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Auto-fluorescent phytoliths: can we detect past fires in tropical and subtropical contexts?

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Abstract To understand human practices and landscape evolution it is crucial to be able to trace evidence of past fires, notably in tropical environments. In such anthromes, phytoliths are generally well preserved and provide local signals on the environment. However, the different approaches to identifying burnt or heated phytoliths have proved inadequate. The recent investigation of auto-fluorescent phytoliths as proxy indicators of fire opens up new possibilities, however, such studies have so far been limited to European temperate regions. The present contribution further explores the potential of this technique, attempting to reconstruct fire histories in tropical and subtropical latitudes. Therefore, we investigated various modern and ancient contexts in Guatemala, Senegal and the Canary Islands using descriptive and quantitative approaches to fluorescence. This comparative process revealed a diversity of fluorescence responses depending on the ecosystems studied, with anthropized areas often producing more fluorescent phytoliths. These results were obtained despite the use of different extraction and mounting methods. In contrast, coloured phytoliths, often considered an indicator of environmental burning,

proved not very informative in some of the samples. This study reinforces our conviction that the auto-fluorescence of phytoliths is a universal phenomenon and is useful as a new proxy for detecting ancient fire in tropical and subtropical archaeological and palaeoenvironmental deposits.

Keywords Phytoliths · Auto-fluorescence · Fire · Tropics · Subtropics · Palaeoenvironment · Human impact

Introduction

Identifying past fires in archaeological and palaeoenvironmental records can improve our understanding of long-term cultural adaptations and human impacts on the environment (Snitker et al. 2022). Fire, whether of natural or human origin, has drastically affected past ecosystems and their composition. It is also a key factor linked to human presence in previously uninhabited territories (McWethy et al. 2009) and it is one of the main signs of the creation and maintenance of cultural landscapes in tropical areas such as the Amazon basin (Bush et al. 2007), Australia (Miller et al. 2005), New Guinea (Fairbairn et al. 2006), Southeast Asia (for example, Hunt et al. 2012; Hamilton et al. 2019) and the Pacific region (Thevenon et al. 2004). Several lines of evidence can be followed to study past natural and human-caused fires. At the macroscopic scale, the occurrence of fires and the material burnt can be studied through charcoal studies (anthracology) (Théry-Parisot et al. 2010) and charcoal quantification (Whitlock and Larsen 2001). At the microscale, the study of microscopic charcoal particles (Finsinger and Tinner 2005), microscopic fire by-products such as calcareous ash pseudomorphs (Canti and Brochier 2017) and fire-affected microfossils can provide valuable data on the type and intensity of fires in the sediment record. Finally, at the molecular scale, polycyclic aromatic hydrocarbons (PAH) (Lima et al. 2005) and charcoal particle elemental composition (through Fourier transform infrared spectroscopy or FTIR) can help assess fire frequency and intensity (Gosling et al. 2019). Each proxy brings opportunities and context-specific limitations related to abundance and degradation in sediments. The study of fire-affected microfossils such as phytoliths is promising due to their potential for preservation and the representation of taxa that rarely leave macroscopic traces in the sedimentary record such as grasses and shrubs.

Phytoliths can remain in soils or sediments for hundreds or thousands of years, but

phytolith taphonomy is poorly understood. Hodson (2018) reviewed this topic and suggested that both the morphology and chemistry of phytoliths could affect their persistence in sediments. There is some evidence that the presence of aluminium compounds may decrease phytolith solubility. It is also possible that cell wall phytoliths with a high carbon content may break down faster in the soil. However, the principal factor determining phytolith dissolution seems to be the ratio of their geometric surface area to volume. Soil factors are also important, and it has been known for many years that phytoliths survive much less well in alkaline soils. Since the early 2000s, several ways have been proposed to use phytoliths to detect fires, such as melting (Brochier 2002; Canti 2003), Raman spectroscopy (Pironon et al. 2001), refractive index (Elbaum et al. 2003) and change in colour (Parr 2006). Specifically, colour changes have been a frequently used indicator by the phytolith community in the last two decades (Gu et al. 2008; Garnier et al. 2013; Aleman et al. 2014; Hensel et al. 2022). However, Evett and Cuthrell (2017) consider three of these indicators (colour, refractive index and Raman spectroscopy) to be inadequate for their study area on the central coast of California and doubt whether they can be used everywhere. Wu et al. (2012) reported significantly different temperatures at which alteration and melting occurred. These appear to be influenced not only by the specific plant taxa and organs but also by environmental factors such as sodium or chlorine content of the soil, which can markedly reduce the melting temperatures. A more detailed review of these aspects is given by Devos et al. (2021) and the references therein. Phytolith auto-fluorescence is the emission of fluorescent light upon illumination with a particular wavelength (Devos et al. 2021). The recent identification of the link between fluorescence and fire opens new research avenues for the identification of fire in the phytolith record. Our first paper in this series (Devos et al. 2021) concerned fluorescent phytoliths in archaeological samples from northern European contexts. We surveyed the small amount of literature available, and only Matthews (1995) appears to have found fluorescent phytoliths in a context outside Europe, from hearth deposits at Abu Salabikh, Iraq. More recently, Vrydaghs et al. (2024) conducted experimental work on the inflorescence bracts of *Avena sativa* (oats) and showed that heating caused all phytolith morphotypes, both lumen and cell wall types, to fluoresce. So far, most work has been on grasses and cereals, but our unpublished observations suggest that the phytoliths

of other taxa also fluoresce when heated.

The present study will explore the potential of phytolith auto-fluorescence as an indicator of heat or fire in various tropical and subtropical areas. We selected some ecological and taphonomical contexts in one subtropical and two tropical regions for a comparative study. This study intends to address three questions: 1, do phytoliths from (sub)tropical plant taxa become fluorescent when heated by fire? 2, do extraction procedures and mounting media affect phytolith fluorescence? and 3, how do observation and counting methods affect the identification of phytolith fire signatures? This analytical effort will allow us to propose a methodology for studying phytolith auto-fluorescence in these geographical regions. It will also enable us to identify potential pitfalls in the study of auto-fluorescence, particularly variations in its intensity. This study is a first step to test the potential of phytolith auto-fluorescence as an accessible and reliable complementary method to other fire proxies in the archaeological and sedimentary record.

Study sites and sampling

Three geographical areas located in the tropical and subtropical areas of the northern hemisphere were selected for this study, the central Maya lowlands in the Petén region of northern Guatemala, the Bédik region of southern Senegal and the island of La Palma in the Canary Islands (Fig. 1). These regions cover a wide range of ecosystems and were inhabited by people who developed long-term recurrent interactions with their environments. The Petén region in Guatemala is covered by a dense, semi-humid rainforest, where the great Mayan cities developed between 1000 BCE and 900 CE (Sharer and Traxler 2005). The impact of ancient Mayan societies on their forest ecosystems and the associated transformation of landscapes are a key issue in current research into environmental change (Dunning et al. 2019; Castanet et al. 2022). The Bédik region of Senegal is covered by wooded savannas, where the Bédik people lived as large agrarian societies (Bocoum and Moriset 2012). The Canary Islands (Islas Canarias) have a diverse island vegetation that is rich in endemics, with five main ecological vegetation formations, coastal scrublands, thermophilous forests, laurel forests, Canary pine forests and highland shrub. The earliest permanent human colonisation by settlers arriving from North Africa probably took place around the

1st–3rd century CE (Morales et al. 2023; Santana et al. 2024).

