FAMeLeS : A multispecies and fully automated method to measure morphological leaf traits

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Received: 12 May 2023 | Accepted: 23 December 2023

DOI: 10.1111/2041-210X.14287

Methods in Ecology and Evolution

PRACTICAL TOOLS

FAMeLeS: A multispecies and fully automated method to measure morphological leaf traits

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Funding information
INTERBIO

Handling Editor: Torbjørn Ergon

Abstract

1. Leaf shape parameters are of key importance to explain the role of energy balance and water economy in plant species distribution, plant productivity and, more generally, in plant–environment interactions. Yet, leaf shape measurements based on image processing are still challenging due to the high diversity of leaf shapes, colours and sizes leading to the development of time-consuming methods with a narrow field of applicability, sometimes species-specific or often limited to a few species.
2. We developed a fully automated method for measuring multiple leaf shape parameters (area, perimeter, length, width, circularity and solidity) based on a large image sampling of leaf diversity (including litter) belonging to 587 species and spread over 232 countries worldwide. To evaluate the accuracy of the method to detect small objects, the sampling particularly targeted Mediterranean ecosystems (32 species and 25,205 leaves), in which small leaves often represent methodological challenges.
3. We compared our approach and found that its mean error in leaf area measurement (+0.46%) was 1.7–148 times lower than four existing methods. It was also the only one capable of detecting and measuring all leaves in the test data set, even variegated and small leaves (less than 1 mm^2). Its reliability was extensively checked on the largest and most diversified data set ever used.
4. Our method, accessible to the broader scientific community, was simple, rapid and effective on multiple image file types and on a high diversity of leaf size, shape and colour. As such, our approach allows measurement not only on fresh leaves but also on dry leaves, such as leaf litter or leaves from herbaria. This tolerance to leaf characteristics is crucial to increase large-scale sampling efforts and paves the way for a standardized multispecies approach to measuring leaf morphological traits for ecological and agricultural studies.

KEYWORDS
fully automated method, leaf shape diversity, litter, morphological leaf traits, multispecies validity

1 | INTRODUCTION

Due to their crucial role in plant development and productivity and their high plasticity, plant leaf traits are key features to understand both plant responses to environmental conditions (i.e. response traits) and impacts on ecosystem functioning (i.e. effect traits) (Violle et al., 2007). Indeed, morphological leaf traits such as leaf length, perimeter, shape and area (see, e.g. Wright et al., 2017) are strongly correlated with perturbations (soil and/or air contamination, fire frequency, herbivory), biophysical processes (see, e.g. leaf surface temperature), plant metabolism (energy, water, nutrients, CO_2 concentration) and resource availability (Anderson et al., 2020). Leaf traits are, in turn, a key component determining resource availability (for herbivory), resource prehension and litter quality with consequences on soil fauna, microbial activity and fungal diversity (Le Bagousse-Pinguet et al., 2021). Thus, the accuracy of leaf shape measurements highly influences our understanding of many processes involved in functional ecology, plant diversity and repartition, ecosystem ecology or crop production (Kumar et al., 2012). However, the predictive capacity of new species distribution models faces challenges related to integrating a diverse range of species (Vesk et al., 2021) while maintaining a high sensitivity to intraspecific variability. This sensitivity is crucial to getting a deeper understanding of the role of phenotypic plasticity in local adaptations to climate change (Benito Garzón et al., 2019).

The leaf shape was traditionally described and measured using the paper weighing method (Pandey & Singh, 2011) or allometric relationships between the area of the leaf and the morphometric parameters such as length and width (Ajayi, 1990). More recently, such allometric relationships have been investigated with new models (Amiri & Shabani, 2017) and equations (Liu et al., 2019) but, in addition to being time-consuming, these methods are only valid on a limited number of species. To avoid manual measurements, different image processing methods were developed. Based on software, algorithm or script, most of methods are not fully automated and needed laborious user interventions and treatments for image processing (e.g. Varma & Osuri, 2013) or algorithm calibration for each species (Easlon & Bloom, 2014). Yet, the fully automated methods published so far have been tested and calibrated on a few numbers of species (up to 61) or a small number of samples (from 4 to 240), raising questions about their genericity across species, leaf colours and leaf shapes.

Others only tested the validity of their method on artificial samples (e.g. geometrical figures cut on paper in Ferreira et al. (2017)). Another method consists of portable leaf area measurement devices such as Li-Cor©3100 (Machado et al., 2016) or the leaf area meter MK2 Delta-T Devices© (Vile et al., 2005). However, these instruments are expensive and do not allow the acquisition of leaf pictures for subsequent verifications (Bylesjö et al., 2008). Additionally, an image sampling provides additional leaf traits (perimeter, length, width, solidity, circularity, shape, margin type) of great importance for leaf classification (Murat et al., 2017), leaf recognition processes (Thyagharajan & Kiruba Raji, 2019), for the relationships between leaf margin and temperatures (Royer et al., 2005), to evaluate the leaf efficiency to trap contaminated particles (Leonard et al., 2016) or to determine the leaf capacity to home phyllosphere microbial communities (e.g. Kembel et al., 2014). Yet, so far, no method is fully automatic, fast, highly accurate, reproducible, easy to implement and can handle a large number and diversity of leaves (shapes and colours), species and image type. In addition, no automated image processing method has successfully measured leaves less than 2 mm^2. Developing such a tool could greatly simplify large-scale measurements by processing a large number of samples using a single process, ensuring standardization of results, while preserving diversity and quality of biological information (leaf shape measurements possibly at intraspecific scale), with the aim of contributing to open science in ecology and evolution.

We aimed to develop a new powerful, versatile, fully automated and free-to-use method to measure morphological leaf traits based on the free Fiji© software (Schindelin et al., 2012): the **F**ully **A**utomated **Me**asurement of **Le**af **S**hape (FAMeLeS hereafter). We focus on improving small leaf detection, accuracy and diversity of shape measurements (leaf area, perimeter, length, width, solidity, circularity) and more generally on the validity range of the method, to avoid the main weaknesses of existing methods.

