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Not all flowers' visitors are pollinators: Combining indicators to identify their role in the cocoa pollination service

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ABSTRACT

Animal pollination is critical for wild plant reproduction and crop yield. However, not all flower visitors are necessarily pollinators. Using cocoa (*Theobroma cacao* L.) as a case study, we explored a combination of indicators to assess the effectiveness of flower visitors as pollinators. We first investigated the potential trade-off between the quality and quantity of pollen deposited on the limitation of pollination success in a hand pollination trial under controlled conditions. We found that the fruit set and ovule fecundation rate are mainly driven by the quantity of pollen grain deposited. We then conducted transects and video monitoring in the field in Ecuador to compare the performance of cocoa flower visitors as pollinators using several indicators. We found that small crawling arthropods, in particular ants and aphids, proved to be promising pollinators, with a higher level of pollination with viable pollen being observed on the flowers they visited. Ants were even more efficient than other visitors in depositing significant numbers of pollen grains on the styles. Instead, stingless bees and microdiptera were less efficient when depositing pollen, but complementary to small crawling arthropods in the time of flower visits. Interestingly, we found that others cocoa flower visitors were detrimental to the pollination service, such as hemipteran nymphs and caterpillars. We conclude that cocoa plantation management focused on increasing the complementarity of pollinator visits, acting at different periods of the day and with distinct behaviours, could improve pollination services, and that the use of multiple indicators should be advocated in future research to assess pollinator effectiveness.

1. Introduction

Animal pollination is critical for wild plant reproduction and crop yield (Potts et al., 2016). However, not all flowers' visitors are necessarily pollinators (King et al., 2013). Some insects can instead be harmful to a plant's reproduction by feeding on the flower or by collecting nectar and pollen without depositing pollen (Saunders et al., 2016). Even among pollinating insects, depending on the morphotype, the quantity or quality of pollen deposited is sometimes insufficient in order to ensure fruit set and development (Ne'eman et al., 2010). Identifying the insect group or community of insects that are the most efficient pollinators for the yield of different crops is currently a major challenge (Földesi et al., 2021; Page et al., 2021), since animal pollination, and in particular that provided by insects, benefits a large majority of cultivated plants and for some crops is even essential (Klein et al., 2007). This is particularly the case for cocoa (*Theobroma cacao* L.), a tropical perennial crop native to the Amazon region in South America (Motamayor et al., 2002). Cocoa trees produce a very large number of

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flowers, ranging from several thousand to up to 125,000 flowers per tree per year (Lachenaud and Mossu, 1995), although paradoxically yielding very few fruits, amounting to less than 80 fruits per tree (McKelvie, 1960; Vanhove et al., 2020). Overall, fruit set does not exceed 10 % in natural cocoa pollination (Groeneveld et al., 2010; Toledo-Hernández et al., 2020; Vansynghel et al., 2022; Young, 1981). The most critical stage driving this low rate seems to be pollination success (Groeneveld et al., 2010; Toledo-Hernández et al., 2017) although significant losses are also caused during the fruit development stage by the cherelle wilt process in cocoa and by fruit pests and diseases, that are themselves affected by climate and agricultural practices (Abdulai et al., 2020; Bos et al., 2007; Melnick, 2016; Toledo-Hernández et al., 2017). Indeed, partial or total hand pollination trials on cocoa trees have observed increases in yield of up to 200 % (Forbes et al., 2019; Groeneveld et al., 2010; Toledo-Hernández et al., 2020; Vansynghel et al., 2022). In different cocoa producing regions, style collections revealed rates of unpollinated flowers ranging from 51 % to 76 % (Lucas, 1981; Parvais et al., 1977; Tovar and Ortíz, 1991). These high rates are due to drivers both internal and external to the plant.

Internal drivers promoting pollination success include the fact that the cocoa flower is hermaphrodite (Glendinning, 1972), produces up to 14,000 pollen grains (Massaux et al., 1976) and its entire style is receptive (Posnette, 1940). However, many internal factors can also limit pollination success. For instance, the sticky nature of cocoa pollen makes wind pollination very unlikely (Claus et al., 2018; Falque et al., 1995; Glendinning, 1972) and classifies cocoa as one of the crops most dependent on animal-mediated pollination (Klein et al., 2007). Furthermore, the morphology of the flower, which has stamens inserted into the petals and a style surrounded by five staminodes, induces a high level of pollinator specialisation (Arnold et al., 2019). Furthermore, the short flower life span, on average only two days (McKelvie, 1958), and its low amount or absence of nectar (Arnold et al., 2019; Kaufmann, 1973) further reduce the probability of being visited by a pollinator. Lastly, fruit set and seed set also depend on the quantity and the quality of the pollen deposited, two criteria in cocoa that have traditionally been studied separately.

The quantity of pollen grains deposited to ensure fruit and seed set is the most frequently documented relationship in the literature. Cope (1962) has shown that the number of fertilised ovules is limited by the number of pollen grains deposited (Glendinning, 1972) because of no evidence of apomixis (i.e. unfertilised ovules developing into seeds). It has long been acknowledged that flower retention requires a minimum threshold of 35–40 pollen grains deposited on the style (De Reffye et al., 1978; Kaufmann, 1975a; Parvais et al., 1977; Tovar and Ortíz, 1991), with ovary saturation (i.e. fecundation of all ovules and seed set) requiring around 50 pollen grains (De Reffye et al., 1978). However, these statements were actually based on the number of ovules per ovary, a total of 40–65 ovules (Falque et al., 1995; Lachenaud, 1991). Falque et al. (1996), (1995) demonstrated that these thresholds depend on the cocoa genotype; they observed fruit development after only 22 pollen grains deposited on the style; and ovary saturation was achieved with a minimum of 3.8 pollen grains per ovule. Unfortunately, the quantity of pollen grains deposited is not a guarantee of pollination success, as quality also plays a critical role (Stavert et al., 2020). The notion of quality of pollen deposit has emerged as a driver of pollination deficit in cacao production due to the early evidence of a high level of self-incompatibility in cocoa crops (Cope, 1962; Glendinning, 1972). In cocoa, this process is based on fusion failure between male and female gametes in some ovules, resulting in ovary abortion (Cope, 1962; Ford and Wilkinson, 2012). To limit these non-fertile pollinations, cocoa plantation farmers can cultivate certain self-compatible cultivars, such as CCN51 (Branco et al., 2018), which is common in Ecuador, or require varietal arrangements (López et al., 2021). For hand pollination, the choice of pollen donor tree avoids this issue of incompatibility, meaning the rates observed by Falque et al. (1996), (1995) depended only on pollen viability, i.e. its ability to germinate and fertilise an ovule. In cocoa, this aspect of effective pollen was mentioned early on by De Reffye et al. (1978), but remains little studied. The few studies dealing with this aspect have considered pollen longevity, i.e. survival time, or viability, i.e. the germination rate just after dehiscence of the pollen lodges. Beyond cocoa, Aizen and Harder (2007) indicated that, from their perspective, studies of pollen limitation should pay more attention to this notion of pollen quality.

Among the external drivers of cocoa pollination success, the effectiveness of flower visitors as pollinators appears to be the main driver, based on the high rates of non-pollinated flowers. Indeed, only 12–25 % of the cocoa flowers were visited by insects according to several studies that used glue traps in Bolivia, Peru and Indonesia (Chumacero De Schawe et al., 2016; Toledo-Hernández et al., 2021; Vansynghel et al., 2022; Zegada Herbas et al., 2020). For decades, microdiptera, and in particular midges of the families Ceratopogonidae and Cecidomyiidae, have been considered as the main pollinators of cocoa and studied extensively in the different cocoa producing regions (Bigger, 2012; Toledo-Hernández et al., 2017). It is evident that pollen transfer has been observed in the case of these microdiptera (Cilas et al., 1987; De Reffye et al., 1980; Kaufmann, 1973, 1975a; O'Doherty and Zoll, 2012). However, their greater effectiveness as pollinators compared to other cocoa flower visiting arthropods has not yet been established. Indeed, different indicators of effectiveness should be considered, including flower visitation frequency, quantity and quality of deposited pollen grains (Ne'eman et al., 2010). Regarding the frequency of visits, the most common cocoa flower visitors are actually aphids, ants and thrips whether in the Americas, where the crop originated, or in Africa and Asia (Chumacero De Schawe et al., 2016; Cilas et al., 1987; De Reffye et al., 1980; Jaramillo et al., 2024; Toledo-Hernández et al., 2021; Vansynghel et al., 2022; Winder, 1978; Zegada Herbas et al., 2020). Monitoring techniques are often limited to assessing the potential complementarity of arthropods' visiting periods (Knop et al., 2018), and only two studies have explored the eventuality of nocturnal pollination of cocoa by performing night sampling of cocoa flower visitors (Frimpong et al., 2009; Jaramillo et al., 2024). Regarding the quantity of pollen grains, some studies have focused on the body pollen load of several cocoa flower visitors (De Reffye et al., 1980; Jaramillo et al., 2024) or investigated the relationship between arthropod abundance and the scarcity of pollen deposited in cocoa plantations (Cilas et al., 1987; Vansynghel et al., 2022). However, cocoa flower visitor performance in terms of pollen deposit has been little studied, with only one trial collecting pollen from the style of flowers visited by diptera and hymenoptera (Adjaloo and Oduro, 2013). Lastly, the indicator of quality of pollen grains deposited by flower visitors has not been studied in cocoa, probably because of the very low natural fruit set (Toledo-Hernández et al., 2020; Vansynghel et al., 2022; Young, 1981), which greatly complicates the assessment of different yield components, as has been achieved in the case of other plants (Ne'eman et al., 2010). Thus, the identity of the most efficient pollinator(s) remains unclear in cocoa (Jaramillo et al., 2024; Toledo-Hernández et al., 2020; Vansynghel et al., 2022).

