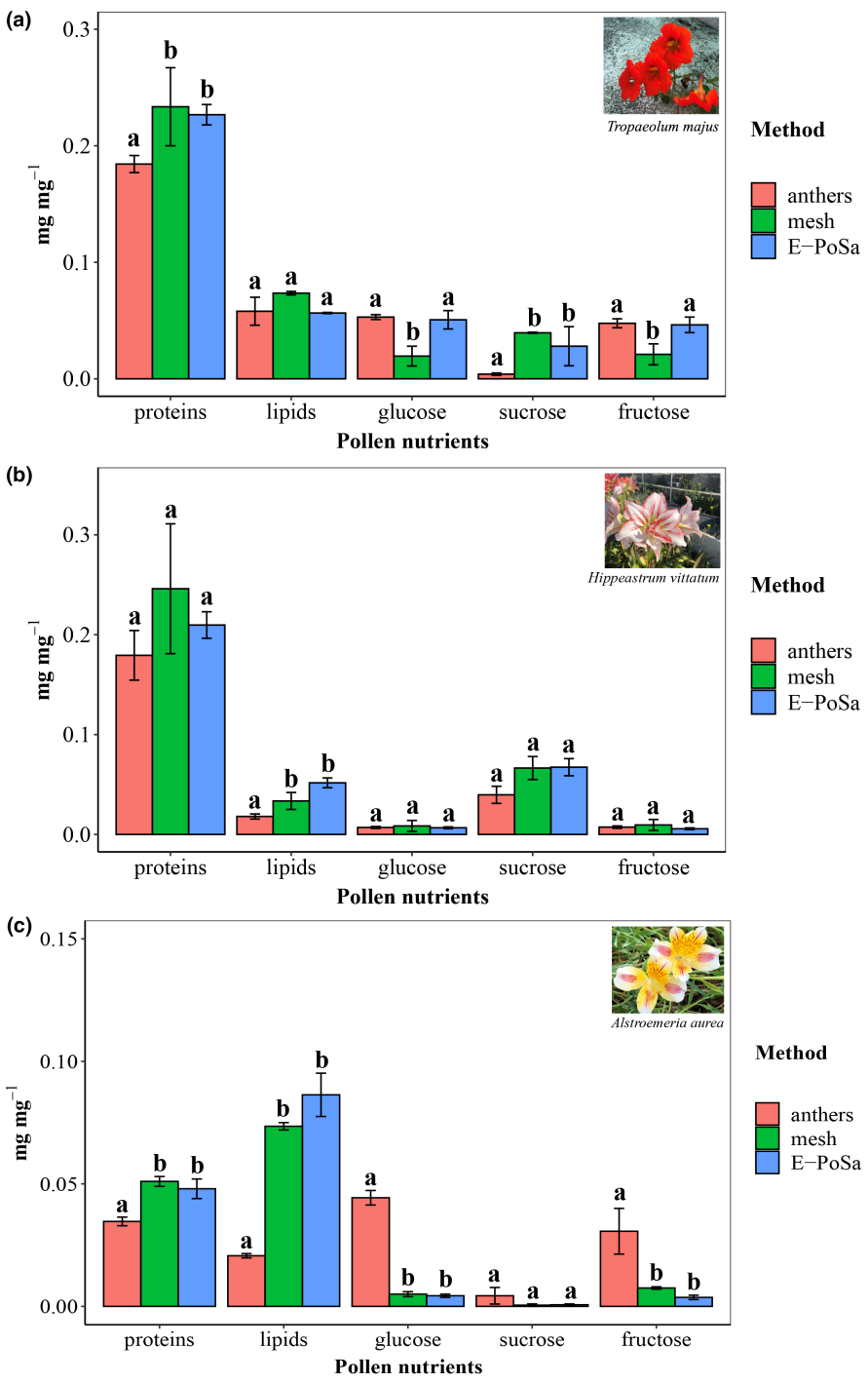

FIGURE 5 Quantified amounts of the macronutrient composition of the samples in (a) *Tropaeolum majus*, (b) *Hippeastrum vittatum*, (c) *Alstroemeria aurea*. Values are reported as the mean ± SEM. For each species, $N = 3$ for anthers, $N = 2$ for mesh and $N = 3$ for Electronic Pollen Sampler samples. For each nutrient, significant differences ($p < 0.05$) among the three sampling methods are estimated through the post hoc Tukey's test and are reported by different letters.