2 | MATERIALS AND METHODS

In order to ensure performance across a high diversity of leaf sizes, shapes and colours, the automated image processing proposed was tested on a mixed sampling of field, databases and web images. To test the ability of the proposed method to detect all leaves (even the smallest) and only leaves, a validation procedure was conducted on 26,034 leaves (including 31 species with needles): The number of leaves detected by the algorithm was compared to manual counts. To assess the accuracy of the method in measuring various leaf traits (such as area, perimeter, length, width, circularity and solidity), a subset of 2218 leaves, including 223 leaf litter samples, was operated (Figure 1). The mean error was estimated by comparing measurements obtained by the automated threshold set by the algorithm with measurements obtained after manual correction of the threshold. The correction process involved creating an overlay in Fiji, where the binary image was superimposed onto the original picture with 50% transparency. The pixels were then added or removed from the binary image with pixel-level precision until a perfect match was achieved between the paired pictures.

Our sampling included images from photographs and scans, taken on a white background, as this is the most common practice for leaf imaging. The length, width, perimeter and surface of the leaves sampled in our study varied by a factor of 187.4; 326.8; 819.6 and 13,118.7, respectively. This high variability in measurements reflects the wide variety of leaf shapes, sizes and colours on which FAMeLeS was tested (Figure 2).

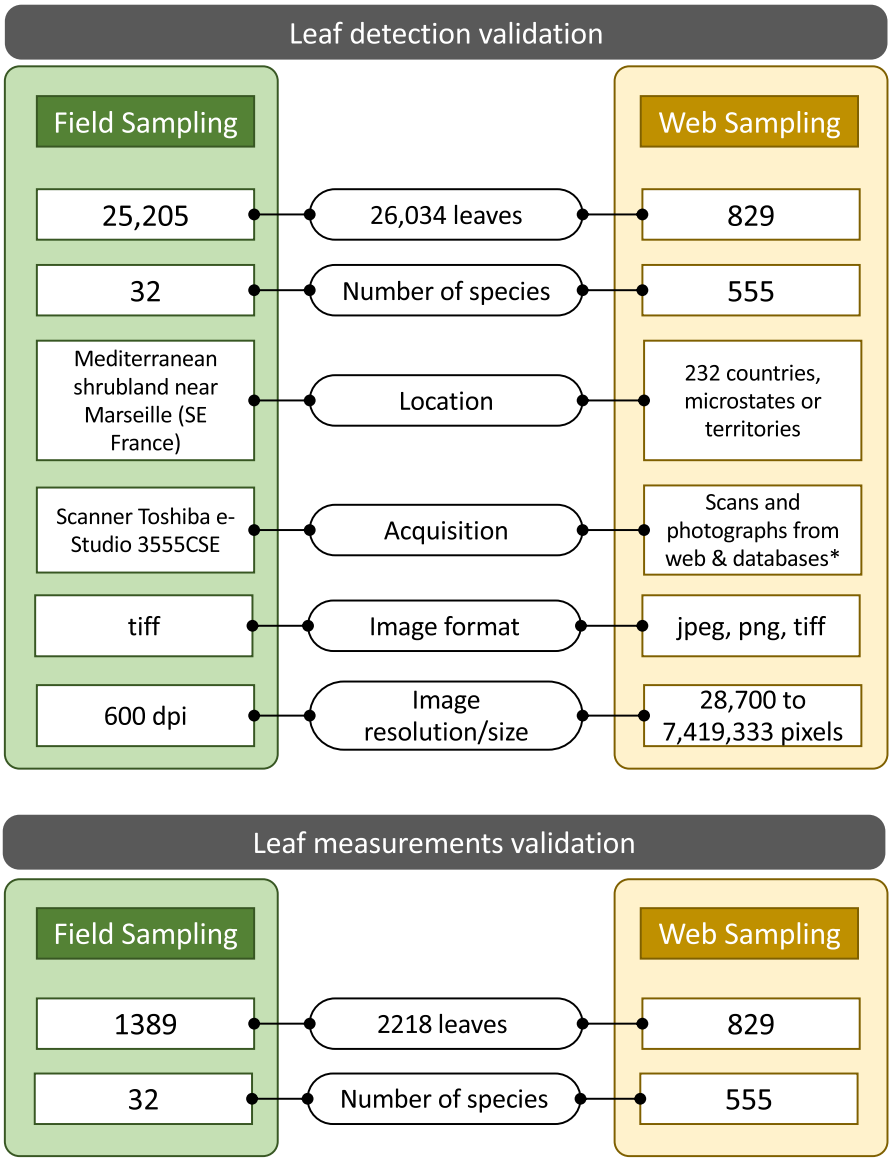

FIGURE 1 The green column (on the left) represents field sampling, and the brown column (on the right) represents web sampling. The accuracy of the method was tested on the complete data set for leaf detection (upper part of the picture) and on a subset for leaf measurements (lower part of the picture).

* Web and database sources:
Flavia leaf dataset (Wu et al., 2007)
Tree leaf Database (MEW, 2014)
Web site: Baumkunde.de (https://www.baumkunde.de, 10 September 2021).
Images from Murat et al. (2017)

These leaves belong to 94 families, 269 genus and 587 species (Appendix S1) from 232 countries (Figure 2). The number of leaves in a single image ranged from 1 to 50.

2.1 | Image processing

The image processing was based on the free and open-source ImageJ(c) v image processing software ImageJ© v. 2.9.0/1.53t (Schindelin et al., 2012).

One of the most common procedures to measure the leaf area on a picture is thresholding, to obtain a two-colour picture: the leaf area and the background (e.g. LAMINA software in Bylesjö et al., 2008). Figure 3 shows a flow diagram of the entire image processing that produces a binarized image from an RGB image.