This study aims to identify the most efficient pollinator(s) among flower visitors, using cocoa as a case study. With this objective in mind, we conducted several trials to assess pollination performance by combining the monitoring of cocoa flower visitors and a count of pollen grains deposited. First, we performed a hand pollination experiment in controlled conditions (i.e. a greenhouse) by varying not only the quantity of pollen grains deposited but also the quality, using an indicator related to the longevity of this pollen, i.e. the time between the flower's harvest and the use of the pollen. This experiment aimed to test a hypothetical trade-off between pollen quality and quantity on the limitation of pollination success as a possible generic assessment for pollination performance in cocoa (Aizen and Harder, 2007; Ne'eman et al., 2010). We then assessed pollination success using two indicators, namely the fruit set and the rate of fecundated ovules during the formation of young fruits. We put forward the hypothesis that if quantity

prevails then the most efficient pollinators would be those depositing a lot of pollen grains or visiting more often. If quality is more important, then pollinators carrying pollen earlier in the day to deposit fresh pollen or pollen from longer distances to avoid self-fertilisation become the most efficient pollinators. The second and the third trials were carried out during the peak flowering period in Amazonian cocoa plantations in Ecuador, a region where cocoa is native (Lanaud et al., 2012). We performed diurnal transects and pollen grain counting to record the diversity of flower visitors and to compare the pollen received by clusters of flowers that have been visited at least once by these visitors. Moreover, we installed video monitoring on previously bagged flowers to record day and night flower visitors and their behaviour. We then aimed to compare the effectiveness of these visitors as pollinators using indicators of visitation frequency, quantity and quality of pollen deposited.

Identifying the most efficient pollinator(s) among flower visitors will help in proposing management recommendations to optimise pollination service and crop yield. Moreover, identifying flower visitors that do not pollinate and that could instead be predators of pollinators or flower pests will facilitate the proposal of suitably adapted management recommendations with regard to the integrated pest and pollinator management framework (Biddinger and Rajotte, 2015; Merle et al., 2022).

2. Material and methods

2.1. Assessing the potential trade-off in quantity-quality pollen deposition

The first trial was conducted under controlled conditions in the CIRAD tropical greenhouse in Montpellier, France during the cocoa flowering period in June 2021. Hand pollinations were performed on a total of 158 flowers from four cocoa trees of the self-incompatible Upper-Amazonian Scavina 6 (SCA 6) clone known to have a high flowering intensity, using flowers from three cocoa trees derived from the ICS1 clone as pollen donors, a self-compatible Trinitario clone selected in Trinidad (Falque et al., 1996; Lanaud et al., 2017). We first removed young or mature fruit to prevent competition. To avoid potential pollination by ants, the trees had no branch contact and resin was spread at the base of the trunks. Throughout the experiment, the average temperature and relative humidity were recorded using a Hobo U23 Pro v2 sensor (HOBO, Onset Corp) given their possible impact on pollen germination (Aneja et al., 1992). The data were stored every 10 minutes.

The first day of the experiment was dedicated to the removal of open flowers, on both trees selected as pollen donors and pollen receivers, in order to carry out three consecutive days of hand pollination from flower buds opened the same day. At the beginning of each day of hand pollination, the flowers of the pollen donor trees were harvested and their anthers used to perform two pollination series per hour from 8:20 am to 4 pm, except on the third day when only one series per hour was performed due to the lower number of open flowers. The time elapsed between harvest of the donor flower, and the use of the flower for pollen deposition was used as an estimate of the age of the pollen, given that anthers burst shortly after flower opening at dawn and that storage conditions, which could affect pollen viability, changed little in the greenhouse (Aneja et al., 1992). Each hand pollination series consisted of four hand pollinations using four different techniques to deposit different amounts of pollen grains (Table S1). Hand pollination was performed by removing two staminodes from the receptor flower to access the style and (i) by lightly touching the style of the receptor flower with an anther from the donor tree, or (ii) by brushing the style with one anther, (iii) with two anthers, or (iv) with three anthers successively (Falque et al., 1996, 1995; Paulin, 1981). Each flower was then tagged with a unique code to monitor pollination success.

In order to estimate the amount of pollen deposited more accurately, the styles of pollinated flowers were collected 24 hours after pollination, which is the time necessary for the pollen tubes to reach the embryo sacs and for the double fertilisation to be completed (Aneja et al., 1992; Bouharmont, 1960). A total of 152 styles were collected 24 hours after hand pollination (excluding fallen flowers, n = 6). Since ovule fecundation takes place within 24 hours, this action did not affect further fruit development and the number of seeds per fruit. We therefore monitored fruit set until 7 days after hand pollination and harvested the 137 remaining fruits in 70 % ethanol to avoid their degradation before dissection. At this development stage, the large and white fecundated ovules could be differentiated from the small and yellow non-fecundated ovules (Lanaud et al., 2017). Fruit dissection for ovules counting was performed following the protocol outlined in Lanaud et al. (2017). The fruits were boiled in water for 20 minutes to soften the fruit husks and facilitate dissection. Using a scalpel and a fine needle, the ovules were removed from each fruit and the fecundated and non-fecundated ovules were counted.

2.2. Field study area in Ecuador and experimental design

The field trial was conducted during the peak flowering period of cocoa, from June to July 2022, in the Amazonian Experimental Station of the INIAP (Instituto Nacional de Investigaciones Agropecuarias), in the province of Orellana in Ecuador (0°20'26.069''S, 76°52'27.472''W). We selected seven plots of cocoa cultivated in agroforestry systems within the experimental station of the *Nacional* cocoa variety. We performed a total of 91 transects during the peak cocoa flowering period (from June to July 2022). Due to the different flowering intensities of the trees, ranging from a few flowers to several hundred, the transects were conducted for a fixed time of 15 minutes rather than a fixed distance (O'Connor et al., 2019). The transects were conducted throughout the flowering season with an average of two to seven transects per day, and at different times of the day (between 8 am and 6 pm) avoiding rainy events and night. An average of 13.0 ± 6.5 transects were performed per plot and, for each plot, they were distributed over each one-hour window between 8 am and 6 pm. The location in the plot to conduct each transect was selected in order to prevent observing trees already studied during a previous transect and to maximise the number of flowering trees and the number of open flowers per tree.

2.3. Visual observations of flower visitor performances

We estimated the number of trees surveyed during the transect and the average number of open flowers per tree. Transects were conducted to a height of 2 m. The pruning of cocoa trees and the particularity of cocoa flowers to develop on the trunk and older branches allowed us to exclude almost no flowers from our observations. The observer walked at a slow pace from tree to tree to carefully inspect the floral reproductive organs of each flower, and the note-taker reported for each flower with a visitor the tree number, flower height, staminode organisation (i.e. converging, parallel, or splayed, Frimpong-Anin et al., 2014) (Fig. S1), the number of visitors present, their order and family if recognisable, and finally the floral organ on which it was located. Only flower visitors observed on or very close to reproductive organs (i.e. the style, staminodes, or petals containing anthers) were considered. Each visit was photographed by the note-taker to enable the identification of visitors to be refined and to record the time of their visit. The observed arthropods were classified in 17 different morphotypes (Fig. 1), mainly according to the order of the arthropod, or even its family in the case of the most abundant and easily identifiable. The visited flowers were also marked with small flags labelled with a unique code mounted on entomological pins (Fig. S2) in order to collect the styles at the end of the transect for pollen counting. During the diurnal transects, the morphotypes were observed visiting a total of 711 flowers. Of these flowers, 700 styles were collected (excluding dropped flowers, n = 3, and flowers eaten by caterpillar visitors, n = 8). The average values of air temperature, relative humidity and wind speed during the transects were recorded using a weather station (Anemometer Thermometer