A total of 19 samples were studied from these three areas (Table 1). Of these, ten were from modern topsoils, four from archaeological contexts and five from fossil sediments. They cover periods ranging from the present day to the Pleistocene. Thus, the diversity of ecological and temporal contexts studied should include a wide range of phytolith auto-fluorescence responses.

The eight samples from the site of Petén, Guatemala, are divided into five samples from present-day ecosystems (GM 1–5) and three sedimentary samples covering the 1st millennium BCE (GF1–3). Among the modern samples, three came from the cultivated areas of the Uaxactun village, where traditional slash-and-burn farming is still being done. GM1 corresponds to recent fallow land rich in palm trees, GM2 to a cultivated garden at the edge of a cleared plot of land and GM3 to a place which had been burnt a few days earlier (Supplementary Material (ESM) Fig. S1). The other two samples (GM4 and 5) represent modern ecosystems close to the Naachtun Maya archaeological site, not cultivated for at least 800 years (Testé et al. 2020). GM4 is from a palm forest at the foot of the slope of a karst depression, while GM5 was collected from a swampy area covered with water and herbaceous plants. The three archaeological samples range from the Middle Preclassic (GF3, 800–300 BC) to the Late Classic (GF1, AD 350–800). They were collected north of the Mayan archaeological site of Naachtun from a sediment section in a seasonal marsh (section CS1–S2c, sedimentary zones E1C, E2A and E2B in Castanet et al. 2022). Palaeoecological interpretations based on the phytoliths showed that this swamp area was used in the Mayan period (Testé 2020).

Five modern and two fossil samples from Senegal have been analysed. The modern samples were sampled in various different contexts, Sudan and Guinea woodland, Sudan woodland, open Sudan tree savanna, Sudan gallery forest (along rivers or wetlands in dry areas), *Oxytenanthera abyssinica* forest in the Bédik region in Sudan and Guinea (Fig. 1, ESM Fig. S1). This area is affected by seasonal fires caused by people. Two fossil samples from the river Falémé deposits in eastern Senegal were also analysed. They came from Holocene (SF1, 1.7 ± 0.1 ka) and Pleistocene deposits (SF2, 19,548–19,202 cal BP) (CP9, samples 16 and 22 in Davidoux et al. 2018).

Four archaeological samples were collected from La Palma, the northwesternmost

of the Canary Islands. These are sediment samples collected from Cueva de Belmaco, a rock shelter site showing pre-Hispanic indigenous occupation, which partially served as an animal pen for approximately 600 years from the 8th to the 14th century CE (Marrero Salas et al. 2016). Radiocarbon dates have been recalibrated using OxCal. v4.4 (Bronk Ramsey, 2021) following the IntCal20 curve (Reimer et al. 2020). During this period of use, periodic burning of dung residues was done to clean it and prepare it for reuse, as shown by micromorphological and biomarker data (Fernández-Palacios et al. 2023, 2024). This kind of burnt stable or animal pen deposit, also known as a *fumier* (Angelucci et al. 2009) consisted of a series of layers of unburnt (or only slightly heated) dung, alternating with black layers showing abundant charred plant material and charred dung, and white layers mainly made up of dung ash, according to the microstratigraphy (Fernández-Palacios et al. 2023). For the present study, samples CA1 and CA3 were collected from dung ash layers, while CA2 and CA4 were collected from black layers.

Methods and counting

Methods of analysis

The samples from Senegal and Guatemala were prepared according to Neumann et al. (2009) and Piperno (2006) and were treated with 30% hydrogen peroxide at 90 °C, followed by deflocculation of clay with sodium hexametaphosphate (20 to 40 g/L), then flotation with sodium polytungstate (specific gravity 2.3). The samples from the Canary Islands were treated using a relatively similar method, with hydrogen peroxide at 90 °C, nitric acid and hydrochloric acid, before flotation using sodium polytungstate (specific gravity 2.3). Heat treatment of the samples in hydrogen peroxide at 90 °C dissolves the organic matter covering the phytoliths, which is essential for observing their auto-fluorescence. As the work carried out by Vrydaghs et al. (2024) indicated that the first fluorescent phytoliths are only found if they have been heated to 200 °C or more, this part of the preparation process should not affect our results. Microscope slides were made using three different media, those from Guatemala and Senegal using microscope immersion oil (O). The samples from the Canary Islands were either mounted with Canada Balsam (B) or with Permout (P).

We studied the phytoliths using polarising microscope Zeiss Axioskope 5 equipped with a fluorescence kit, in plane polarized light (PPL), blue fluorescence (blue-fluo) (filter set: 450-490, FT510, LP 520) and ultraviolet (UV-fluo) (filter set: G365, FT395, LP420) at 500× magnification. The photomicrographs were taken with a Deltapix Invenio 6EIII camera incorporated into the microscope.

Counting

As the present research is an exploratory work, the counting was limited to a minimum of 50 diagnostic phytolith morphotypes for each of the 19 samples. (Fig. 2; ICPT 2019). Counting was organised according to three parameters that are possibly related to the heating or firing of the samples: 1, translucent phytoliths, 2, dark or coloured phytoliths and 3, auto-fluorescent phytoliths. Opal phytoliths are generally colourless in plane polarised light (PPL), which is what is meant by translucent. The colour is in the coating of all phytoliths resulting from the charring of the organic material surrounding them (Parr 2006), and possibly also by the burning of carbohydrates within cell wall phytoliths (Hodson 2019). It does not take into account inclusions that can sometimes be seen within lumen phytoliths (Vrydaghs and Devos 2020).

For the counts, each phytolith was assigned to its morphotype as described in ICPN 2.0 (ICPT 2019) and also to one of six categories coded as T-NF; T-MF; T-HF; C-NF; C-MF; C-HF. The first letter of a code, T or C, refers to the colouration of a morphotype observed in PPL (Fig. 2), T for translucent phytoliths and C for coloured or dark. The last two letters of a code refer to phytolith auto-fluorescence, which has been divided into three categories according to its intensity, NF for non-fluorescent, MF for moderately fluorescent and HF for highly fluorescent (Fig. 3). The intensity of auto-fluorescence can be defined as the ability of a phytolith to emit photons and therefore to glow. So far, we have not been able to establish a standard method for measuring fluorescence. Our division of fluorescence intensity into categories is therefore based on observable characteristics. The HF category corresponds to very bright morphotypes that create a luminous halo around them (Fig. 3). MF includes phytoliths whose auto-fluorescence is duller, or where only part of the phytolith fluoresces, and NF corresponds to phytoliths that do not fluoresce at all.