The issue with thresholding was to remove shadows created by the thickness of leaf and/or petiole, a difficulty that grows with the chromatic diversity of leaves (at interspecific, specific, individual or even leaf scale in the case of variegated leaves) and which explains why most of the leaf surface area measurement methods have been limited to a few numbers of species (Appendix S2).

However, the spectral properties of leaves can differ from the spectral properties of shadows or background, depending on the considered channel (Varma & Osuri, 2013). Although leaf and shadow spectral properties can partially overlap in the green and red channels, we were able to reduce the overlap between the

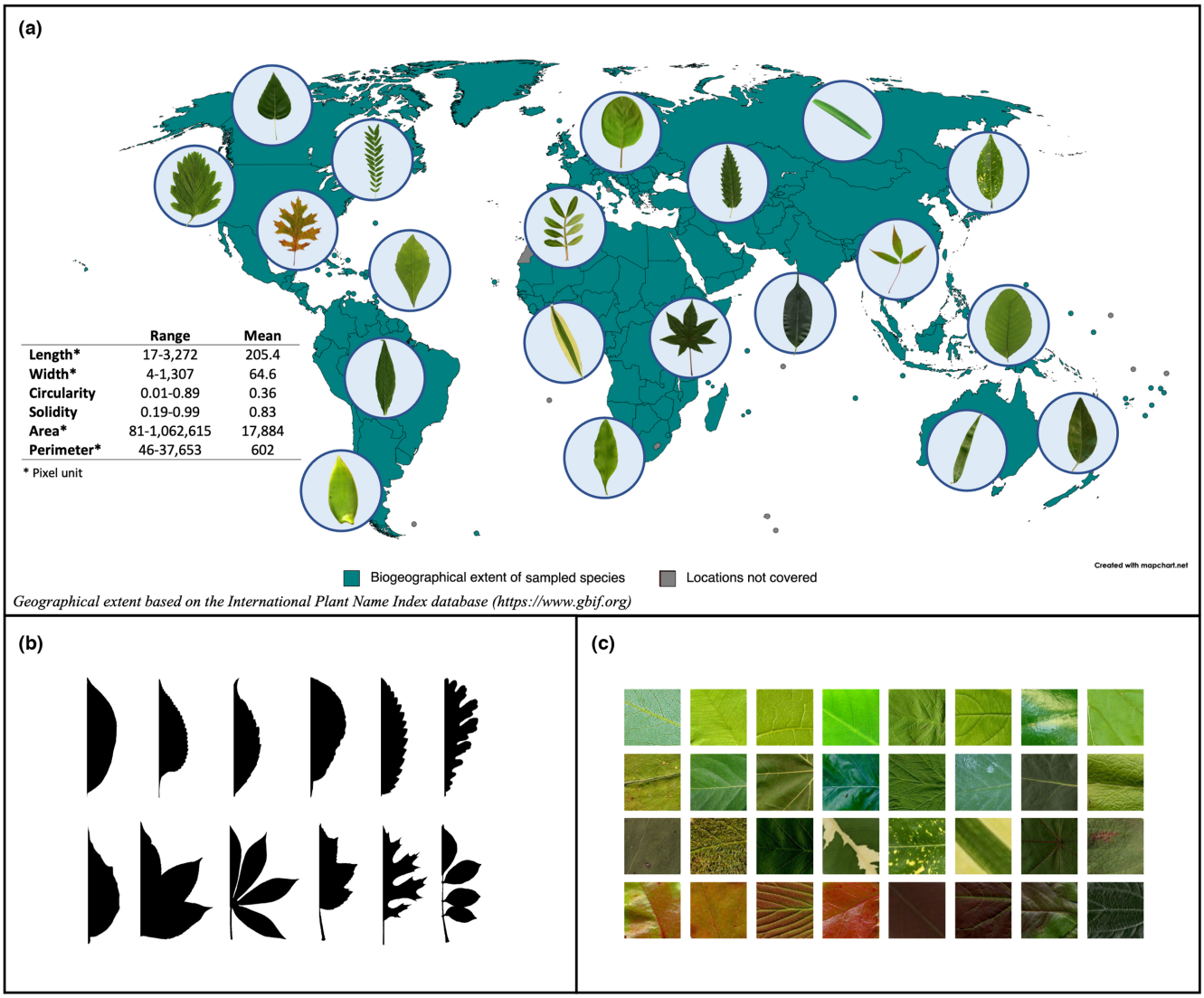

FIGURE 2 Diversity of geographical locations and leaf size (a), leaf margins and lobation (b) and leaf colour and texture (c) considered in the whole leaf data set ($n = 26{,}034$).

high reflectance values of leaves and the low reflectance values of shadows/background in the blue channel (Appendix S3) (Bylesjö et al., 2008). The originality of our method lies in the use of these reflectance differences, which allowed us, after fine-tuning on a separate set of leaves, to remove light and dark shadows in two separate steps:

From the three layers of the RGB image, a Bandpass filter was applied, allowing the removal of the darkest shadows, those closest to the leaf. This step can lead to losing the brightest or lightest parts of the leaves, which could be assimilated to the white background. Another image was created from the blue layer of the raw image. The blue layer was thresholded to separate the leaf from the background. This step ensures that all parts of the leaf are preserved, especially the brightest and lightest parts. During this thresholding, shadows (especially the darkest ones) can be selected along with the leaf. Combining this image, where light shadows were removed but dark shadows may have remained, with the previous one, where dark shadows were removed, will result in an image where all parts of the leaf are preserved, and all shadows are removed.

The method presented in this paper consists of a full automation of image processing coupled to batch processing on multiple files. The FAMeLeS source code (Fiji macro), documentation and trial set can be freely downloaded on figshare (https://doi.org/10.6084/m9.figshare.22354405) and can be used without specific skill, since after ImageJ and the plugins were installed, the user just has to specify the folder locations when prompted. The step-by-step procedure is available in Appendix S4.

