# ANIMAL SEED DISPERSAL: AN ECOSYSTEM SERVICE IN CRISIS

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PUBLISHED IN: Frontiers in Ecology and Evolution

frontiers Research Topics

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ISSN 1664-8714
ISBN 978-2-88976-506-5
DOI 10.3389/978-2-88976-506-5

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# ANIMAL SEED DISPERSAL: AN ECOSYSTEM SERVICE IN CRISIS

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**Citation:** Traveset, A., Rogers, H. S., Donoso, I., Fricke, E., eds. (2022). Animal Seed Dispersal: An Ecosystem Service in Crisis. Lausanne: Frontiers Media SA.
doi: 10.3389/978-2-88976-506-5

# Table of Contents

frontiers
in Ecology and Evolution

REVIEW
published: 06 April 2021
doi: 10.3389/fevo.2021.641411

# The Critical Importance of Old World Fruit Bats for Healthy Ecosystems and Economies

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OPEN ACCESS

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**Specialty section:**
This article was submitted to
Population and Evolutionary
Dynamics,
a section of the journal
Frontiers in Ecology and Evolution

**Received:** 14 December 2020
**Accepted:** 08 March 2021
**Published:** 06 April 2021

**Citation:**
Aziz SA, McConkey KR,
Tanalgo K, Sritongchuay T, Low M-R,
Yong JY, Mildenstein TL,
Nuevo-Diego CE, Lim VC and
Racey PA (2021) The Critical
Importance of Old World Fruit Bats
for Healthy Ecosystems
and Economies.
Front. Ecol. Evol. 9:641411.
doi: 10.3389/fevo.2021.641411

Despite extensive documentation of the ecological and economic importance of Old World fruit bats (Chiroptera: Pteropodidae) and the many threats they face from humans, negative attitudes towards pteropodids have persisted, fuelled by perceptions of bats as being pests and undesirable neighbours. Such long-term negativity towards bats is now further exacerbated by more recent disease-related concerns, particularly associated with the current COVID-19 pandemic. There remains an urgent need to investigate and highlight the positive and beneficial aspects of bats across the Old World. While previous reviews have summarised these extensively, numerous new studies conducted over the last 36 years have provided further valuable data and insights which warrant an updated review. Here we synthesise research on pteropodid-plant interactions, comprising diet, ecological roles, and ecosystem services, conducted during 1985-2020. We uncovered a total of 311 studies covering 75 out of the known 201 pteropodid species (37%), conducted in 47 countries. The majority of studies documented diet (52% of all studies; 67 pteropodid species), followed by foraging movement (49%; 50 pteropodid species), with fewer studies directly investigating the roles played by pteropodids in seed dispersal (24%; 41 pteropodid species), pollination (14%; 19 pteropodid species), and conflict with fruit growers (12%; 11 pteropodid species). Pteropodids were recorded feeding on 1072 plant species from 493 genera and 148 families, with fruits comprising the majority of plant parts consumed, followed by flowers/nectar/pollen, leaves, and other miscellaneous parts. Sixteen pteropodid species have been confirmed to act as pollinators for a total of 21 plant species, and 29 pteropodid species have been confirmed to act as seed dispersers for a total of 311 plant species. Anthropogenic threats disrupting bat-plant interactions in the Old World include hunting, direct persecution, habitat loss/disturbance, invasive species, and climate change, leading to ecosystem-level repercussions. We identify notable research gaps and important research priorities to support conservation action for pteropodids.

**Keywords: bat-plant interactions, double mutualism, ecosystem services, Palaeotropics, pollination, Pteropodidae, seed dispersal**

## INTRODUCTION

Discrepancies between human perceptions of an animal, and the importance of the animal to broader human well-being, is perhaps most profoundly unbalanced for bats. Old World fruit bats (family Pteropodidae; Simmons and Cirranello, 2020; also referred to as "pteropodids") are particularly threatened, facing multiple environmental pressures not only as a result of exclusion from statutory protection policies, but also due to a lack of necessary conservation attention, even for legally protected species (Aziz et al., 2016). In addition, the COVID-19 pandemic is the latest and worst disease-related concern to have reinforced long-held fears and negative attitudes towards bats (López-Baucells et al., 2018; Rocha et al., 2020; Tuttle, 2020; Zhao, 2020). Bats are persecuted due to misguided fears of viral transmission (Tuttle, 2018; Rocha et al., 2020; Lu et al., 2021), the noise, smell and mess associated with roosts in residential areas (Aziz et al., 2017a), and for their consumption of fruit crops (Aziz et al., 2016); the latter has even induced mass culls of ∼50% of the endemic flying fox (*Pteropus niger*) population in Mauritius (Florens and Baider, 2019). Fruit bats also continue to be intensively harvested for consumption (Mildenstein et al., 2016), despite often dwindling populations, and despite zoonotic disease concerns.