The quantitative approach integrates several kinds of data which are shown in

distribution diagrams (Figs. 4, 5, 6). For each sample, the percentages of non-fluorescent (NF) and fluorescent phytoliths (MF+HF) for each morphotype are given. The relative distributions of the six categories presented above are also given for all morphotypes. Finally, there are two overall indices which integrate all morphotypes for each sample. A fluorescence index (hereafter IF) was calculated using the following ratio: $IF = (\text{all MF} + \text{all HF}) / (\text{all phytoliths})$. A colour index (hereafter IC) shows the ratio between coloured and colourless phytoliths following the formula $IC = (\text{all C}) / (\text{all phytoliths})$. The purpose of these ratios is to allow a quantitative comparison between the two fire proxies. Finally, sponge spicules and/or diatom frustules were also noted but not included in the counts. These siliceous opal remains can also become auto-fluorescent (Wyche 2012) and thus provide additional information on soils, sediments or taphonomic contexts.

Results

Auto-fluorescent phytoliths were seen in all the studied samples (Table 1, ESM Table S1). They include BILOBATE, SADDLE, RONDEL, SPHEROID (ornate and echinate), CONICAL, ELONGATE, BLOCKY, BULLIFORM, ACICULAR (psilate and nodulate), HAIR BASE, and ARTICULATED POLYGONAL (Fig. 2). Besides demonstrating that a large range of phytoliths can become auto-fluorescent, it also establishes that the various extraction methods and mounting media allow us to study auto-fluorescence in them.

Samples from Guatemala

The modern and fossil soil samples showed significant variations in phytolith auto-fluorescence and colour. Overall, the IF ratio varied between 0.24 and 0.87. The three samples from the agricultural areas (GM1, 2 and 3) all had values above 0.5. The recently burnt soil surface (GM3) had the highest IF value (0.75) for modern samples. Old-growth forest samples GM4 and GM5 had lower values (respectively 0.24 and 0.36). In these samples, the IC was lower than 0.07, except for GM5, which had an IC value of 0.12 (Fig. 4). In the fossil samples, both IF and IC values were relatively high, with IF ranging from 0.73 (GF3) up to 0.87 (GF2) and IC values varying between 0.10 (GF3) to 0.19 (GF2).

Variations in auto-fluorescence have also been found at morphotype level, within

and between samples. Within the same sample, the percentage of auto-fluorescent phytoliths can vary greatly from one morphotype to another. The percentages of auto-fluorescent phytoliths for each morphotype can also differ between samples. For example, in GM2, the percentage of auto-fluorescent SPHEROID ORNATE phytoliths was only 19.2%, compared with 90.9% for SPHEROID ECHINATE. In GM3, the percentage of auto-fluorescent SPHEROID ORNATE phytoliths reached 78.5%, while the percentage of auto-fluorescent phytoliths in SPHEROID ECHINATE remained high at 76.4%. Each morphotype reached the level of 50% auto-fluorescent phytoliths in at least one sample. Sponge spicules and diatoms were mostly non-fluorescent, except for two opal remains identified in GM3 and GM5. We found that there were more highly fluorescent (HF) phytoliths present in fossil than in present day samples. HF phytoliths were most abundant in the SPHEROID ORNATE and GSSCP morphotypes.

Samples from Senegal

The samples from Senegal also showed variations in phytolith auto-fluorescence, albeit less distinctly than those from Guatemala (Fig. 5). In all modern samples, IF values ranged from 0.58 (SM5) to 0.83 (SM1). The two fossil samples showed greater variability, with the Holocene sample SF1 showing an IF ratio of 0.79, while the Pleistocene sample SF2 had a value of 0.49. The IC values were significantly higher compared to the samples from Guatemala. The modern samples with the minimum (IC = 0.30 for SM5) and maximum (IC = 0.56 for SM1) values correspond to the samples with the minimum and maximum IF values. For samples SM2, SM3, and SM4, the distribution of IC and IF values showed the opposite. Finally, the two fossil samples also showed a pronounced deviation in the distribution of IC values. Sample SF1 had an IC value of 0.65, while for the Pleistocene sample SF2 it was 0.24.

All the samples studied from Senegal had morphotypes of which at least 50% of the phytoliths were auto-fluorescent. Only morphotypes classified as 'other' in sample SF2 had only 33.8% auto-fluorescent phytoliths. Within all morphotypes, HF phytoliths were observed, albeit in varying proportions. In modern samples, grass silica short-cell phytoliths (GSSCP: BILOBATE, SADDLE and RONDEL) showed higher fluorescence compared to the other morphotypes. However, for the fossil samples, highly fluorescent ELONGATE and BULLIFORM types were recorded

in greater quantities (Fig. 5).

Samples from the Canary Islands

The samples from the Canaries showed much less variation in phytolith auto-fluorescence, whether they were mounted with Canada balsam or Permount (Fig. 6). The IF values were all above 0.80, reaching a maximum of 0.91 in sample CA2.B. Similarly, the distribution of coloured phytoliths indicated by the IC ratio showed very little variation and were particularly low, never exceeding 0.05 (CA1.P) and even reaching zero (CA3.P).

All morphotypes show cohorts dominated by auto-fluorescent phytoliths. Only few samples contained highly fluorescent (HF) phytoliths which were mainly SPHEROID and GSSCP morphotypes. There were a few HF phytoliths among the ACICULAR NODULATE and BULLIFORM morphotypes. HF phytoliths were mainly found in slides mounted with Canada balsam. Only two HF phytoliths were recorded in the CA2 sample which used Permount (CA2.P).

Discussion

Fluorescence in tropical and subtropical samples

Phytolith auto-fluorescence is a phenomenon common in both tropical and subtropical regions, as well as in northern Europe (Devos et al. 2021). Auto-fluorescent phytoliths occur in plants growing in a variety of modern ecosystems, tropical forests, wooded savannas and wetlands as well as in places affected by human activities such as cultivated fields, fallow land, ancient woodlands, wetlands, and alluvial sedimentary deposits. Fluorescent phytoliths have also been found in all types of sedimentary and archaeological samples covering the Holocene and the late Pleistocene. These repeated observations reinforce the idea that phytolith fluorescence is widespread.

We also established that all the following morphotypes can become fluorescent: SPHEROID (ORNATE and ECHINATE), grass silica short-cell phytoliths (BILOBATE, SADDLE and RONDEL), CONICAL, ELONGATE, BLOCKY, BULLIFORM, ACICULAR (PSILATE and NODULATE), HAIR BASE and articulated POLYGONAL. What is more, the morphotypes found in our samples generally included at least one auto-fluorescent phytolith (Fig. 2).

Importantly, we found that not all the phytoliths within a sample showed fluorescence. As recent experiments showed (Vrydaghs et al. 2024), this does not necessarily mean that the plant material they originated from had never been heated, which shows the importance of combining different strands of evidence with fluorescence, such as the colour index.