2.2 | Comparisons with existing methods

To evaluate the effectiveness of FAMeLeS, the measurements were compared with six other existing methods (the only ones that were free and still available among the 26 partially or fully

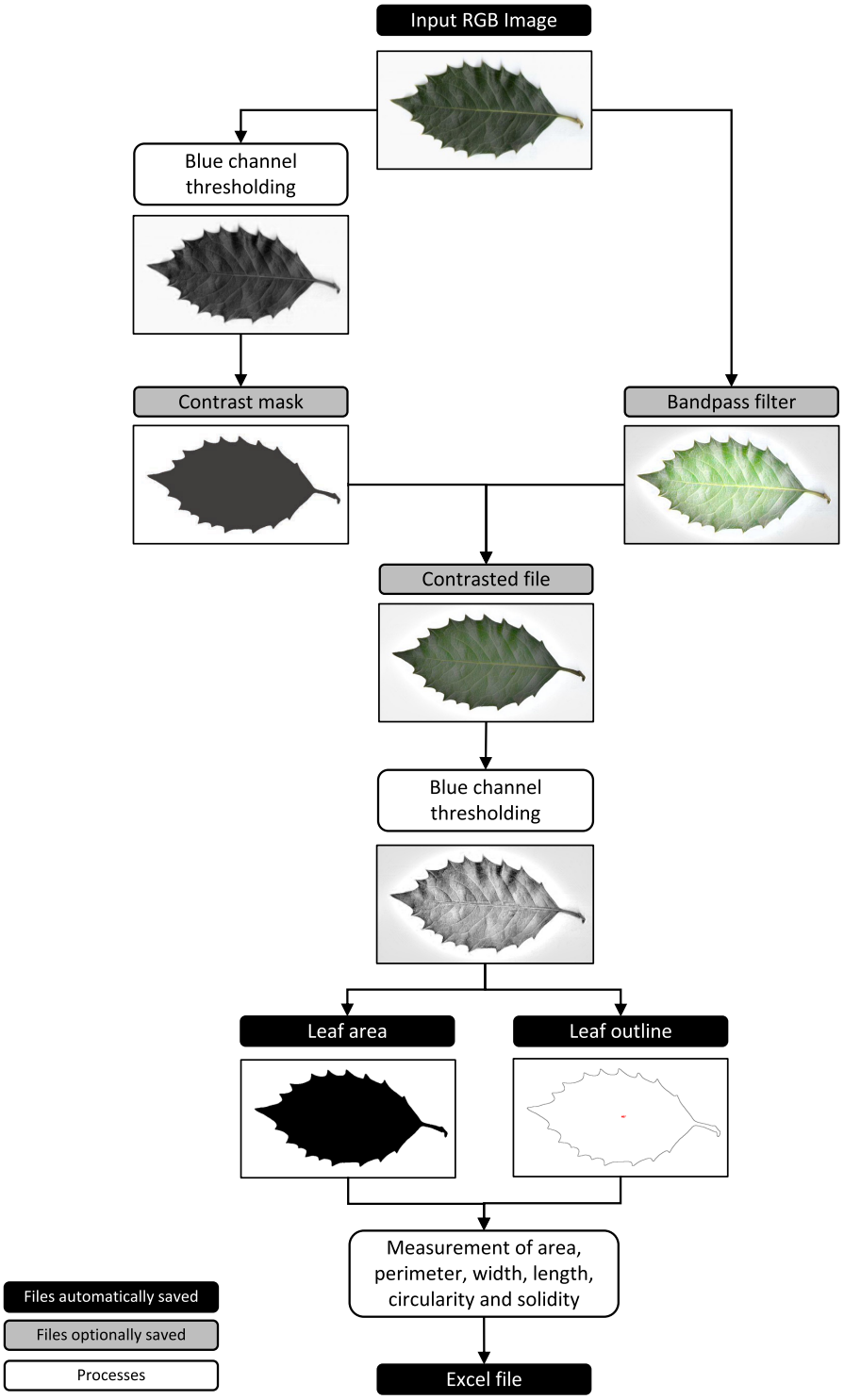

FIGURE 3 Flow diagram of the image processing by FAMeLeS: From the raw image, two different treatments are conducted in parallel. The blue channel is used to create a mask that preserves the brightest and lightest parts of the leaves, while the three channels of the RGB image are used to remove the darkest shadows.

automated methods found in the literature (Appendix S2)). Leaf area measurements on a subset of 145 leaves, obtained by LeafByte (Getman-Pickering et al., 2020), BlackSpot (Varma & Osuri, 2013), LeafArea R package (Katabuchi, 2015) and Easy Leaf Area (Easlon & Bloom, 2014) on 69 species were compared to FAMeLeS and the true leaf area obtained after leaf shape was manually checked and corrected (see above). None of the compared methods had prior exposure to the data set used as it was new to all of them. We also investigated an AI tool called 'Segment Anything' to compare it with FAMeLeS. This tool is capable of separating objects from their backgrounds, even if they are heterogeneous. However, we ultimately decided not to include it in our comparison because it could not handle batch processing on multiple image files, struggled to accurately segment small leaves in groups, required manual selection of different parts of compound leaves and was not providing leaf trait measurement.

3 | RESULTS

3.1 | Leaf detection

Among the 26,034 leaves considered, 61 detection errors were attributable to image processing (0.23%) and were partially due to overdetection bias (0.18%) because of leaf image fragmentation created during image processing. This was the case for species with silvery trichomes, light basal leaves or small compound leaves. The underestimation bias of leaf number (0.05%) was due, on the one hand, to very small leaves that were filtered because they were considered as dust, and, on the other hand, to bright leaves that were assimilated to the white background.

3.2 | Variables of leaf shape and size

The wide range of leaf dimensions considered in the entire data set (see Figure 2 for range and mean values of leaf shape variables) led to remarkably high values of the standard deviation for all variables. The smallest leaves had a leaf area of less than 100 pixels squared (with a width of 4 pixels). Circularity ranged from 0.01 to 0.89 and solidity ranged from 0.19 to 0.99.