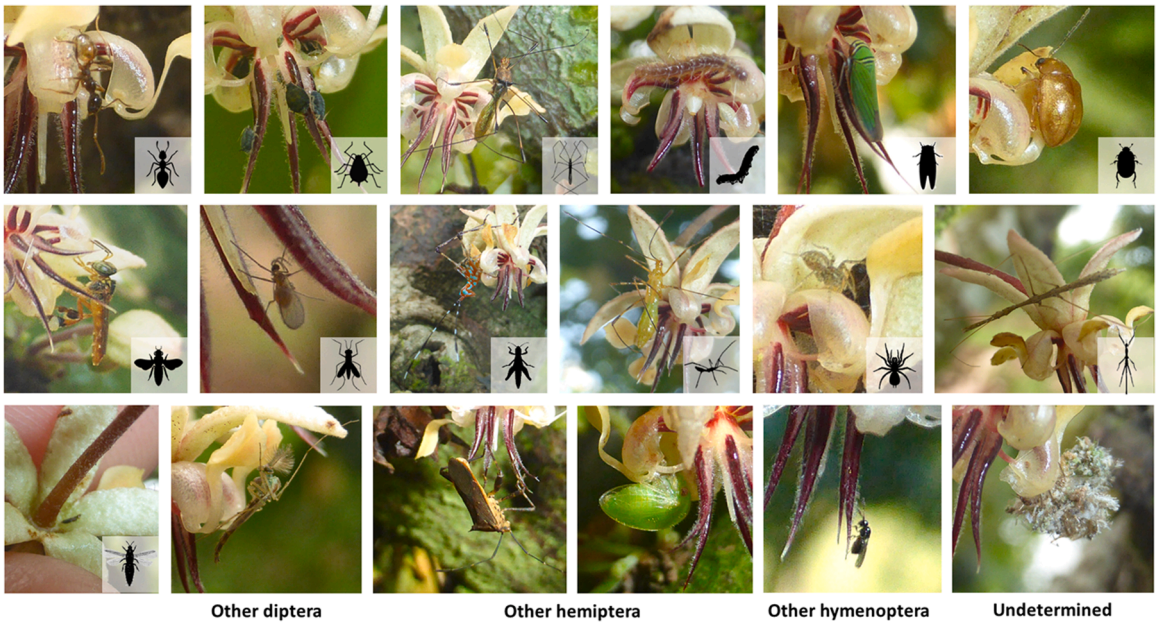

Fig. 1. Main cocoa flower visitors observed in the Ecuadorian Amazon region (icons from www.flaticon.com and www.vecteezy.com). From left to right and from top to bottom: ants, aphids, berytids, caterpillars, cicadellids, coleoptera, stingless bees, microdiptera (mainly Cecidomyiidae), orthoptera (Tettigoniidae), Hemipteran nymphs (probably from Berytidae or Reduviidae family), spiders, stick insects, thrips, other Diptera, other Hemiptera, other Hymenoptera and undetermined arthropods.

Hygrometer Atmos, Air et Aventure) placed in the plot on a telescopic base at a height of 1.3 m.

2.4. Video monitoring of diurnal and nocturnal flower visitors

Three of the seven cocoa plots were equipped with cameras. To monitor freshly hatched and unvisited flowers, fine-mesh exclusion bags (Alt'Droso Maraichage, 0.8 × 0.8 mm mesh) were placed on clusters of flower buds at the end of the first day between 5:00 pm and 6:00 pm, as cocoa flower buds hatch during the night. These bud clusters were selected in a way to maximise the number of buds ready to hatch and any open flowers and arthropods present were removed. On the second day, between 7 am and 8 am, one PICT camera (Droissart et al., 2021) was installed on each of the two clusters of covered buds with the highest number of freshly hatched flowers. Each camera was installed at 40 cm from the flower cluster (fixed focal length) and horizontally, at the height of the flower cluster still covered so as to be able to film the flower's key organs (i.e. petals, staminodes and styles), which are oriented downwards, while avoiding glare from the sun or the accumulation of raindrops on the sealed box in front of the camera lens. Once the device was powered, the recording started and the plastic mesh bag could be removed. In order to observe flower visitors during the day and at night with the help of an infrared light, the camera was removed between 5 pm and 6 pm on the second day after collecting the style of the flowers still present in the cluster for the pollen count. In order to associate the visits received by each flower with the quantity of pollen grains counted on the style, a unique code was given to each flower and this code was reported on the slide of the collected style. The choice of a monitoring period of approximatively 32 hours allowed the monitoring of two cohorts of flowers per recording with the flowers opening during the night in the same cluster, and also prevented the exclusion of the period from flower emergence to the time of camera installation. However, by using a total of four cameras to carry out this monitoring over two consecutive days, a maximum of two cameras could be installed each day, as the two cameras installed on the second day could not be installed on another batch on the third day. We then used a motion detection program to provide a history of arthropod visits for each flower (Droissart et al., 2021).

When analysing the day and night data provided by the cameras, the small size of the cocoa flowers and their clustering on the trunks had the advantage of being able to record several flowers at a time, but did not permit the optimisation of the video analysis time using the motion detection program. Indeed, an excessive number of animal movements in the trees, arthropods on the trunks and movements of leaves due to wind were detected. However, this program should have helped to reduce the size of the videos to only the detection sequences. In addition, the very small size of some flower visitors required an increase in detection sensitivity and thus did not result in a significant reduction in video length. Finally, when comparing the flower visitation history obtained from the videos from the detection to that obtained by watching the full videos in time-lapse, detection was superior in the second case and provided a better estimate of the duration of each visit for visitors with low mobility in the flower. As a result, the work of determining the history of flower visits was carried out on two videos for each of the three plots among the total of 38 videos. These six videos recorded the visits received by 62 flowers and were selected according to criteria of quantity of flowers present during the video monitoring and quantity of pollen counted on the 34 collected styles (excluding fallen flowers, n = 28) to maximise the chances of observing visits.

2.5. Quantity and viability of pollen deposited

The collected styles of the flowers hand-pollinated in the greenhouse (n = 152), the flowers visited during the transects (n = 700), and the flowers monitored by camera (n = 34) were placed directly on a slide in a drop of modified Alexander dye (Alexander, 1969) and the unique code of the corresponding flower was reported in order to associate each style with a precise flower. This dye helped to differentiate aborted pollen from non-aborted pollen by staining and thus to estimate the viability of the deposited pollen. The pollen grains were then counted under an optical microscope at × 400 scale and using a hand tally counter (Fig. S3).

2.6. Statistical analysis

All analyses were conducted using R software, version 4.4.1 (R Core

Team, 2024). In the following paragraphs, we describe the analyses carried out and the packages used for each of the datasets from the three trials, i.e. hand pollination, diurnal transects and video-monitoring.

2.6.1. Assessing the potential trade-off in quantity-quality pollen deposition

We studied the effect of pollen age, pollen quantity, temperature and relative humidity during hand pollination on fruit set and on the ovule fecundation rate on the 7th day, i.e. percentage of fecundated ovules. For the fruit set and the ovule fecundation rate we used generalised linear models with a binomial distribution of the *lme4* package version 1.1–35.4 (Bates et al., 2014).

2.6.2. Visual observations of flower visitor performances

2.6.2.1. Diurnal visitation frequency and floral reproductive organ visited. We first compared morphotypes by examining their visitation frequency, i.e. the probability of being visited (Ne'eman et al., 2010). This indicator was calculated per transect using the average number of flowers observed and the number of arthropods present on the petals, staminodes or styles of these observed flowers. The average number of flowers observed for each transect was estimated using the number of trees surveyed during the transect and their average number of open flowers per tree. The visitation frequency was expressed as a count per 1000 flowers observed and was fitted to a negative binomial distribution using a generalised linear mixed model of the *lme4* package version 1.1–35.4 (Bates et al., 2014) with the plot and the day of the transect as random factors. The average values of air temperature, relative humidity and wind speed recorded during transects were also considered as independent variables in this first model. The differences in visitation frequency between morphotypes were then compared using a Tuckey post-hoc test from the *lsmeans* package version 2.30–0 (Lenth, 2016). We then compared the behaviour of the main cocoa flower visitors in terms of floral reproductive organ visited using a contingency table and the chi-square test.

2.6.2.2. Quantity and viability of pollen deposited. Given the low probability of visit of cocoa flowers, we used the diurnal transect sampling method that does not allow us to compare single visits but rather batches of flowers that have received at least one visit by the same morphotype. Assuming that flower visits are rare, this technique provides an estimate of the observed visitor's pollen deposition and, by using an active visitor tracking technique rather than a passive one, allows us to study a greater diversity. However, in order to take into account the probability that a flower has been visited previously, we have included the time of observation in the model of pollen grain abundance that we will describe in this section. A first selection of the most promising morphotype was carried out with regard to the pollen deposition performances of the observed morphotypes using a non-metric multidimensional scaling (NMDS) representation from the *vegan* package version 2.6–6.1 (Oksanen et al., 2022). Four performance levels were created: no pollen grains deposited, pollination but no viable pollen grain, less than 22 viable pollen grains and more than 22 viable pollen grains (Falque et al., 1995). Since some arthropod morphotypes displayed disparities in size, we then grouped the arthropods according to their size and their ability to fly (Table S2), as these are important criteria for pollination. To assess the potential relationship between these particular morphological characteristics of flower visitors and their performance as pollinators, we studied the dependency between these two factors using a contingency table and the chi-square test. To compare the most abundant morphotypes according to the pollen grain quantities collected on the style of the flower they visited, we used the decimal logarithm transformation of the pollen grain count that we fitted to a negative binomial distribution using a generalised linear model. In this model, we also tested the effect of the observation time (as an indicator of previous visitor pollen deposit), the number of individuals present in each flower for each of the selected arthropod morphotypes, the average number of flowers observed, the staminode opening angle (Frimpong-Anin et al., 2014), the height of the flower in the tree, and the average values of air temperature, relative humidity and wind speed recorded during the transects.