The occurrence of fluorescent phytoliths in soils and sediments that had clear evidence of burning or heating supports their use as a proxy for fire (Devos et al. 2021). The presence of numerous auto-fluorescent phytoliths in ancient material also supports the use of fluorescence analysis for studying past fire events and fire uses in the tropics and subtropics.

The presence of fluorescence in some samples and morphotypes raises questions. For instance, the discovery of auto-fluorescent phytoliths in contexts of old growth forests with no trace of fire for 800 years (Guatemala, GM4) or perennial wetlands (GM5) may have little to do with a response to burning. It is therefore necessary to consider the possibility that other physical or chemical factors could also affect the auto-fluorescence of phytoliths. However, the virtual absence of fluorescence in sponge spicules and diatoms from soils in Guatemala may suggest that this is not something that happened after deposition. These siliceous remains can fluoresce in the same temperature range as phytoliths (Wyche 2012). The post-depositional (taphonomic) factor that caused the auto-fluorescence of the phytoliths (such as fire, heating, etc.) should therefore have caused the diatoms and spicules to auto-fluoresce as well. At the very least, we can assume that the phytolith auto-fluorescence reaction comes from the phytoliths themselves rather than an outside agency.

The occurrence of fluorescent phytoliths in our contexts also contrasts with the uneven distribution of coloured ones. The IC ratio shows significant variations in the proportion of coloured phytoliths in the samples from Senegal and it is particularly low in samples from Guatemala and the Canary Islands. Despite the repeated use of the “coloured phytolith” method in recent years (Garnier et al. 2013; Aleman et al. 2014; Meng et al. 2022), it seems that this is not applicable in all contexts (Evetts and Cuthrell 2017). Furthermore, our results even indicate a potential underestimation of (past) fires in some cases, given that all samples show higher IF values than IC values. These observations reinforce the idea that fluorescence can be a universal proxy for heating or fire, but it is necessary to

calibrate it.

Quantification of auto-fluorescence and its relevance for indicating burning

The index of fluorescence of individual samples or morphotype groups can show variations that could be informative about the use of fire in the environment. For the modern Petén samples, the maximum IF value (0.75) corresponds to a burning which happened a week before sampling (GM3). The average IF values of between 0.54 and 0.59 in samples GM1 and GM2 correspond to fallow ground and gardens on land that had been cleared by slash-and-burn practices some 20 years ago. Finally, the lowest IF values were obtained from environments that had not been inhabited for at least 800 years (GM4 and GM5). Modern samples from Senegal also had IF values between 0.58 and 0.83 from environments regularly affected by fire. Without clear histories of the places studied in Senegal, it is impossible to establish a link between the fluorescence ratios and fire regimes. However, it appears that variations in the IF ratio are comparable to variations in the coloured phytolith ratios.

Quantifying auto-fluorescence at the morphotype level also provides information on vegetation burning. The samples from similar environments in Guatemala and Senegal had percentages of auto-fluorescent phytoliths of the same morphotype which were higher or lower from one sample to another. Take sample GM3, for example, which had large values of auto-fluorescent SPHEROID ORNATE, while samples GM1 and GM2 showed lower values for this morphotype (Fig. 4). All three samples were collected from forest ecosystems similarly disturbed by farming; GM1 and GM2 were still fallow land, while GM3 had just been burnt at the time of sampling. Therefore, by examining the percentages of fluorescent phytoliths in each morphotype, it may be possible to provide additional ecological information to the overall IF ratio.

Finally, quantifying phytolith auto-fluorescence in archaeological and fossil samples could provide information on the degree of human influence at a site, reflecting local fire use. Archaeological and fossil samples showed relatively high IF values always above 0.70, except for the SF2 sample which dates from the Pleistocene. Similarly, for Guatemala, IF values were slightly higher for GF1 and GF2 which correspond to the period of occupation of the site.

The archaeological samples from the Canary Islands showed high and similar IF values for the different dung layers at Cueva de Belmaco. Previous work carried out on these samples showed the presence of darkened spherulites in CA1 and CA3 dung ash layers (Fernández-Palacios et al. 2023), implying that temperatures may have not exceeded 600–700 °C (Canti and Nicosia 2018). The discovery of auto-fluorescent phytoliths in this same sample appears to agree with this observation, as prolonged heating above 700 °C can melt phytoliths (Gur-Arieh et al. 2014) and cause them to lose their fluorescence (Devos et al. 2021; Vrydaghs et al. 2024). Samples CA2 and CA4, on the other hand, are rich in charred matter and thus only partially burned according to the micromorphology, and still showed high IF values. Some fluorescent phytoliths occur when the heating reaches only 200 °C (Vrydaghs et al. 2024). This could be the case for CA2 and CA4, as black layers in *fumier* dung deposits can reach temperatures of 400-500 °C (Carrancho et al. 2016, 2024; Herrejón-Lagunilla et al. 2022).

The high proportions of fluorescent phytoliths in the archaeological samples from our three study sites could also reflect the role of taphonomy. Although post-depositional processes do not appear to have directly affected the fluorescence of phytoliths and biogenic silica, they may have influenced them in the soil (Madella and Lancelotti 2012). The micromorphological study of phytoliths and the quantitative counting of the prepared microscope slides should help to provide detailed interpretations (Devos and Vrydaghs 2023).

Concerning preparation and counting methods

Effects of physical and chemical preparation methods and mounting media

The various preparation methods did not affect the recovery of fluorescent phytoliths in our study. The same applies to the different mounting media used, immersion oil, Canada balsam or Permout, and fluorescent phytoliths were identified in large numbers in all these media.

However, the mounting medium could affect the observation of fluorescence. Permout, in particular, seems to react under UV light, making the whole slide fluoresce for the first few minutes of observation, which happened with all the samples from the Canary Islands that used it (Fig. 7). Fluorescent phytoliths were

finally observed and counted on these slides after only a few minutes of UV exposure, when this overall fluorescence had ceased. In blue light, the use of Permout posed no problems for studying fluorescence in phytoliths.

Limits of fluorescence intensity

In our counts we chose to divide the fluorescent phytoliths into two categories, highly fluorescent (HF) and moderately fluorescent (MF) (Fig. 3). However there are several reasons why this division may not be relevant. For example, the highest percentages of HF phytoliths were not found in the samples with the highest IF values. On the other hand, some morphotypes such as SPHEROID and GSSCP had more HF phytoliths than others. The intensity of auto-fluorescence may be connected with the process of mineralisation of these morphotypes (Vrydaghs et al. 2024) rather than with the taphonomic processes.

Therefore, fluorescence behaviour as a function of time of exposure to light and also the particular mounting medium convinces us not to consider fluorescence intensity as a useful character. We selected three samples mounted in different media (Canada balsam, Permout and immersion oil) and photographed, at regular intervals, the observation fields under blue and ultraviolet light (Fig. 7). The results confirm that the intensity of fluorescence differs according to the mounting medium, so it is necessary to adapt the way of recording fluorescence in phytoliths, because the fluorescence intensity depends upon the preparation and observation methods. Our analysis also shows that the time of exposure to light can affect the intensity of fluorescence intensity and thus the possibility of dividing phytoliths into groups according to their intensity.