3.3 | Quality of automated measurements and influence of foliar traits

The mean errors (mean percentage of error between estimated and manually measured values) were 2.3 ± 0.06% for leaf area, −1.12 ± 0.08% for leaf perimeter, −0.47 ± 0.02% for leaf length, 1.18 ± 0.05% for leaf width, 0.82 ± 0.04% for leaf solidity and 6.12 ± 0.18% for leaf circularity (Table 1).

The same pattern was observed in the leaf type with the highest values of mean error for circularity and the lowest for length. The absolute error was almost similar for leaf area, width and solidity and a little bit higher for perimeter. However, differences in magnitude can be observed between leaf types: Compound leaves had the highest values of mean error, while simple leaves and litter had the lowest.

Spearman correlations, used to assess how algorithm performance was impacted by variation in leaf morphology, reveal that the mean errors of the automated measurements were negatively correlated with their respective variables. This indicates that the accuracy of the measurements increases with leaf size (Appendix S5).

Mean (± standard error) of automated measurement of leaf area (81,247 ± 3306 pixels), circularity (0.392 ± 0.004) and solidity (0.820 ± 0.003) were significantly higher compared to manual measured leaf area (80,893 ± 3345 pixels), circularity (0.378 ± 0.004) and solidity (0.815 ± 0.003) ($p < 0.001$). Automated measurement of the leaf perimeter (1336 ± 34 pixels) was significantly inferior to manual measurement of the leaf perimeter (1392 ± 37) ($p < 0.001$). No significant differences were detected between automated and manual measurements of leaf length (430 ± 8 and 431 ± 8 pixels, respectively; $p = 0.841$) and width (190.9 ± 5 and 190.5 ± 5 pixels, respectively; $p = 0.636$).

3.4 | Comparison with existing methods

With a mean error of +0.46%, FAMeLeS was up to 148 times more accurate than other methods tested (−0.8%, −2.6%, −19.2% and −68.4%, for LeafArea, LeafByte, BlackSpot and Easy Leaf Area, respectively) and was the only one able to detect all the 145 leaves considered (Figure 4). In comparison, eight of the 145 leaves were not detected by LeafByte (*Asparagus acutifolius*, *C. monspeliensis*, *Fumana ericifolia* (Wallr.), *Fumana laevipes*, *Fumana thymifolia*, *Fumana viridis* (Ten.) Font Quer, *H. hirtum*, *T. vulgaris*) and one was not detected by BlackSpot (*Viburnum x bodnantense* Aberc. Ex Stearn), Leaf Area (*Lavandula latifolia* Medik.) and Easy Leaf Area (*Sedum anopetalum* DC.), the latter failing to measure all the leaves of the litter.

Of the 26 automated methods found in the literature (Appendix S2), only five were fully automated, but only BlackSpot and LeafArea were free and still available.

TABLE 1 Mean and standard deviation ($n = 2218$) of the percentage of error between estimated and manually measured values of leaf area, perimeter, circularity, length, width and solidity for the different leaf types (terminology based on Ellis et al., 2009).

Mean errors	Area	Perimeter	Length	Width	Solidity.	Circularity	Mean
Compound	3.20% ± 0.03	−10.45% ± 0.07	−0.07% ± 0.01	−0.06% ± 0.01	3.26% ± 0.03	32.38% ± 0.29	4.71%
Palmately lobed	1.67% ± 0.05	−4.78% ± 0.10	0.08% ± 0.03	0.21% ± 0.03	1.62% ± 0.09	16.32% ± 0.34	2.52%
Palmatisect	−2.32% ± 0.13	4.76% ± 0.43	−2.42% ± 0.08	2.85% ± 0.08	−3.18% ± 0.15	13.80% ± 0.56	2.25%
Pinnately lobed	4.93% ± 0.14	−3.13% ± 0.20	0.56% ± 0.03	2.87% ± 0.11	1.30% ± 0.01	16.61% ± 0.21	3.86%
Pinnatisect	1.36% ± 0.03	−4.24% ± 0.04	0.02% ± 0.00	−0.01% ± 0.01	1.48% ± 0.03	13.73% ± 0.15	2.06%
Simple	2.38% ± 0.06	−0.36% ± 0.05	0.60% ± 0.02	1.39% ± 0.05	0.66% ± 0.04	3.79% ± 0.13	1.41%
Litter	0.99% ± 0.05	1.58% ± 0.11	0.13% ± 0.03	0.59% ± 0.03	0.03% ± 0.02	−1.53% ± 0.10	0.30%
Whole data set	2.3% ± 0.06	−1.12% ± 0.08	0.47% ± 0.02	1.18% ± 0.05	0.82% ± 0.04	6.12% ± 0.18	1.66%

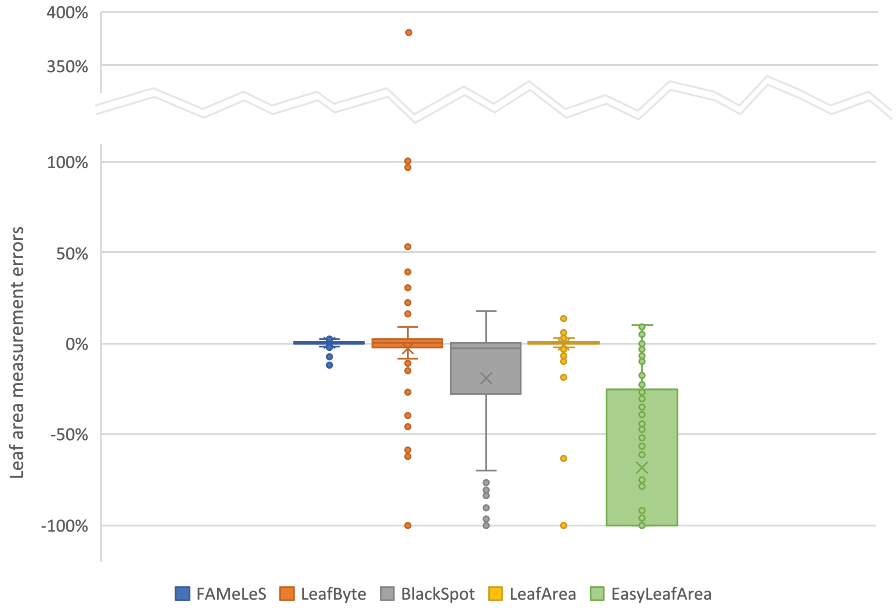

FIGURE 4 Comparisons of leaf area measurements errors between methods on a set of 145 leaves belonging to 69 species.