2.6.3. Video monitoring of diurnal and nocturnal flower visitors

2.6.3.1. Diurnal and nocturnal visitation frequency and duration. For each hour of video, we extracted the number of flowers present in the observed cluster, the flower falls, the arthropods that visited the flowers, the time and duration of each visit and the floral reproductive organ touched by the visitor. Due to the quality of the videos, it could not be determined whether visitors touching the staminodes were in contact with the style or not. From this extracted data, we first described the abundance of the different arthropods visiting the flowers in order to form groups whose behaviour was then studied. The aim was to determine potential complementarities in terms of circadian cycle, duration of visits to flowers and contact with floral reproductive organs. Using the generalised additive models of the *mgcv* package version 1.9–1 (Wood, 2017), we identified visitor groups whose frequency of visit depends on the time of day and represented their circadian cycle.

We used the decimal logarithm transformation of the visit duration pertaining to the different visitor groups that we fitted to a linear model including the visitor groups and the time of the visit as independent variables.

2.6.3.2. Quantity and viability of pollen deposited. From the six videos analysed, of the 62 flowers monitored, the styles of 34 flowers could be collected for pollen grain counting because the others had fallen. Of these 34 flowers, only 13 received at least one pollen grain, an insufficient amount to compare the performance of different flower visitors on the indicators of quantity and viability of the deposited pollen in the case of video monitoring.

3. Results

3.1. Assessing the potential trade-off in quantity-quality pollen deposition

The pollen quantity deposited on the styles displayed a slightly quadratic positive effect on the fruit set (Fig. 2A, $P < 0.001$). It also affected the flower fecundation rate since a lower rate of fertilised ovules was observed in the fruits for quantities lower than 200 pollen grains deposited (Fig. 2B, $P < 0.001$). The fruit set model indicated a 50 % probability of fruit formation from 64 pollen grains deposited. The minimum number of pollen grains where a fertilisation rate of 100 % of ovules was observed was 221. Pollen age had no influence on the two pollination success indicators ($P = 0.129$ and $P = 0.718$ on the fruit set and the ovule fecundation rate, respectively). Regarding microclimate variables, the temperature recorded at the moment of hand pollination did not significantly affect the fruit set ($P = 0.677$) and the ovule fecundation rate ($P = 0.109$). Furthermore, relative humidity did not affect fruit set ($P = 0.434$) and ovule fecundation rate ($P = 0.152$).

3.2. Visual observations of flower visitor performances

3.2.1. Diurnal visitation frequency and floral reproductive organ visited

During the transects, a total of 1142 visitors were observed on the 711 flowers that received one or more visitors. Of these, only 167 flowers were observed with more than one visitor present at the time of observation, with a maximum of 19 visitors. The flower visitation frequency was affected by the morphotype of the arthropod visiting the flowers ($P < 0.001$) (Fig. 3A). Indeed, 7 morphotypes emerged as common visitors of cocoa flowers compared to the 10 others. Aphids were the most commonly reported, with an average of 38 individuals per

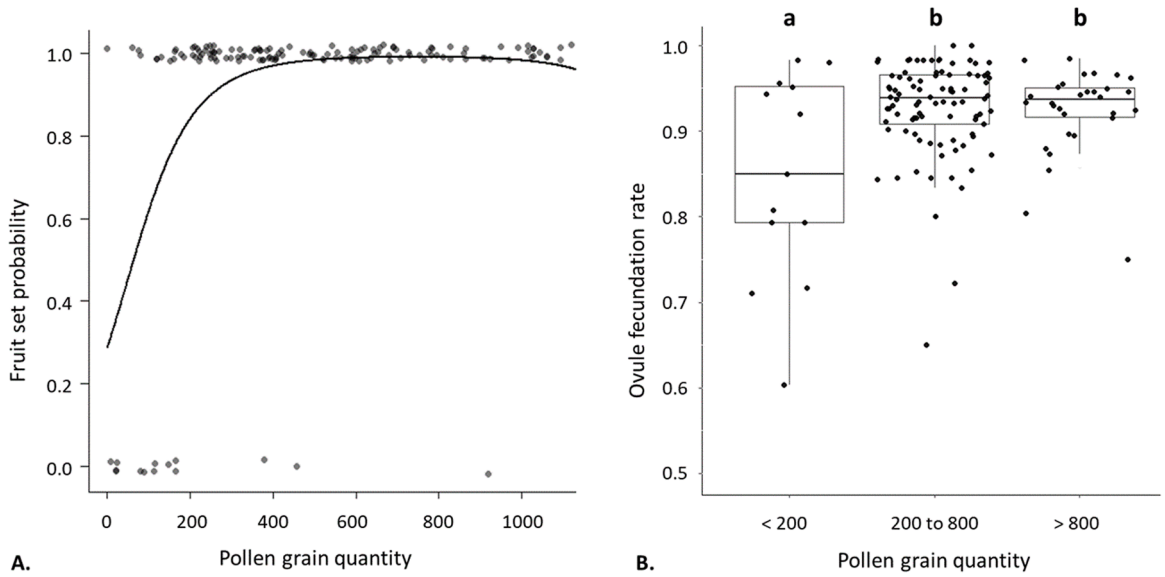

Fig. 2. Effect of pollen grain deposited on (A) fruit set probability and (B) ovule fecundation of cocoa, experimented by means of hand pollination in controlled greenhouse conditions. Significant differences in the fecundation rate are indicated by different letters.

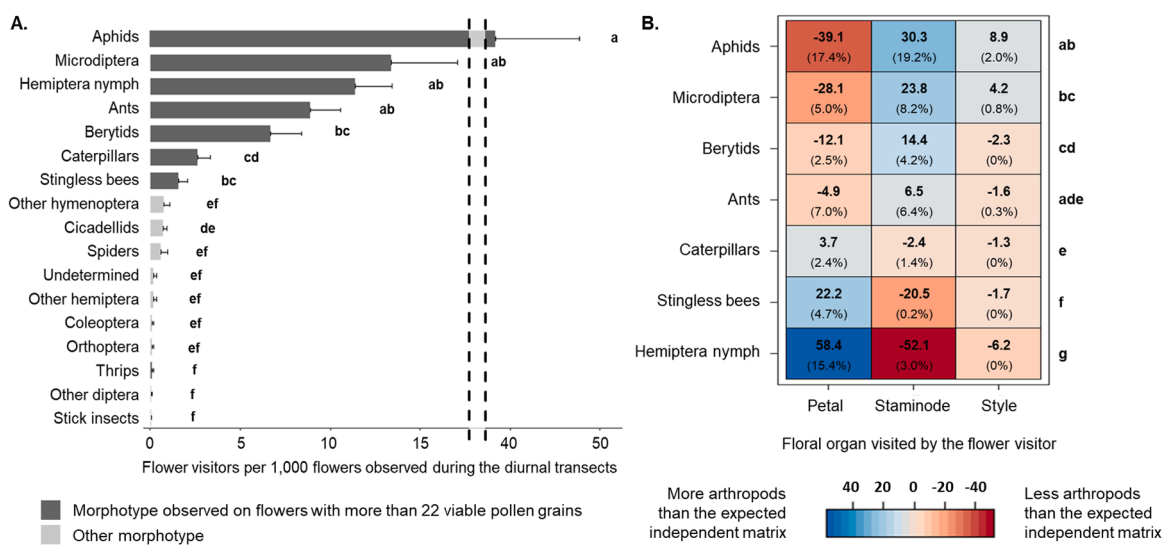

Fig. 3. (A) Frequency of cocoa flower visitors observed during the diurnal transects in the Ecuadorian Amazon region and classified according to their morphotypes. (B) Floral organ (petal, staminode, style) visited by the most common cocoa flower visitors observed during the diurnal transects. The numbers in brackets represent the observed relative arthropod abundances in each combination. Significant differences are indicated by different letters on the bar plot (A) or at the end of the table rows (B).

1000 flowers. On average, 13 microdiptera, 11 hemipteran nymphs, 8 ants, 6 berytids, 2 caterpillars, and 1 stingless bee were also observed per 1000 flowers, compared with less than 1 individual for each of the remaining 10 categories. The frequency of flowers visited by arthropods was not influenced by microclimate variables, i.e. mean air temperature ($P = 0.504$), relative humidity ($P = 0.954$) and wind speed ($P = 0.643$) during transects.

The main cocoa flower visitors display different behaviours in terms of the floral reproductive organs they visit (Fig. 3B). On the one hand, we observe aphids and microdiptera, whose behaviour is not significantly different ($P = 0.147$), as they are more often found on staminodes than petals, and visit the style of flowers, unlike berytids, caterpillars, stingless bees and hemipteran nymphs. On the other hand, the latter three morphotypes are mainly present on petals and only occasionally visit staminodes. However, this behaviour is less pronounced in caterpillars than in stingless bees ($P < 0.001$) and hemipteran nymphs ($P < 0.001$). We then observed visitors whose behaviour is intermediate, such as ants, which behave no differently from aphids ($P = 0.164$) and that also visit the flower style occasionally, nor differently from berytids and caterpillars ($P = 0.060$ and $P = 0.283$ respectively). Berytids, meanwhile, behave differently to visitors who prioritise a floral organ between the petals and the staminodes (i.e. aphids ($P = 0.038$), caterpillar ($P = 0.014$), stingless bees ($P < 0.001$) and hemipteran nymphs ($P < 0.001$)).