Conclusions

Our new data presented here confirm that auto-fluorescent phytoliths occur widely in material from the tropics and subtropics. Auto-fluorescent phytoliths of various morphotypes have been found in both ancient and modern samples which had been exposed to heat or not, collected from diverse tropical and subtropical ecosystems. The various different methods used for extracting and studying phytoliths do not affect the recognition of auto-fluorescence under the microscope. The quantitative approach tested in this study, in the form of a fluorescence index (IF)—calculating the ratio of auto-fluorescent to non-auto-

fluorescent phytoliths—showed significant variations depending on the contexts studied. In particular, present day and archaeological samples with clear evidence of human firing from the three areas studied had the highest IF values. These data support the idea that heating is a significant factor in phytolith auto-fluorescence, although we do not discount other physical and chemical alterations that might have led to a similar result. However, our attempts to record the degree of fluorescence intensity do not appear to be conclusive. The distribution of strongly fluorescing phytoliths does not follow any particular pattern, and classification into broad categories of intensity is determined by the particular methods of preparation and observation. Finally, this exploratory work contributes to the development of a practical framework for the study of auto-fluorescence as a proxy for vegetation burning. Our encouraging results on a quantitative approach to phytolith auto-fluorescence convince us to pursue this methodological development, drawing in particular on recent studies on the onset of auto-fluorescence (Puppe et al. 2022; Vrydaghs et al. 2024). Future research could be based on experimental field data, for example from fire maps and comparisons with other proxies indicative of fire such as microcharcoal.

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Figure and Table legends

Fig. 1 Locations and biomes of the sites from which samples were obtained to study their phytolith auto-fluorescence

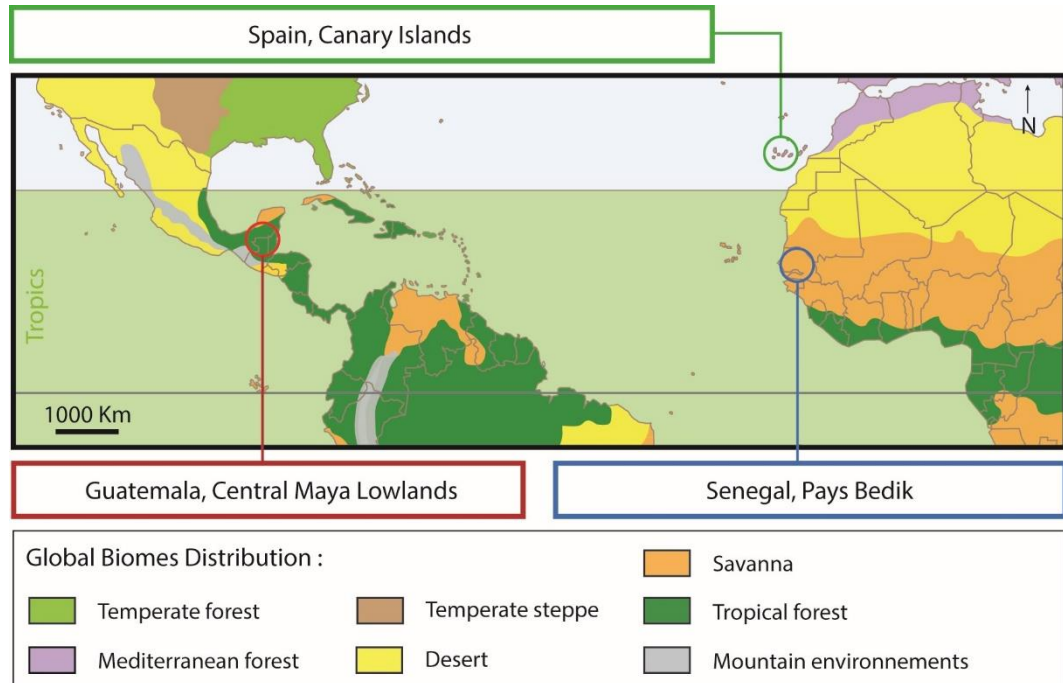


Fig. 2 Examples of translucent, coloured and auto-fluorescent phytolith morphotypes found in the samples