4 | DISCUSSION

Here, we aimed to develop a powerful, broadly applicable, fully automated and free-to-use method to measure morphological leaf traits. Our results highlight that, compared to other available existing methods, FAMeLeS is an accurate and efficient fully automated method, able to cope with a wide range of species and a large set of samples and the only one able to cope with very small leaves.

Although most of the available methods require strong programming skills (PHP language in Can et al. (2012) or Visual C++ in Haqqiman Radzali et al. (2016)) or user adjustments (e.g. Schrader et al., 2017), the full automation of FAMeLeS enabled high-precision measurements on thousands of leaves, including isolated leaves or multiple leaves scanned at once, regardless of the number of leaves in the image.

FAMeLeS focussed on a strict preservation of the integrity of the leaf image by avoiding the numerous isolated pixels generated by the binarization process. Out of the 26,034 leaves in the dataset, only 47 supernumerary objects were counted (0.18%), and none on the 145 leaves tested in the subset, while the existing methods tested failed to preserve the integrity of the leaf image, generating multiple objects for each leaf (+210% and +238%, respectively, for Blackspot and LeafArea). The algorithm of FAMeLeS was tuned to eliminate outliers by smoothing the picture and removing noise, resulting in leaf margin erosion that negatively affected the accuracy of the smaller leaf measures. Thus, we recommend prioritizing a sufficient image resolution to ensure maximum accuracy. However, it allowed accurate measurements of numerous foliar traits such as leaf area, with errors ranging from 1.7 to 148 times lower than the four existing methods used for comparison.

FAMeLeS is the only method that allows dealing with a very large set of leaves, with 26,034 leaves used to test the detection accuracy and 2218 leaves used to validate leaf traits measurements. On the contrary, previously published leaf area measurement methods have only been tested on a small set of leaves, which may be problematic for species with a high polymorphism. Among the 26 methods listed in Appendix S2, the maximum number of leaves that were used to validate the accuracy of methods was 240 (Karatassiou et al., 2015), and the lowest number of leaves was found in Patil and Bodhe (2011) with only four leaves to validate their method.

An overview of the existing methods points to the fact that, in addition to being tested on a low number of leaves, they are based on a small number of species. The highest number of species used (when given) to test the method was 61 (Amiri & Shabani, 2017), but with only one leaf per species, and the mean number of species used in the 26 methods was 10. Furthermore, most existing methods have limitations on leaf shape and are not suitable for compound leaves (Getman-Pickering et al., 2020) or complex leaf margins (Schrader et al., 2017). Although the precision of FAMeLeS tends to decrease for compound leaves, it remains high and is relatively stable for leaves with different levels of complex margins.

Our field sampling focussed on small leaves since no automated method has yet demonstrated its effectiveness on leaves less than $2\,mm^2$. The four methods tested failed to detect all leaves, which is a major obstacle to studying leaf traits in Mediterranean regions, arid ecosystems or warm deserts, as well as cold high-elevation regions, where species with small leaves are dominant (Wright et al., 2017) and ecological issues are of particular concern in a context of global warming. Red petiole or red parts of lamina were also often missed by existing methods and generated underestimates, especially EasyLeaf which failed to measure the completely red or brown leaves (fresh or litter). In contrast, FAMeLeS allows one to detect and accurately measure leaves whatever their colour (Figure 2c), and has proven its accuracy to measure dead leaves and litter. As such, our method could be useful to measure leaf features from the herbarium in order to study leaf morphology changes over time and space. Moreover, the accuracy of FAMeLeS to detect and measure small objects can be used to determine the number, shape and size of litter particles.

The accuracy of FAMeLeS on images from field sampling mixed with web images attests to the efficiency of the method even with images from multiple devices (scanner or photographs). While some authors warned about the limitation of their method depending on image quality (Meira et al., 2020) or brightness (Haqqiman Radzali et al., 2016), FAMeLeS has proven to be very tolerant of the diversity of image sizes (compressed or not), resolutions and lighting conditions which makes it possible to work on existing leaf image databases or to aggregate data sets from different sources. The combination of such a high tolerance to image type and leaf characteristics offers the opportunity to build standardized database of leaf binary images from any biome, and to produce high amounts of data to address functional ecology questions, to study biogeographical patterns and drivers of morphological leaf traits and to feed allometric models and algorithms of plant leaf classification.

AUTHOR CONTRIBUTIONS

Nicolas Montès designed the research, developed the algorithm, performed web sampling and wrote the first draft of the paper. Lorène Tosini, Isabelle Laffont-Schwob and Hélène Folzer performed field sampling and image acquisition. Nicolas Montès and Lorène Tosini measured validation data. Lorène Tosini, Nicolas Montès and Yoann Le Bagousse-Pinguet performed statistical analysis and figures. Nicolas Montès, Lorène Tosini, Yoann Le Bagousse-Pinguet, Isabelle Laffont-Schwob and Hélène Folzer improved the manuscript. Funding acquisition for data collection was achieved by Isabelle Laffont-Schwob, Hélène Folzer and Lorène Tosini. All authors contributed critically to the drafts and gave final approval for publication.