3.2.2. *Quantity and viability of pollen deposited*

The seven most common morphotypes were also the most effective pollinators in the NMDS analysis, with the exception of thrips (Fig. 4A). Flowers visited by spiders and other undetermined hemipterans or undetermined arthropods carried no pollen grains. Of the remaining 14 flower-visiting morphotypes, none fell exclusively into the category of pollinators never depositing viable pollen grains (Fig. 4A, Fig. S4).

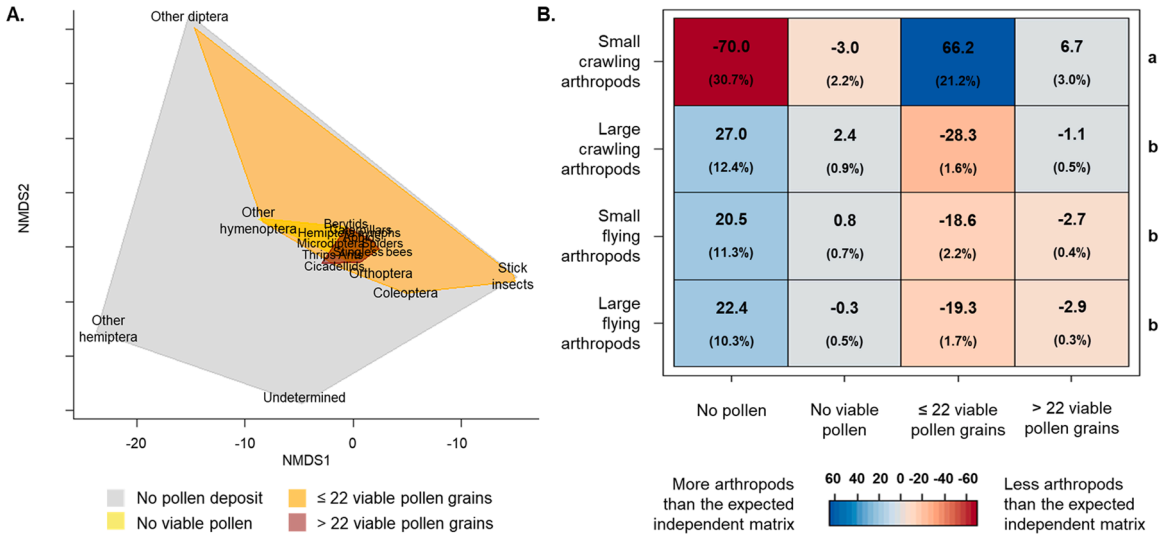

Fig. 4. (A) Performance of cocoa flower visitors observed during the diurnal transects in terms of quantity and quality of the pollen collected on the style (no pollen grains, no viable pollen grain, less than 23 viable pollen grains and more than 22 viable pollen grains) and (B) the difference of repartition of the arthropod categories visiting cocoa flowers (Table S2) between these categories of performance using the diurnal transect data. The numbers in brackets represent the observed relative arthropod abundances in each combination. Significant differences in repartition are indicated by different letters at the end of each row. and (B) their performance as pollinators.

However, for only eight of the morphotypes the estimated pollen grain deposit was sufficient for a fruit to be developed. These eight morphotypes are ants, aphids, berytids, caterpillars, some hemipteran nymphs, stingless bees, microdiptera and thrips (Fig. S4). By grouping all arthropods into four categories based on their size and ability to fly, we found that the small crawling arthropods presented a significantly different distribution to the other three groups ($P < 0.001$), with fewer individuals from whom the flowers visited received either no pollen grain at all or non-viable grains, and therefore more individuals for whom the flowers received viable pollen despite a low quantity (Fig. 4B). On the other hand, large crawling arthropods, large flying arthropods and small flying arthropods displayed no difference in distribution according to performance in terms of the quantity and quality of the pollen collected on the style ($P = 0.692$ between large crawling arthropods and large flying arthropods, $P = 0.931$ between large flying arthropods and small flying arthropods and $P = 0.551$ between large crawling arthropods and small flying arthropods).

The quantities of pollen collected on the styles of the visited flowers increased significantly over the day ($P < 0.001$) (Fig. 5). Variables related to flower visitor identity, abundance and the floral reproductive organ they touched during the visit also influenced these amounts of pollen collected. Indeed, if flower visitors touched the style during their visit, more pollen grains were present on the collected styles (Table 1, Fig. 5). Regarding visitor identity and abundance, ant presence and their number in the flower increased the number of pollen grains on the styles from the visited flowers ($P = 0.004$), while in the case of hemipteran nymphs the number of pollen grains decreased ($P = 0.015$). The presence or absence of the other six morphotypes of interest had no impact on this quantity of pollen (Table 1). The variables linked to flowering or flower morphology did not affect the quantity of pollen collected ($P = 0.169$, $P = 0.849$ and $P = 0.933$ for the average number of flowers observed, the height of the flower in the tree and the flower staminode opening angle, respectively). Finally, none of the microclimatic variables tested on the quantity of pollen grains collected on the styles of the flowers visited exhibited any effect ($P = 0.808$, $P = 0.671$ and $P = 0.681$ for the mean air temperature, relative humidity and wind speed, respectively).

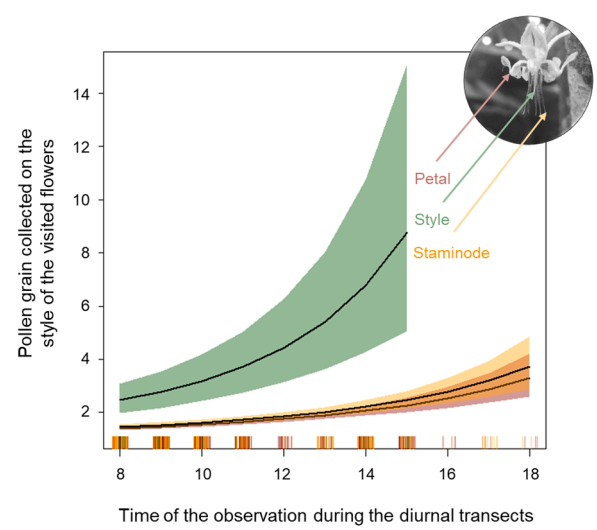

Fig. 5. Pollen grain collected on the style of the visited flowers depending on the time of the observation and the flower part touched by the visitor observed during the diurnal transects. The coloured areas represent the confidence interval of the model. The bars on the x-axis represent the observed data for the time of observation, and their colour refers to the part of the flower visited.

3.3. *Video monitoring of diurnal and nocturnal flower visitors*

3.3.1. *Diurnal and nocturnal visitation frequency and duration*

On the 62 flowers monitored by camera we recorded a total of 675 arthropod visits, i.e. an average of 11 visits per flower. Overall, eight of these flowers received no visits at all, while the three most visited flowers received more than 50 visits. During the night recording hours, from 5 pm to 5 am, only 55 flower-visiting arthropods were observed, compared with 620 during the day. Of the 17 morphotypes observed during diurnal transects, we did not observe aphids, caterpillars, cicadellids, orthopterans, thrips and stick insects. In some cases, this is probably due to their slow movement speed or low abundance. Image quality may have hampered thrips detection, but was adequate to detect the very small ants and microdiptera observed during the transects. Ants

Table 1
Summary of the negative binomial GLM assessing the effect of flower morphology, visitor morphotypes and time of observation on pollen collected on the style of the visited cocoa flowers. Significant effects are presented in bold.

Model parameter	Complete model estimate ± SE	Z	P-value
Intercept	**−2.713 ± 0.354**	**−7.653**	**< 0.001**
Observation time	**0.126 ± 0.027**	**4.703**	**< 0.001**
Flower part visited: style	**0.973 ± 0.262**	**3.719**	**< 0.001**
Flower part visited: staminode	0.096 ± 0.171	0.564	0.573
Ant number in the flower	**0.288 ± 0.099**	**2.903**	**0.004**
Aphid number in the flower	0.043 ± 0.031	1.387	0.165
Berytid number in the flower	−0.610 ± 0.326	−1.872	0.061
Caterpillar number in the flower	0.327 ± 0.316	1.036	0.300
Hemipteran nymph number in the flower	**−0.527 ± 0.216**	**−2.437**	**0.015**
Stingless bee number in the flower	−0.606 ± 0.395	−1.532	0.125
Microdiptera number in the flower	−0.234 ± 0.174	−1.341	0.180
Thrip number in the flower	1.202 ± 0.743	1.619	0.105

and stingless bees were the most common visitors, with 369 and 225 visits respectively, compared with less than 16 visits for the other morphotypes. The latter were therefore grouped into the four categories based on their size and ability to fly (Fig. 6A). Two individuals could not be classified in any of these categories because, although small, their ability to fly could not be determined. Thus, they were excluded from the rest of the analysis. Generalised additive models revealed the effect of time of the day on visit frequency for ants ($P < 0.001$), stingless bees ($P < 0.001$) and large flying arthropods ($P = 0.005$) (Table 2). Visits by ants were spread over all hours of the day and night with a maximum of visits at 2 pm while large flying arthropods were observed between 5 am and 7 pm with a peak at 12 am and stingless bees between 8 am and 4 pm with a maximum at 11 am (Fig. 6B).