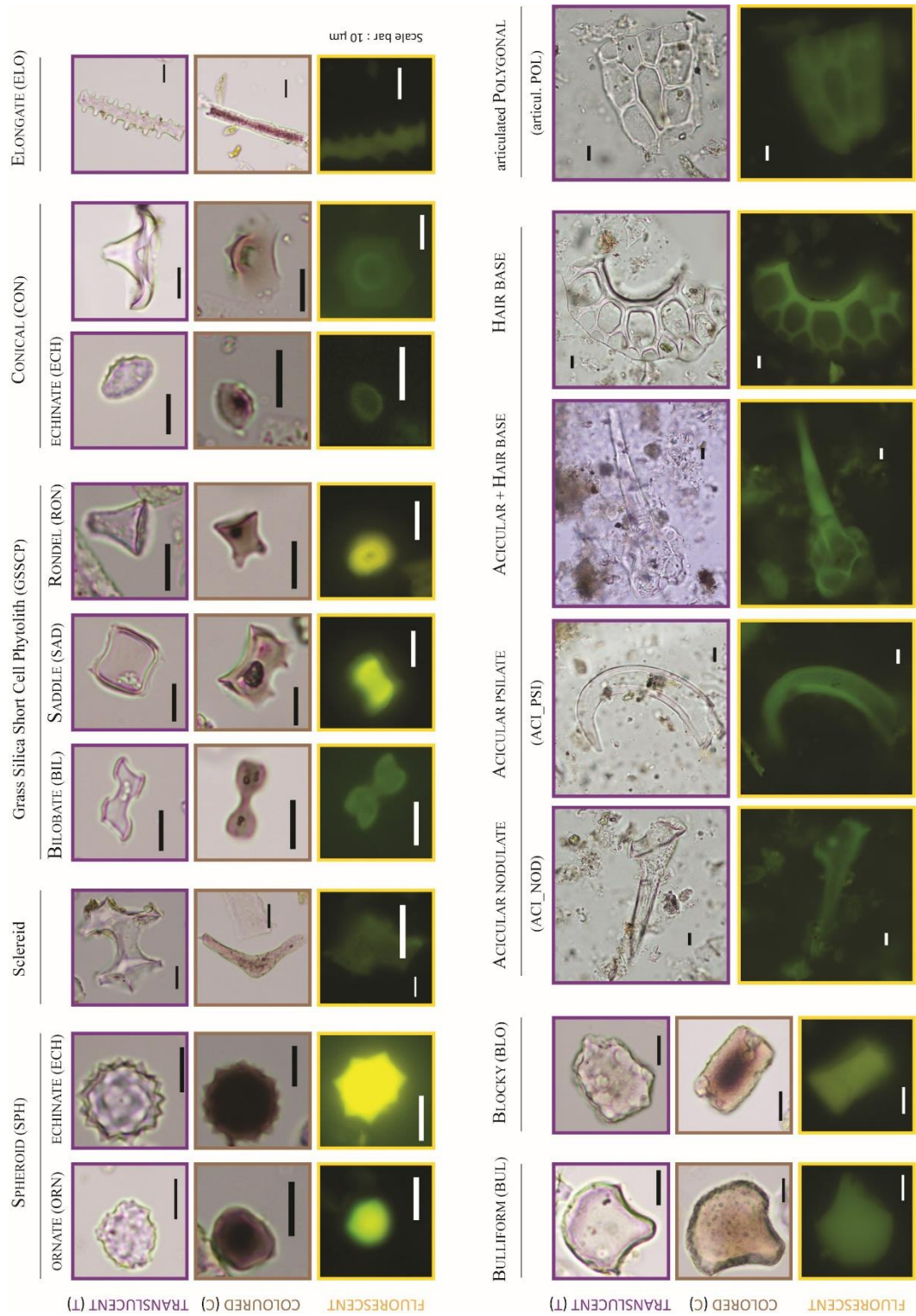


Fig. 3 SPHEROID ECHINATE phytoliths showing different fluorescence intensities in the study