ACKNOWLEDGEMENTS

The authors thank the Calanques National Park for having authorized this research work and leaf harvest within the Calanques National Park (DI_2021_049_AMU_Isabelle_LAFFONT-SHWOB) and for funding the project research INTERBIO. The doctoral school ED 251 in Environmental Sciences from Aix-Marseille University is acknowledged for the PhD grant of Lorène Tosini. Many thanks to Manuel Cartereau, Pascale Prudent, Margot Cardenas Saavedra, Laurine Dariel, Roberto Alex Arencibia Celestrin and Laura March for their technical help during field sampling and/or leaves scanning, and to the ImageJ forum members for their help and advice on writing and improving the script.

CONFLICT OF INTEREST STATEMENT

All authors declare that they have no conflict of interest.

FUNDING INFORMATION

Project research named INTERBIO, funded by the Calanques National Park.

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.14287.

DATA AVAILABILITY STATEMENT

The Fameles script, a trial set of pictures and a user guide are freely available on Figshare: https://doi.org/10.6084/m9.figshare.22354405.

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REFERENCES

Ajayi, N. O. (1990). Rapid determination of leaf area in ovate vegetable leaves by linear measurements. *Journal of Horticultural Science*, *65*(1), 1–5. https://doi.org/10.1080/00221589.1990.11516020

Amiri, M. J., & Shabani, A. (2017). Application of an adaptive neural-based fuzzy inference system model for predicting leaf area. *Communications in Soil Science and Plant Analysis*, *48*(14), 1669–1683. https://doi.org/10.1080/00103624.2017.1373801

Anderson, C. G., Bond-Lamberty, B., & Stegen, J. C. (2020). Active layer depth and soil properties impact specific leaf area variation and ecosystem productivity in a boreal forest. *PLoS ONE*, *15*(12), e0232506. https://doi.org/10.1371/journal.pone.0232506

Benito Garzón, M., Robson, T. M., & Hampe, A. (2019). ΔTraitSDMs: Species distribution models that account for local adaptation and phenotypic plasticity. *New Phytologist*, *222*(4), 1757–1765. https://doi.org/10.1111/nph.15716

Bylesjö, M., Segura, V., Soolanayakanahally, R. Y., Rae, A. M., Trygg, J., Gustafsson, P., Jansson, S., & Street, N. R. (2008). LAMINA: A tool for rapid quantification of leaf size and shape parameters. *BMC Plant Biology*, *8*(1), 82. https://doi.org/10.1186/1471-2229-8-82

Can, M., Gursoy, O., Akcesme, B., & Akcesme, F. B. (2012). Leaf area assessment by image analysis. *Southeast Europe Journal of Soft Computing*, *1*(2), Article 2. https://doi.org/10.21533/scjournal.v1i2.54

Easlon, H. M., & Bloom, A. J. (2014). Easy leaf area: Automated digital image analysis for rapid and accurate measurement of leaf area. *Applications in Plant Sciences*, *2*(7), 1400033. https://doi.org/10.3732/apps.1400033

Ellis, B., Daly, D. C., Hickey, L. J., Johnson, K. R., Mitchell, J. D., Wilf, P., & Wing, S. L. (2009). *Manual of leaf architecture*. Cornell University Press.

Ferreira, O. G. L., Rossi, F. D., Vaz, R. Z., Fluck, A. C., Costa, O. A. D., & Farias, P. P. (2017). Leaf area determination by digital image analysis. *Archivos de Zootecnia*, *66*(256), 593–597.

Getman-Pickering, Z. L., Campbell, A., Aflitto, N., Grele, A., Davis, J. K., & Ugine, T. A. (2020). LeafByte: A mobile application that measures leaf area and herbivory quickly and accurately. *Methods in Ecology and Evolution*, *11*(2), 215–221. https://doi.org/10.1111/2041-210X.13340

Haqqiman Radzali, M., Ashikin Mohamad Kamal, N., & Mat Diah, N. (2016). Measuring leaf area using Otsu segmentation method (LAMOS). *Indian Journal of Science and Technology*, *9*(48), 1–6. https://doi.org/10.17485/ijst/2016/v9i48/109307

Karatassiou, M., Ragkos, A., Markidis, P., & Stavrou, T. (2015). A comparative study of methods for the estimation of the leaf area in forage species. *Proceedings of The7th International Conference on Information and Communication Technologies in Agriculture*, pp. 326–332.

Katabuchi, M. (2015). LeafArea: An R package for rapid digital image analysis of leaf area. *Ecological Research*, *30*(6), 1073–1077. https://doi.org/10.1007/s11284-015-1307-x

Kembel, S. W., O'Connor, T. K., Arnold, H. K., Hubbell, S. P., Wright, S. J., & Green, J. L. (2014). Relationships between phyllosphere bacterial communities and plant functional traits in a neotropical forest. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(38), 13715–13720. https://doi.org/10.1073/pnas.1216057111

Kumar, U., Singh, P., & Boote, K. J. (2012). Chapter two—Effect of climate change factors on processes of crop growth and development and yield of groundnut (*Arachis hypogaea* L.). In D. L. Sparks (Ed.), *Advances in agronomy* (Vol. *116*, pp. 41–69). Academic Press. https://doi.org/10.1016/B978-0-12-394277-7.00002-6

Le Bagousse-Pinguet, Y., Gross, N., Saiz, H., Maestre, F. T., Ruiz, S., Dacal, M., Asensio, S., Ochoa, V., Gozalo, B., Cornelissen, J. H. C., Deschamps, L., García, C., Maire, V., Milla, R., Salinas, N., Wang, J., Singh, B. K., & García-Palacios, P. (2021). Functional rarity and evenness are key facets of biodiversity to boost multifunctionality. *Proceedings of the National Academy of Sciences of the United States of America*, *118*(7), e2019355118. https://doi.org/10.1073/pnas.2019355118