Around 90 % of visit durations were shorter than one minute, but visit durations of several hours with a maximum of 5 hours were also recorded. Stingless bees performed significantly longer visits than ants ($P = 0.049$), with 50 % of visits lasting more than 13 seconds, compared

Table 2
Summary of the GAM assessing the effect of time of visit and morphotype categories on visitation frequency through video monitoring. One GAM was run per morphotype category. Significant effects are presented in bold.

GAM model	F	P-value
Ants	**7.381**	**< 0.001**
Stingless bees	**18.01**	**< 0.001**
Large crawling arthropods	1.895	0.169
Large flying arthropods	**3.357**	**0.005**
Small crawling arthropods	1.554	0.126
Small flying arthropods	0.279	0.598

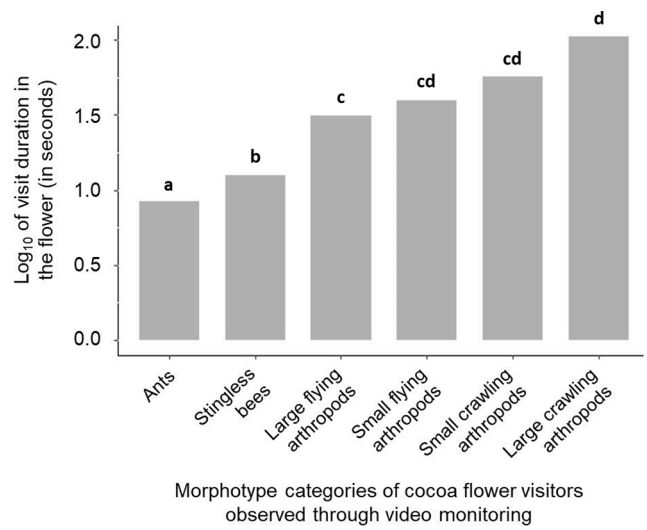

Fig. 7. Decimal logarithm (Log_{10}) of the cocoa flower visit duration (in seconds) of the different categories of flower visitors observed through video monitoring. Significant differences in visit duration are indicated by different letters.

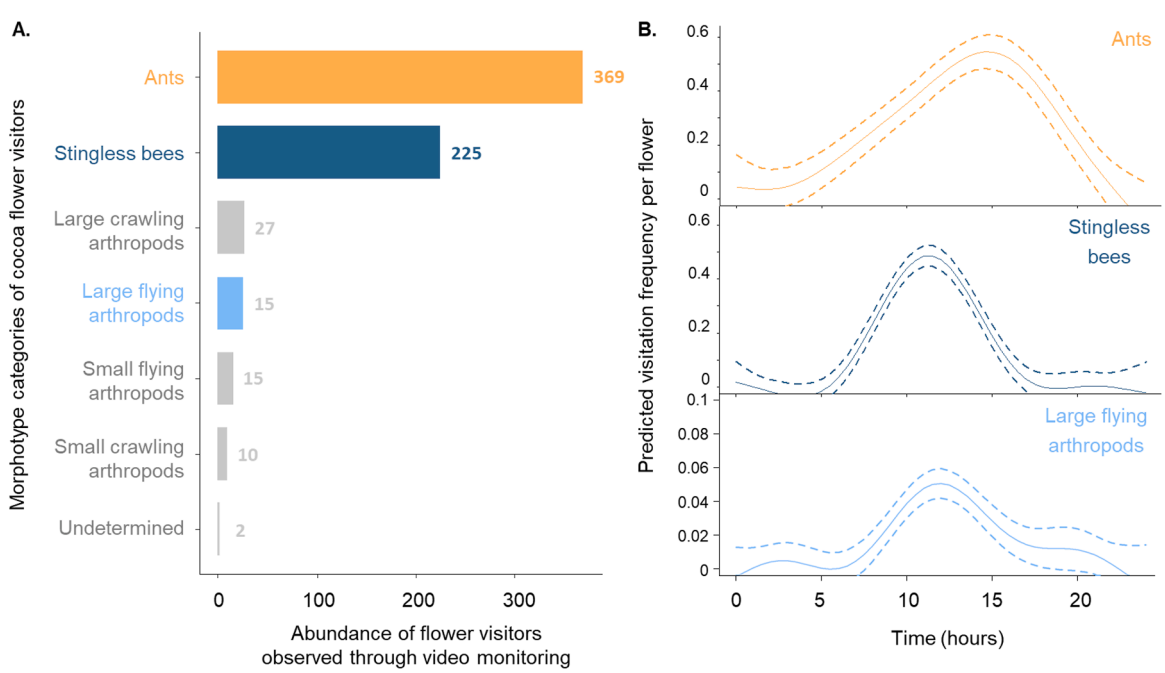

Fig. 6. (A) Abundance of cocoa flower visitors observed through video monitoring and classified by morphotype category and (B) time series of their visitation frequency predicted by GAM from the video monitoring data (Table 2). The three coloured bar charts represent morphotypes for which the time series were significant (i.e. significant time effect in GAM).

with 8 seconds for ants (Fig. 7). The other four categories spent more time in the flowers than ants ($P = 0.002$, $P = 0.001$, $P = 0.001$ and $P < 0.001$ for small flying arthropods, large flying arthropods, small crawling arthropods and large crawling arthropods, respectively) and stingless bees ($P = 0.047$, $P = 0.012$, $P = 0.004$ and $P < 0.001$ for small flying arthropods, large flying arthropods, small crawling arthropods and large crawling arthropods, respectively), while only two of these four categories displayed differences between them: large flying arthropods and large crawling arthropods ($P = 0.010$). The time of day had a slight negative effect on visit duration ($P < 0.001$).

3.3.2. Quantity and viability of pollen deposited

Of the 62 flowers monitored by video, 8 were not visited and 19 were only visited by individuals of the same arthropod morphotype, mostly stingless bees with 15 flowers, followed by ants on the remaining 4 flowers. The average number of visits per flower was 11 arthropods, taking into consideration differences of flower life span, with some flowers opening or falling during the video. Among the 34 flowers that did not fall, the pollen grain count on the style showed that the most visited flower received 63 visits but no pollen was deposited, while the two flowers that received only one visit received 0 and 44 pollen grains respectively. The 13 pollinated flowers received from 1 to 67 pollen grains, with a median of 16 grains. Of the 13 pollinated flowers, four were visited exclusively by stingless bees, while the majority were visited by multiple morphotypes including ants.

4. Discussion

We assessed the effectiveness of cocoa flower visitors as pollinating agents by combining indicators. Our hand pollination trial in the greenhouse revealed that cocoa pollination success is better driven by the quantity of the pollen deposited than by its quality. Indeed, both the fruit set and the rate of fecundated ovules depended only on the quantity of pollen grain deposited. Based on this result, we wanted to compare in the field the effectiveness of different visitors to cocoa flowers in terms of pollen grain deposition. Due to the very low natural pollination rate of cocoa, we counted pollen grains on flowers visited by different morphotypes in order to compare them. Although the absence of pollen on the styles can be linked to a non-effective visit, the presence of pollen remains an estimate of the pollen deposition made by the observed visitor, as we only used the time of observation as an indicator of potential previous visit to consider this bias of the technique. Our results suggest that ants may be better pollinators in our field trial, as they are the only visitors for which more pollen grains have been counted on the styles of flowers visited. In addition, this indicator of estimated quantity of pollen deposited also highlighted that one of the most frequent flower visitors in our plots, a yellowish hemipteran nymph, is not a pollinator and even disrupts pollination by its presence in the flowers. If we focus on the rate of pollinated flowers with sufficient viable pollen, then the category of small crawling arthropods emerges as the most effective. As a result, it seems that the small size of the visitors is an advantage for the pollination of cocoa flowers while their ability to fly is not. Therefore, other arthropods such as aphids could deposit pollen grains on the styles and have the advantage of being more abundant in the flowers than all the other visitors. Among the most frequent visitors were microdiptera, which were also observed on the styles, such as ants and aphids. In consequence, the presence of visitors on the flower style was naturally linked to an increase in the amount of pollen counted on these styles. But the presence of microdiptera in the flowers could not be linked either to a greater quantity of pollen on the styles or a better rate of pollination with viable pollen, meaning that their identity as pollinators cannot be established. For visitors frequently observed in flowers but whose identity as a pollinator could not be confirmed by the quantity and viability of pollen collected on the styles, behavioural indicators, i.e. the floral organs visited, the duration and the moment of the visit, can provide information on their role. This is the case for caterpillars, which were mostly observed eating petals or staminodes and even in the case of certain flowers which no longer had a style. This is also the case for stingless bees, which briefly visit the flowers, going through several or even all the petals in order to collect pollen grains. The indicators of the quantity of pollen present on the styles and that of the rate of flowers pollinated with viable pollen did not reveal them to be pollinators, nor showed any detrimental effect of their presence on the pollination service and therefore a potential role as pollen thieves. The video monitoring of cocoa flowers showed that the monitoring method had an influence on the visitation frequency indicator, since some arthropods that were abundant in the transects, such as aphids and caterpillars, were not observed at all on the recorded flowers. This monitoring method did not reveal any nocturnal cocoa pollinators, but it demonstrated that stingless bees, ants and certain large flying arthropods have a circadian cycle in terms of visiting cocoa flowers.