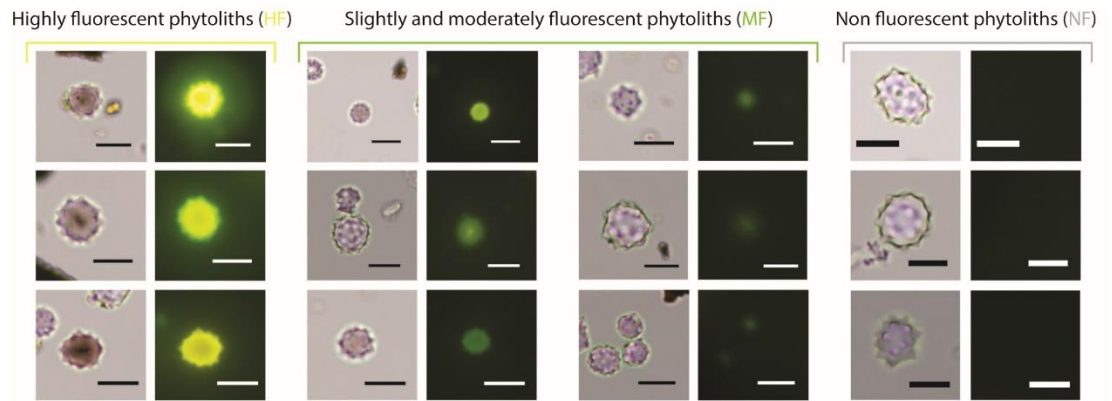


Fig. 4 Distribution diagram of fluorescent phytoliths in samples from Guatemala

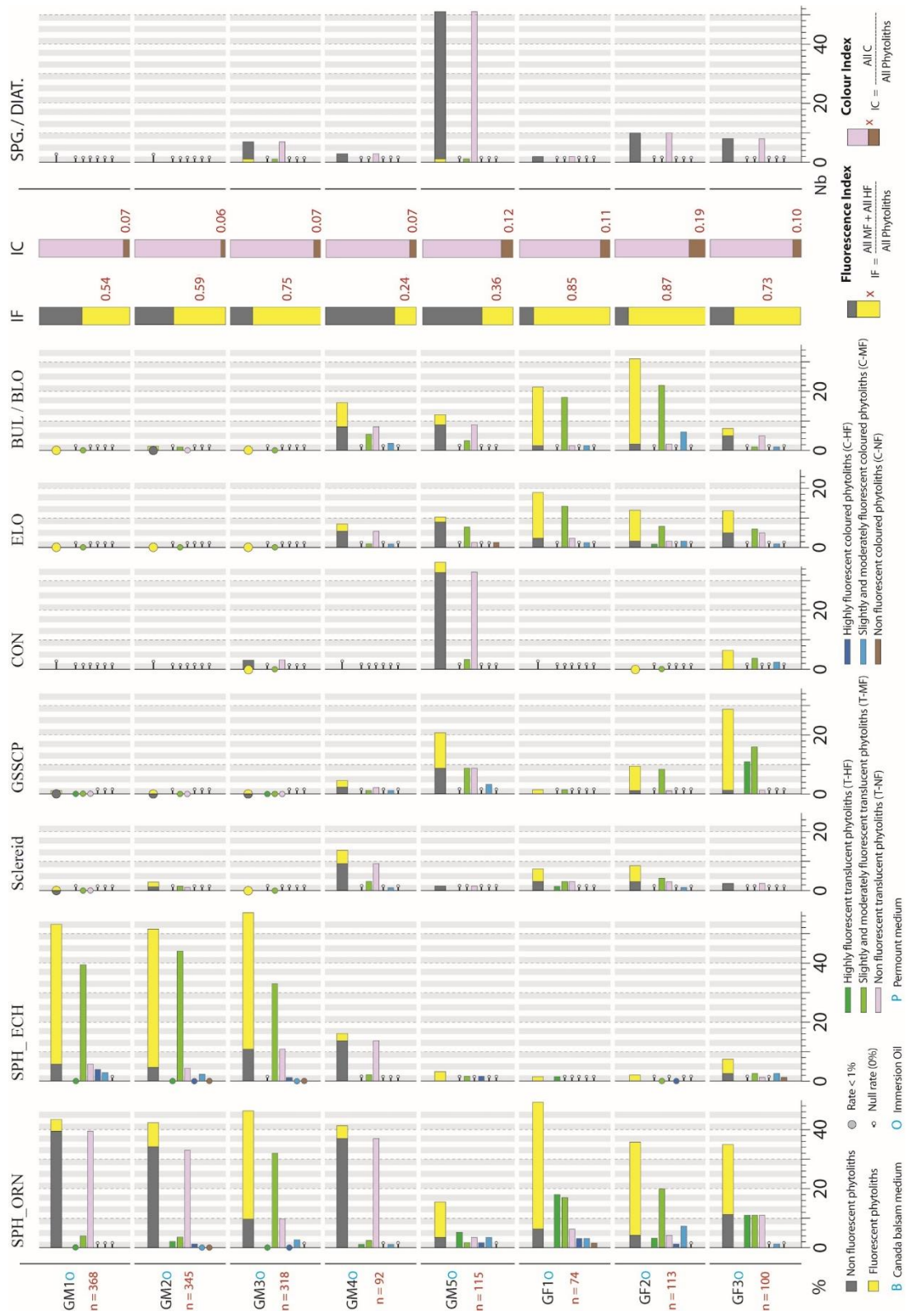


Fig. 5 Distribution diagram of fluorescent phytoliths in samples from Senegal

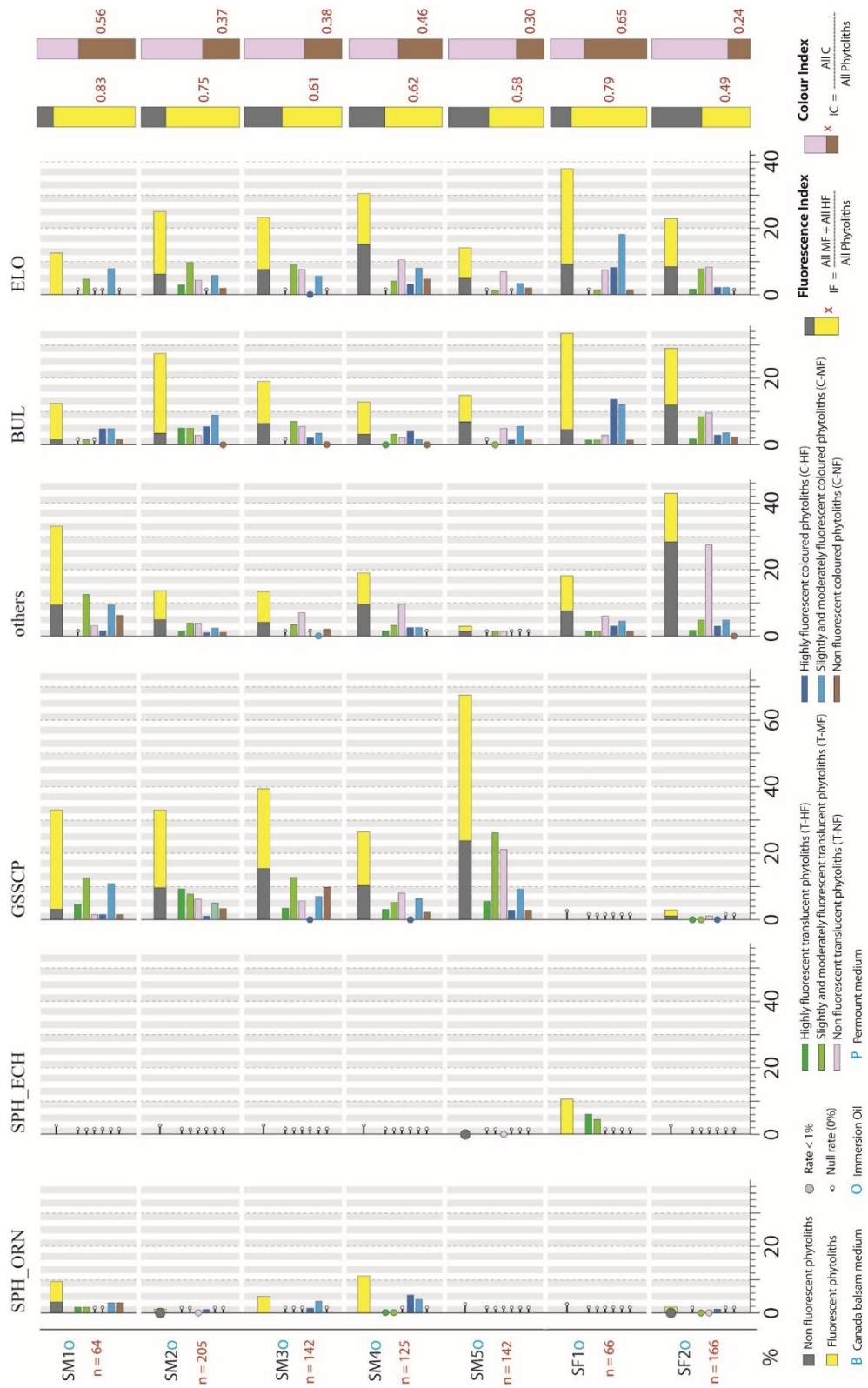


Fig. 6 Distribution diagram of fluorescent phytoliths in samples from the Canary Islands