The 201 species of pteropodids range from Africa, the eastern Mediterranean, Madagascar, Indian Ocean islands, across South and Southeast Asia, southern East Asia including Hong Kong and Taiwan, and throughout islands of the Pacific from the Ryukyu Archipelago, to coastal eastern Australia (including Christmas Island), Melanesia, Micronesia, and Polynesia excluding New Zealand and Hawai'i (Mickleburgh et al., 1992). Available data on the status of species suggest serious population declines for many, mainly due to habitat loss and overhunting, with 37% of assessed species being threatened (IUCN, 2020). Pteropodid declines will also result in plant declines, with ecosystem-wide repercussions.

Complex inter-relationships between Pteropodid bats and plants over millennia have resulted in "bat flowers" and "bat fruits" that are reliant on bats for pollination and/or seed dispersal (Marshall, 1983). Bat-plant interactions were first recorded in 1772 and compiled in the mid-1980s (Marshall, 1983, 1985), showing how pteropodids carry out vital ecological functions in diverse habitats, and are thus essential for the healthy functioning of ecosystems and economies (Marshall, 1985; Fujita and Tuttle, 1991; Mickleburgh et al., 1992; Richards, 1995; Lacher et al., 2019). On faunally depauperate islands, pteropodids play keystone roles as principal pollinators and seed dispersers (Elmqvist et al., 1992; Shanahan et al., 2001; Fleming and Racey, 2009; McConkey and Drake, 2015; Florens et al., 2017), while their high abundance on some continents ensures they are important providers of ecosystem services (Redford et al., 2013; Baker et al., 2018; van Toor et al., 2019; Laurindo et al., 2020). Many plants visited by pteropodids are utilised by humans, and thus have economic importance (Fujita and Tuttle, 1991; Kunz et al., 2011; Scanlon et al., 2014).

Although recent decades have seen a huge growth of papers on pteropodid diet and function, their role has likely been underestimated compared to the much more widely studied birds, primates, and large terrestrial mammals (Seltzer et al., 2013; Baker et al., 2018). Further, pteropodid species that can maintain plant populations in degraded areas regularly forage in isolated trees (Schmelitschek et al., 2009), and can be key to catalysing restoration in disturbed habitats (Sritongchuay et al., 2014; Oleksy et al., 2015). A comprehensive understanding of bat-plant interactions in the Old World is essential for defining their importance, and their benefits to humans – which can help foster a much-needed and more favourable balance in public opinion, and direct research to important gaps in knowledge.

The goal of this paper is to synthesise the breadth of our latest knowledge on pteropodid diet and pteropodid-plant interactions, building from previous reviews and spanning more than three decades of research (1985–2020). We use this dataset to: (i) describe the confirmed and potential ecological roles that pteropodids perform, by evaluating studies on diet, foraging movement, pollination, and seed dispersal; (ii) summarise the main threats to these roles; and (iii) identify the most critical research gaps. This review thus covers the current state of knowledge regarding pteropodid-plant interactions.

## METHODS

In this review of more recent bat-plant interaction studies, we included interactions described in post-1985 reviews along with the many new studies since. We also included studies that investigated ecosystem services and disservices by pteropodids (Zhang et al., 2007).

With the exception of Pacific island nations and Papua New Guinea, data have been organised according to country and geopolitical regions (United Nations, 2020). We grouped together island nations of the regions Melanesia, Micronesia, and Polynesia as "The Pacific." For Africa, we treated insular and continental nations as separate regions.

Although this review covers all pteropodids across their range, for which the common term "fruit bats" is sometimes used, we use the terms "large pteropodid" and "large fruit bat" to refer only to species with either body weight $\geq$250 g or forearm length $>$110 mm (following Pierson and Rainey, 1992; Kunz and Pierson, 1994), which includes the genera *Acerodon, Aproteles, Desmalopex, Dobsonia, Eidolon, Hypsignathus, Pteralopex, Pteropus*, and *Styloctenium* (Pierson and Rainey, 1992; Kunz and Pierson, 1994; genera list compiled by Mildenstein, 2002). We make this distinction as large fruit bats have distinctly different roles and ecosystem interactions compared to the small fruit bats (Richards, 1995), and also tend to be disproportionately targeted by hunters (Mildenstein et al., 2016).

We reviewed research articles worldwide, during the period 1985-2020, that dealt specifically with the following topics:

(1) diet (studies that investigated, identified and documented food plants consumed by pteropodids, including food choice experiments);

(2) foraging movement (studies involving either long-distance tracking of movements, or feeding behaviour at food plants);
(3) pollination (studies involving exclusion experiments, effective pollen transfer, or effect of bat visitors on fruit set);
(4) seed dispersal (studies involving observations of dispersal distances, modelling of seed shadows, direct observations of bats carrying off fruit, ingestion of viable seeds, gut passage times, seed germination experiments, seedling/sapling recruitment, or seed predation observations);
(5) conflict between pteropodids and fruit growers, including owners of non-commercial backyard trees, which can be considered a form of ecosystem disservice (Zhang et al., 2007; Shackleton et al., 2016).