Leonard, R. J., McArthur, C., & Hochuli, D. F. (2016). Particulate matter deposition on roadside plants and the importance of leaf trait combinations. *Urban Forestry & Urban Greening*, *20*, 249–253. https://doi.org/10.1016/j.ufug.2016.09.008

Liu, H., Ma, X., Tao, M., Deng, R., Bangura, K., Deng, X., Liu, C., & Qi, L. (2019). A plant leaf geometric parameter measurement system based on the android platform. *Sensors*, *19*(8), 1872. https://doi.org/10.3390/s19081872

Machado, B. B., Orue, J. P. M., Arruda, M. S., Santos, C. V., Sarath, D. S., Goncalves, W. N., Silva, G. G., Pistori, H., Roel, A. R., & Rodrigues-Jr, J. F. (2016). BioLeaf: A professional mobile application to measure foliar damage caused by insect herbivory. *Computers and Electronics in Agriculture*, *129*, 44–55. https://doi.org/10.1016/j.compag.2016.09.007

Meira, L. A., Pereira, L. E. T., Santos, M. E. R., & Tech, A. R. B. (2020). USPLeaf: Automatic leaf area determination using a computer vision system. *Revista Ciência Agronômica*, *51*(4), 2–10. https://doi.org/10.5935/1806-6690.20200073

Murat, M., Chang, S.-W., Abu, A., Yap, H. J., & Yong, K.-T. (2017). Automated classification of tropical shrub species: A hybrid of leaf shape and machine learning approach. *PeerJ*, *5*, e3792. https://doi.org/10.7717/peerj.3792

Pandey, S. K., & Singh, H. (2011). A simple, cost-effective method for leaf area estimation. *Journal of Botany*, *2011*, 1–6. https://doi.org/10.1155/2011/658240

Patil, S. B., & Bodhe, S. K. (2011). Betel leaf area measurement using image processing. *International Journal on Computer Science and Engineering*, *3*(7), 5.

Royer, D. L., Wilf, P., Janesko, D. A., Kowalski, E. A., & Dilcher, D. L. (2005). Correlations of climate and plant ecology to leaf size and shape: Potential proxies for the fossil record. *American Journal of Botany*, *92*(7), 1141–1151. https://doi.org/10.3732/ajb.92.7.1141

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.-Y., White, D. J., Hartenstein, V., Eliceiri, K., Tomancak, P., & Cardona, A. (2012). Fiji: An open-source platform for biological-image analysis. *Nature Methods*, *9*(7), 676–682. https://doi.org/10.1038/nmeth.2019

Schrader, J., Pillar, G., & Kreft, H. (2017). Leaf-IT: An android application for measuring leaf area. *Ecology and Evolution*, *7*(22), 9731–9738. https://doi.org/10.1002/ece3.3485

Thyagharajan, K. K., & Kiruba Raji, I. (2019). A review of visual descriptors and classification techniques used in leaf species identification. *Archives of Computational Methods in Engineering*, *26*(4), 933–960. https://doi.org/10.1007/s11831-018-9266-3

Varma, V., & Osuri, A. M. (2013). Black spot: A platform for automated and rapid estimation of leaf area from scanned images. *Plant Ecology*, *214*(12), 1529–1534. https://doi.org/10.1007/s11258-013-0273-z

Vesk, P. A., Morris, W. K., Neal, W. C., Mokany, K., & Pollock, L. J. (2021). Transferability of trait-based species distribution models. *Ecography*, *44*(1), 134–147. https://doi.org/10.1111/ecog.05179

Vile, D., Garnier, E., Shipley, B., Laurent, G., Navas, M.-L., Roumet, C., Lavorel, S., Díaz, S., Hodgson, J. G., Lloret, F., Midgley, G. F., Poorter, H., Rutherford, M. C., Wilson, P. J., & Wright, I. J. (2005). Specific leaf area and dry matter content estimate thickness in laminar leaves. *Annals of Botany*, *96*(6), 1129–1136. https://doi.org/10.1093/aob/mci264

Violle, C., Navas, M. L., Vile, D., Kazakou, E., Fortunel, C., Hummel, I., & Garnier, E. (2007). Let the concept of trait be functional! *Oikos*, *116*, 882–892.

Wright, I. J., Dong, N., Maire, V., Prentice, I. C., Westoby, M., Díaz, S., Gallagher, R. V., Jacobs, B. F., Kooyman, R., Law, E. A., Leishman, M. R., Niinemets, Ü., Reich, P. B., Sack, L., Villar, R., Wang, H., & Wilf, P. (2017). Global climatic drivers of leaf size. *Science*, *357*(6354), 917–921. https://doi.org/10.1126/science.aal4760

SUPPORTING INFORMATION

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

Appendix S1: List of the 587 species of the leaf dataset.

Appendix S2: Comparative analysis of leaf area measurement methods considering the number of species and leaves used for the validation process, the automation level of the method (level 1: image processing requires user adjustments, level 2: image processing is automated but only for one file, level 3: image processing is automated on multiple files with a batch process), the measured features (A, P, L, W, C, S referred respectively to Area, Perimeter, Length, Width, Circularity and Solidity) and whether or not the software was free. Availability/Reproducibility was set to "No" when methodology was incompletely described, complete algorithm not given or software no longer available.

Appendix S3: Examples of spectral reflectance of leaf, shadow and background in red, green and blue channels of RGB images.

Appendix S4: Step by step procedure to use the macro Fameles.

Appendix S5: Spearman correlations between foliar traits values and measurement errors (mean percentage of error between estimated and manually measured values).

How to cite this article: Montès, N., Tosini, L., Laffont-Schwob, I., Le Bagousse-Pinguet, Y., & Folzer, H. (2024). FAMeLeS: A multispecies and fully automated method to measure morphological leaf traits. *Methods in Ecology and Evolution*, *15*, 484–492. https://doi.org/10.1111/2041-210X.14287