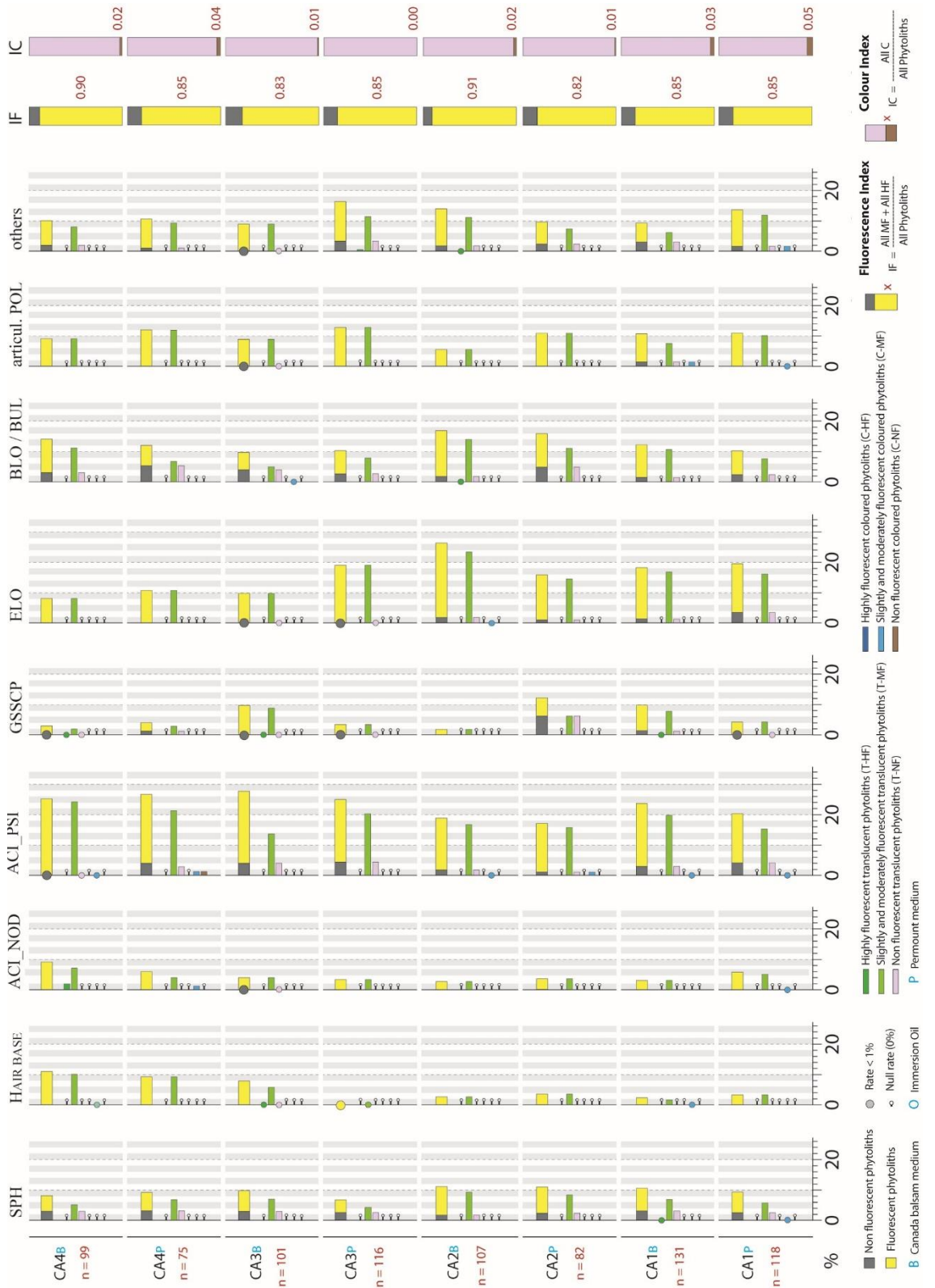


Fig. 7 Different intensities of fluorescence shown by phytoliths seen in the three different mounting media, UV and blue light, and for varying times

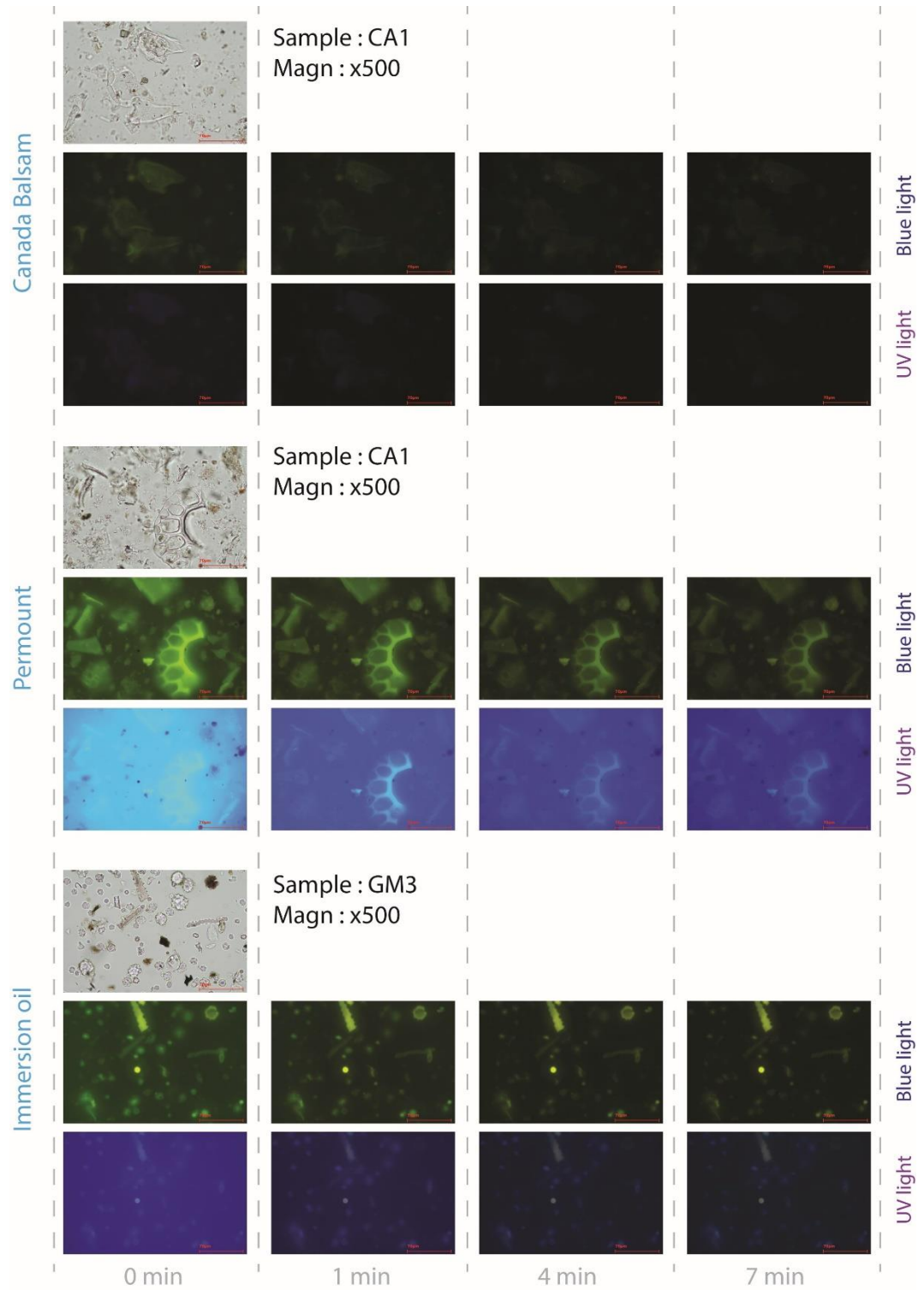


Table 1 List of samples studied according to geographical location, GM and GF, Guatemala; SM and SF, Senegal; CA, Canary Islands

Name	Type	Age	Environment/context	Last fire
GM1	Surface soil	Modern	Palms on fallow land	>20 years
GM2	Surface soil	Modern	Agroforestry – extensive garden	>15 years
GM3	Surface soil	Modern	Recent slash and burn clearing	<1 year
GM4	Surface soil	Modern	Natural low palm forest	>800 years
GM5	Surface soil	Modern	Natural herbaceous swamp	>800 years
GF1	Fossil sediment	350–800 ce	Previously swamp forest	N/A
GF2	Fossil sediment	100 bce–450 ce	Previously swamp forest	N/A
GF3	Fossil sediment	800–300 bce	Previously cultivated savanna	N/A
SM1	Surface soil	Modern	Sudan woodland	<15 years
SM2	Surface soil	Modern	Sudanian tree savanna	<10 years
SM3	Surface soil	Modern	Sudan gallery forest	N/A
SM4	Surface soil	Modern	Sudan-Guinea forest	N/A
SM5	Surface soil	Modern	<i>Oxytenanthera abyssinica</i> forest	< 10 years
SF1	Fossil sediment	1.7±0.1 ka	N/A	N/A
SF2	Fossil sediment	19,548–19,202 cal bp	N/A	N/A
CA1-CA4	Archaeological	1,179–555 cal bp	Rock shelter, pre-Hispanic	>600 years *

*This time period represents the latest use by indigenous herders; it is unknown if the deposit was burned after the European conquest of the island or reused after the indigenous occupation ended.

Conflicts of interest

The authors declare no conflict of interest.

Author contributions

MT, EFP, AG and LV designed the study and managed the protocols. MT, EFP, AG and ACB performed laboratory analyses and acquired phytolith data. MT, EFP, AG, LV analysed the data. MT designed the figures. ACB, MJH, YD provided external advice throughout the study. MT, EFP, AG, ACB, MJH, YD and LV wrote the article.