4.1. A cocoa fruit set and ovule fecundation success better driven by pollen quantity than pollen age in compatible mating

Our results in terms of pollination intensity required for flower retention and ovule fecundation rate are in line with previous studies. Indeed, the threshold of 221 pollen grains to reach 100 % development of ovules into seeds is close to those observed by Falque et al. (1996), (1995), i.e. 238 pollen grains for the ICS39 genotype crossed on UPA 409, and more than 200 pollen grains in the case of the cross of the genotype IFC5 on SCA6. The negligible effect of pollen age on pollination success also suggests that in the case of cocoa, the most efficient pollinators would be those carrying and depositing the highest amount of pollen, regardless of the time of day they visit the flowers. However, our result may have been influenced by the genotype used. For instance, Aneja et al. (1992) observed an average longevity of pollen grains of 12 hours after dehiscence around 8 am. More recently, García Talledo et al. (2019) found that pollen longevity depended on the genotype studied as pollen from *Nacional* type cocoa trees did not germinate 4 hours after collection while pollen from the CCN-51 genotype had a longevity of 24 hours. Even the initial pollen viability rate seems to be different depending on the genotype (García Talledo et al., 2019; Omolaja et al., 2010). In a study of cocoa pollination, Glendinning (1972) highlighted that field studies did not agree on the optimal pollination period. Although pollination seems to take place mainly in the morning during the first day of the flower's life (Entwistle, 1958; Posnette, 1942; Van der Knaap, 1955), some studies reported a better pollination between dawn and 9 am (Entwistle, 1958, 1957) while others mentioned the period from 9 am to 10 am, since anthers did not burst until 9 am (Saunders, 1958). Mena and García (2014) and Young et al. (1987) indicated that regardless of the quantity and quality of pollen deposited, the style could have a period of high receptivity in the morning and early afternoon of the first day, while a recent study identified a stigma receptivity peak around 6 am the second day (Jaramillo et al., 2024). Learning more about the longevity of pollen and the optimal stigma receptivity period would reveal the period, from the dehiscence of the pollen lodges, that is the most favourable for pollination. As a consequence, this period should be the one when pollinator visits would be the most efficient.

4.2. The community of arthropods visiting cocoa flowers using different monitoring methods

There are several reasons for the very low frequency of visits, which was less than 10 % of the flowers observed during the transects. The first is related to the method used, since the aim was to record the situation at a given moment, rather than to trap all flower visitors, as in the case of glue trapping and camera tracking. However, even with the same method, the results can show differences, since glue trapping rates for flower visitors have been observed in different studies ranging from 12 % to 30 % (Chumacero De Schawe et al., 2016; Toledo-Hernández

et al., 2021; Vansynghel et al., 2022). The total frequency of visitors also depends on crop management, which provides more or less food and habitat, but above all on the abundance of flowers. Indeed, pollination rates can reach 50 % or more when flowers are scarce (Entwistle, 1957; Posnette, 1942). Using a method for monitoring flower visitors by diurnal flower observations, we found that aphids, microdiptera and ants were very frequent cocoa flower visitors. This assessment is in line with previous studies that collected visitors directly from flowers of trees from the Theobroma genus (including *Theobroma cacao* and *Theobroma bicolor*), either manually or using glue, in Ecuador (Armijos Vásquez et al., 2020; Ponce-Sánchez et al., 2021; Wright, 1984) and other cocoa-producing countries on different continents (Adjaloo and Oduro, 2013; Chumacero De Schawe et al., 2016; De Reffye et al., 1980; Jaramillo et al., 2024; Toledo-Hernández et al., 2021; Vansynghel et al., 2022; Winder, 1978; Zegada Herbas et al., 2020). In addition to aphids, we found large numbers of two other morphotypes belonging to the order Hemiptera: the family Berytidae and yellowish hemipterans in the juvenile stages of nymphs, probably from the same family or from that of Reduviidae. The Berytidae family has been documented as a leaf pest (Bigger, 2012; Delgado et al., 2023) and as a very occasional flower visitor (Zegada Herbas et al., 2020), but not found in abundance in cocoa flowers until now. Among the seven most common morphotypes we found were caterpillars as reported in previous studies (De Reffye et al., 1980; Vansynghel et al., 2022; Winder, 1978; Zegada Herbas et al., 2020). Finally, with a frequency of one individual for every 1000 flowers observed, stingless bees, most likely the species *Tetragonisca angustula* in our study (Jaramillo et al., 2024; Maia-Silva et al., 2024), seem to be attracted to cocoa flowers (Frimpong et al., 2011). We observed a large number of visits by these stingless bees using video monitoring, since they were the second most frequent morphotype after ants. The method used to monitor flower visitor communities therefore has an influence on the visitation frequency observed (O'Connor et al., 2019). Studies that monitored by means of glue trapping in cocoa flowers generally observed more thrips or hymenoptera other than ants and stingless bees (Chumacero De Schawe et al., 2016; Ponce-Sánchez et al., 2021; Toledo-Hernández et al., 2021; Vansynghel et al., 2022; Zegada Herbas et al., 2020) than studies that monitored through observations and manual or suction collection in flowers (Armijos Vásquez et al., 2020; Frimpong et al., 2009; Jaramillo et al., 2024; Winder, 1978). Finally, another bias generated by the different methods used is the period of trapping. The use of cameras to track pollinators is a recent technique (Droissart et al., 2021) and should be a more robust sampling method than others, allowing to know the complete history of visits and to link directly to the pollen deposited or even to fruiting monitoring. To compare the efficiency of cameras with other methods, we invite future studies to use the same sampling effort between methods and to perform the monitoring on the same flowers. The advantage of glue trapping and video monitoring is that they cover both day and night, but glue trapping does not reveal whether cocoa pollination might take place at night. Few studies to date have investigated nocturnal visitors to cocoa flowers (Frimpong et al., 2009; Jaramillo et al., 2024). We observed that a proportion of flower visits do indeed take place at night, but there do not seem to be any visitors who visit exclusively at night and in abundance, whereas stingless bees have only been observed during the day (Jaramillo et al., 2024). Whether using diurnal transects or video monitoring, certain visitors belonging to the orders Arachnids, Coleoptera, Orthoptera, Phasmatodae or certain morphotypes of Diptera, Hemiptera and Hymenoptera such as chalcid wasps appear to be far too occasional flower visitors in order to represent potential cocoa pollinators (Armijos Vásquez et al., 2020; Chumacero De Schawe et al., 2016; De Reffye et al., 1980; Toledo-Hernández et al., 2021; Winder, 1978; Zegada Herbas et al., 2020). The visitation frequency of flowers is thus an important indicator to consider in the identification of potential insect pollinators, but does not provide a basis for comparing them in terms of effectiveness on pollination services (King et al., 2013). In fact, other processes are involved in achieving effective flower pollination, as a visit does not always mean pollen deposition, and pollen deposition does not always lead to ovule fertilization and fruit set.

4.3. Some promising cocoa pollinators with different features

Our study confirms that ants are promising candidates as insect pollinators for cocoa, since their presence and numbers increase the amount of pollen collected from the styles of the flowers they visit. This efficiency in pollen deposition can probably be explained by different aspects of their behaviour. They visit cocoa flowers very frequently, and over a long period of the day and night. There is therefore a good chance that they will visit the flowers and pollinate them when the style is most receptive (Jaramillo et al., 2024; Mena and García, 2014; Young et al., 1987). The duration of their visit is short: in 50 % of cases it lasts no longer than eight seconds, but they are highly mobile (Jaramillo et al., 2024) and often explore the petals and staminodes. When their size is sufficiently small, they are able to collect pollen on the anthers hidden in the petal and deposit pollen on the style protected by the five staminodes. However, a previous study did not find any pollen grains on the style of the cocoa flowers visited by ants (Adjaloo and Oduro, 2013) and they were observed carrying few pollen grains on their body (Jaramillo et al., 2024). In addition, despite their mobility, ants could mostly perform self-pollination (Toledo-Hernández et al., 2017). They could therefore prove to be pollinators only in the case of plantations using self-compatible cultivars. Given the high level of self-incompatibility in cocoa plantations, they have not been considered as effective pollinators of cocoa in some previous studies (Cope, 1962; Glendinning, 1972). Furthermore, it seems that in other crops ants negatively affect the viability of pollen grains and their germination rate by secreting antibiotic substances necessary for their nest-building and brood-rearing habits (Beattie et al., 1984). However, a positive effect of ants on cocoa pollination and yield has already been documented by Wielgoss et al. (2014), although they suggested an indirect effect on pollination by disturbing pollinators, which enhances their movement from flower to flower (Bisseleua et al., 2017).