4 | DISCUSSION

The recent advances in pollination nutritional ecology require the definition of standardized sampling methods for pollen (Jeannerod et al., 2022). The analysis of pollen from wild plants needs to be fine-tuned for an accurate definition of its role in the nutritional balance of pollinators' diets (Lau et al., 2022). It is well known that the recovery of pollen grains from wild plants is a difficult task to perform since the anthers of enthomogamous plant species usually produce low amounts of pollen (e.g. Jeannerod et al., 2022; Palmer-Young et al., 2019). Obtaining a significant amount of pollen without contamination originating from other floral parts can be very time-consuming, reducing the efficiency and the feasibility of nutritional ecology studies. The results of the nutritional analyses of pollen performed on the three target species show that this strategy avoids sample contaminations, since the pollen collected by the E-PoSa did not show any significant difference in terms of macronutrient composition compared to the pollen sampled by sieving. In this framework, we suggest the adoption of the E-PoSa for the non-invasive collection of a satisfactory amount of pollen in a relatively short time. The adoption of the E-PoSa does not require any expertise or training for its usage due to the ease of assembly and the getting

of the equipment. Furthermore, it let the sampling of pollen free of contaminants possibly impairing further nutritional analyses. This feature makes it possible to conceive its exploitation in multiple contexts, such as in the framework of citizen science activities, plant breeding programmes and/or in contexts requiring the need to optimize the logistics and human resources available. Furthermore, we emphasize the significance of E-PoSa for studies dealing with pollen chemical defence. The swiftness of this sampling approach enables to capture a snapshot of the chemical profile in a specific moment, facilitating the investigation of short-term plant responses to experimental treatments (e.g. herbivory and abiotic stressors). In addition, the E-PoSa sampling accuracy allows to obtain well-separated pollen samples, thus minimizing mischaracterizations linked to the considerable variations occurring in the chemical profile across different floral tissues and rewards (possibly reflecting their ecological roles). Moreover, the elimination of the drying step required by the sieving method would additionally be essential for the analysis of more labile components (e.g. antioxidants and volatiles). At the same time, we underline that steel mesh, Eppendorf tubes and filter paper need to be prepared before each sampling, especially when pollen from different plant species is sampled. This might be particularly important when chemical analyses are carried out, and chemical contaminations among the pollen of different plant species should be avoided.

5 | CONCLUSIONS

The field of nutritional ecology in the context of plant–pollinator interactions is growing in importance to address the issues of pollinator safeguarding and conservation. The novel E-PoSa can provide a cheap and easy-to-assemble tool, encouraging its future use in the field of pollen research, including breeding, interactions with herbivores and ecological studies dealing with the characterization of the nutritional landscapes for pollinators. The effectiveness of the recovery allows the sampling to be performed on a wide range of species considering the availability of floral resources usually occurring in meadows (Table 2) with a high degree of purity, as proved by the nutritional characterization of samples.

AUTHOR CONTRIBUTIONS

Emiliano Pioltelli, Luca Campone, Luca Tonietti and Andrea Galimberti conceived the ideas and designed the methodology; Emiliano Pioltelli, Lorenzo Guzzetti and Andrea Copetta collected the data; Emiliano Pioltelli and Lorenzo Guzzetti analysed the data; Emiliano Pioltelli, Lorenzo Guzzetti, Paolo Biella, and Andrea Galimberti led the writing of the manuscript. All authors critically contributed to the draft and gave their final approval for publication.

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CONFLICT OF INTEREST STATEMENT

None of the authors have any conflicts of interest.

DATA AVAILABILITY STATEMENT

All data can be accessed at the following FigShare link: https://doi.org/10.6084/m9.figshare.24291169.v1.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/2041-210X.14241.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1. Price of the necessary hardware for E-PoSa assembly.

Table S2. Quantified amounts of mg pollen retrieved per flower unit and mg of nutrients per mg of pollen.

Video S1. Video demonstrating E-PoSa assembly and field application.

Appendix S1. Pollen nutritional analysis.

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