Aphids are often the most frequently observed arthropods in cocoa flowers (Vansynghel et al., 2022; Winder, 1978; Zegada Herbas et al., 2020), sometimes even very numerous in the same flower. In our study, we observed up to 17 aphids in a single flower. Their small size enables them to access the anthers, and they were observed several times on the style, but compared with other visitors, they tend to visit the staminodes more than the petals. They may therefore collect less pollen and move very slowly within the same flower, as previously documented (Jaramillo et al., 2024). This behaviour probably explains why their presence and number did not contribute to a better pollination of the flower in terms of the quantity of pollen collected on the visited styles. However, 44 % of flowers visited by aphids were pollinated and 88 % of these pollinated flowers received viable pollen. This suggests that aphids may play an indirect role in pollination. Indeed, they represent a source of food for ants tending honeydew-excreting aphids. In one third of pollinated flowers visited by aphids, ants were also seen in the flowers at the time of observation. Despite the damage caused to flowers by their sap-sucking diet (Armijos Vásquez et al., 2020; Delgado et al., 2023), aphids may contribute to improve cocoa pollen transfer by attracting ants to these flowers.

4.4. An undetermined role for certain flower visitors

Although microdiptera were the most observed arthropods after aphids and were found in contact with the styles of some flowers in our field trial, their pollinating behaviour and effectiveness in depositing sufficient pollen were not confirmed. For instance, they were seen throughout the day and sometimes even by night, as evidenced by the video monitoring, most often immobile on the staminodes or petals (Armijos Vásquez et al., 2020), although a few were observed crawling up the staminode towards its base. According to Kaufmann (1975a),

their purpose is to feed on substances found on the staminodes, which explains why they are occasionally observed on the petals without exploring them and why they only carry an average of three pollen grains, which is not sufficient for a specialised pollinator (Jaramillo et al., 2024). Microdiptera are classed as small flying arthropods and did not demonstrate any superior ability in comparison with large crawling or flying arthropods in pollinating flowers with viable pollen. Flight does not therefore appear to be an important characteristic for pollinating cocoa flowers. Furthermore, their presence and number in the flowers were not associated with greater quantities of pollen collected on the styles of the flowers visited, contrary to the observations of Adjaloo and Oduro (2013) of mass pollen deposition by *Forcipomyia* spp. and Cecidomyiids. Finally, many studies agree that although microdiptera can pollinate cocoa flowers, they are not effective enough to be the only arthropod pollinating this crop (Cilas et al., 1987; De Reffye et al., 1980; Kaufmann, 1975b; Toledo-Hernández et al., 2017; Vansynghel et al., 2022). Characteristics of microdiptera such as their presence as flower visitors in most of the studies conducted on cocoa, their ability to visit all the floral organs and their mobility, which enables cross-pollination, remain major advantages for cocoa pollination. Although our study did not demonstrate a higher effectiveness of microdiptera as cocoa pollinators, the evaluation of other indicators such as flower retention could reveal them to be effective pollinators not by the quantity of pollen deposited or its viability, but by the ability of this pollen to induce fruit set.

Stingless bees emerged as one of the seven most frequent visitors during the transects, and video monitoring resulted in them being observed even more frequently, as they were the second most frequent morphotype after ants. An interference of the experimenters during the transects may have hindered the foraging behaviour of these bees, or the short duration of their visit may have reduced the chances of observing them during the transects compared with slower arthropods. For each flower visited, stingless bees almost always visit the different petals and collect pollen by inserting the front of their body into these petals. This pollen is visible in the white balls on their hind legs. As pollen collectors and transporters, stingless bees are very efficient indeed, carrying more than 50 pollen grains (Bezerra et al., 2020; Jaramillo et al., 2024). Nevertheless, they were never observed in contact with the style because their body is too large to allow them to pass between the staminodes. If they land on the staminodes when they arrive in the flower, their feet may touch the style, as observed in a previous study, but this is a very occasional occurrence (Jaramillo et al., 2024). The few studies that have investigated their effectiveness have been unable to demonstrate their role as pollinators (Frimpong et al., 2009) or even concluded that they may be detrimental to the pollination service by acting as pollen thieves (Young, 1981). Our study also showed them to be ineffective as pollinators, as the flowers visited during the transects did not receive more pollen grains and we did not observe a higher proportion of pollinated flowers with viable pollen. Although more visits by stingless bees were recorded using the cameras, we were unable to track a sufficient number of pollinated flowers to draw conclusions as to their effectiveness. However, the 50 % rate of pollinated flowers in the case of the eight flowers visited only by these stingless bees encourages further study of their behaviour. Moreover, there ability to release large amount of pollen in the petal during the collection was mentioned as an advantage for pollination by small crawling arthropods (Maia-Silva et al., 2024). Finally, many stingless bees share their distribution area with cocoa's area of origin and its current production zone in South America, with some of them being small enough to reach the style of cocoa flowers. If these stingless bees are proven to pollinate cocoa, they could be considered for targeted cocoa pollination (Heard, 1999; Maia-Silva et al., 2024).

The role of insects of the Berytidae family in cocoa flowers is unclear. Their large size prevents them from accessing either the anthers embedded in the petals or the style of the cocoa flower. Moreover, they are almost as abundant as ants according to the transect trial, but their behaviour is more akin to a predator waiting in the flower, since they can sometimes stay there for several hours according to the video monitoring. Lastly, the flowers visited did not have a higher rate of pollination with viable pollen. However, the negative effect of their presence in the flower on the quantity of pollen collected on the style was not significant and therefore does not suggest a detrimental role with regard to pollination service.

4.5. Flower visitors that are detrimental for pollination service

The yellowish hemipteran nymphs that we observed abundantly in the cocoa flowers during the transects varied in size from individuals small enough to access the style to individuals that were too large. However, they were never observed on the style. The smallest individuals of this morphotype belong to the category of small crawling insects whose flowers visited have the highest proportion of pollinated flowers. However, the presence of these nymphs has a negative impact on the quantity of pollen counted on the style of the flowers visited. Their role is therefore detrimental to pollination service. They are not pests that attack the plant directly, but their behaviour as predators waiting for hours for prey on the cocoa flower petals probably prevents the visit of promising pollinators.

Regarding caterpillars, although their presence did not affect the quantity of pollen collected on the style of the flowers visited, their role however was not to pollinate but to damage the flowers, since 20 % of the flowers visited by them could not develop into a fruit, as the styles had been eaten. Their florivory behaviour helps them to collect pollen by eating the petals and sometimes to deposit a large amount on the style, as we observed up to 239 grains of pollen on the style of a flower visited by a caterpillar. However, very few were observed on the staminodes, i.e. close to the style.

5. Conclusion

In order to identify the most efficient pollinators among cocoa flower visitors, we combined the use of several commonly accepted indicators such as the visitation frequency, the duration of the visit and the quantity of pollen deposited (Ne'eman et al., 2010). We also tested the quality of the pollen deposited by assessing its viability, since the greenhouse trial revealed that about three times as many pollen grains as ovules were necessary to fertilise all of them. Some indicators, such as the frequency and duration of visits, are not sufficient to conclude that a flower visitor is in fact a pollinator. In addition, we observed that by using different monitoring techniques, i.e. transects and video, the visitation frequency was different within the same plots probably due to the monitoring duration, and the mobility and behaviour of flower visitors. Other indicators, such as the quantity of pollen deposited, provide information with regard to the pollinator's identity, as in the case of ants visiting cocoa flowers, and can also highlight a behaviour detrimental to pollination, such as the hemipteran nymphs. However, this indicator is not suitable for comparing the effectiveness of different pollinators. Indeed, a flower visitor that deposits few pollen grains but visits the flowers frequently, such as aphids in cocoa flowers, may be more effective than a pollinator that can deposit a large number of pollen grains but only very occasionally, such as stingless bees. Lastly, flower retention and yield are important indicators when working on the pollination service, but remain difficult to study due to the very low natural cocoa fruit set. Therefore, using video monitoring over extended periods could provide new insights on the effectiveness of cocoa pollinators.

Our study did not identify one pollinator as being more efficient than the others in the case of cocoa, and suggests that the cocoa pollination service may benefit from the complementarity of several pollinator visits, taking place at different periods of the day and with varying behaviour, i.e. some visitors by attracting others, others by releasing pollen into the petals. Finally, pollen deposition does not imply ovule

fertilization and fruit formation. Although our study investigated the viability of deposited pollen, we did not collect data on fruit formation on flowers monitored by video. To increase the possibility of observing fruit formation, it would be interesting to repeat the video monitoring, which would provide information on all visits received, outside the peak flowering period, as the pollination rate can be over 50 %. Another approach to consider genetic incompatibility in cocoa when studying the effectiveness of flower visitors would be to study the origin of the pollen deposited. A few studies have approached this notion of pollen origin using marked elements. Lucas (1975) and Massaux et al. (1976) evaluated pollen transport between different trees, but did not study flower visitors. It would be interesting to assess whether these techniques could be used to determine the origin of pollen transported or even deposited by the promising candidate arthropods.

CRediT authorship contribution statement

Merle Isabelle: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Argout Xavier:** Validation, Resources, Investigation. **Paredes Andrade Nelly Judith:** Resources, Methodology, Investigation, Conceptualization. **Requier Fabrice:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Pico Rosado Jimmy Trinidad:** Resources, Methodology, Investigation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2025.109716.

Data Availability

Data will be made available on request.

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