We collated a preliminary list of studies by performing a Boolean search with relevant wildcards on the ISI Web of Science database and Google Scholar (**Supplementary Information 1**).

We then used this database to conduct comprehensive analyses of research trends and information gaps. Other potentially relevant studies may be overlooked because they were unobtainable or inaccessible (e.g., behind paywalls, or in books, workshop proceedings, and local repositories inaccessible to the international community, or in a language other than English).

Scientific and common names of bat species follow the IUCN (2020). We made every effort to update all plant species names to the most current accepted taxonomic revisions rather than using obsolete names listed in the original studies; we used the online database The Plant List (2013) to verify the latest botanical nomenclature. However, we acknowledge that discrepancies still exist, particularly if further taxonomic revisions have occurred during the course of this review. Unresolved plant names, which could not be identified within current taxonomic databases (e.g., misassigned/misspelled names, obsolete names without contemporary equivalents, or synonyms with several matches), were indicated with question marks.

## Data Analysis

Research trends from 1985 to 2020 were analysed and visualised based on regional and geopolitical boundaries, in order to understand research efforts across different countries and to inform future priorities and targets. The proportion of plant parts (e.g., fruits, flowers, and leaves) consumed by pteropodids was analysed at the levels of pteropodid genus and plant family. Data visualisation was conducted using the ggplot package in R 3.6.3 (R Core Team, 2021) and Inkscape 1.0.1 (Inkscape Project, 2021).

The overall bat-plant interaction networks were visualised using the bipartite package in R 3.6.3 (R Core Team, 2021). For each network, interactions were summarised as a bipartite matrix, with each cell containing the number of interactions between each plant family and pteropodid genus.

Relationships among body size (in g), foraging distance, and fruit size were assessed using Spearman Rank correlations.

# RESULTS AND DISCUSSION

We obtained 311 relevant studies on bat-plant interactions in the Old World published during 1985-2020: 204 from Web of Science, an additional 73 from Google Scholar, and 33 opportunistically. Of the 201 pteropodid bat species, 37% ($n = 75$) have been studied, in 47 countries. Most of the studies documented diet (52% of all studies; 67 pteropodid species), followed by foraging movement (49%; 50 pteropodid species). Relatively few studies have directly investigated the roles played by pteropodids in seed dispersal (24%; 41 pteropodid species), pollination (14%; 19 pteropodid species), and conflict with fruit growers (12%; 11 pteropodid species) (**Figure 1**). The database on all recorded pteropodid-plant interactions from 1985 to 2020 is in **Supplementary Information 2**. Detailed regional summaries of these studies are in **Supplementary Information 3**.

Pteropodids display unique feeding behaviours, and researchers have employed a variety of methods to study them. Pteropodid bats feed by squeezing out the juices of the plant part, which they swallow, and then spit out wads known as "ejecta" that contain the fibres and often seeds of the plant. Nectar is accessed by lapping with the bats' long tongues. Because only small seeds and juices are swallowed, morphological investigation of faecal samples on their own can generate biased diet accounts. To overcome this, some researchers use direct observations of feeding bats, collect dropped fruits and seeds, and/or record seedlings/saplings under feeding roosts and parent trees, to supplement information collected from faeces under day roosts.

Bat ejecta and partially eaten dropped fruits are distinctive, and provide reliable accounts of diet (**Supplementary Information 4**). Researchers have also used microscope analyses to identify the flower/nectar portion of the diet. More recently, molecular methods have been developed that can putatively detect all plant parts, although the limitation of this technique is that it does not distinguish the plant parts consumed, and therefore should not be used in isolation; these methods can only supplement morphological/microscopic methods but not replace them (Aziz et al., 2017b). As not all studies employed all methods equally, our results are necessarily biased by the different methodological approaches used, and variation in sampling effort (e.g., time period, seasonality, etc.).

## Pteropodid Diet

Studies have recorded $\geq$1072 food plant species from 493 genera and 148 families (**Supplementary Information 2**). Most plant taxa were recorded as being consumed for their fruit (71% of species), followed by flowers (28.6%) and leaves (8.9%). For 16 pteropodid genera (including 80% of all pteropodid species) that had sample sizes exceeding 10 consumed plant species, nectarivory (defined as $>$50% of diet species comprising floral resources following Fleming et al., 1987) was the least common diet type, found in three genera and four species (*Eonycteris spelaea, Macroglossus minimus, M. sobrinus, Syconycteris australis*) (**Figure 2**), all of which are small-bodied (16-59 g) (**Supplementary Information 2**). The large-bodied *Pteropus* ($n = 67$ species) are considered frugivores ($>$50% of diet

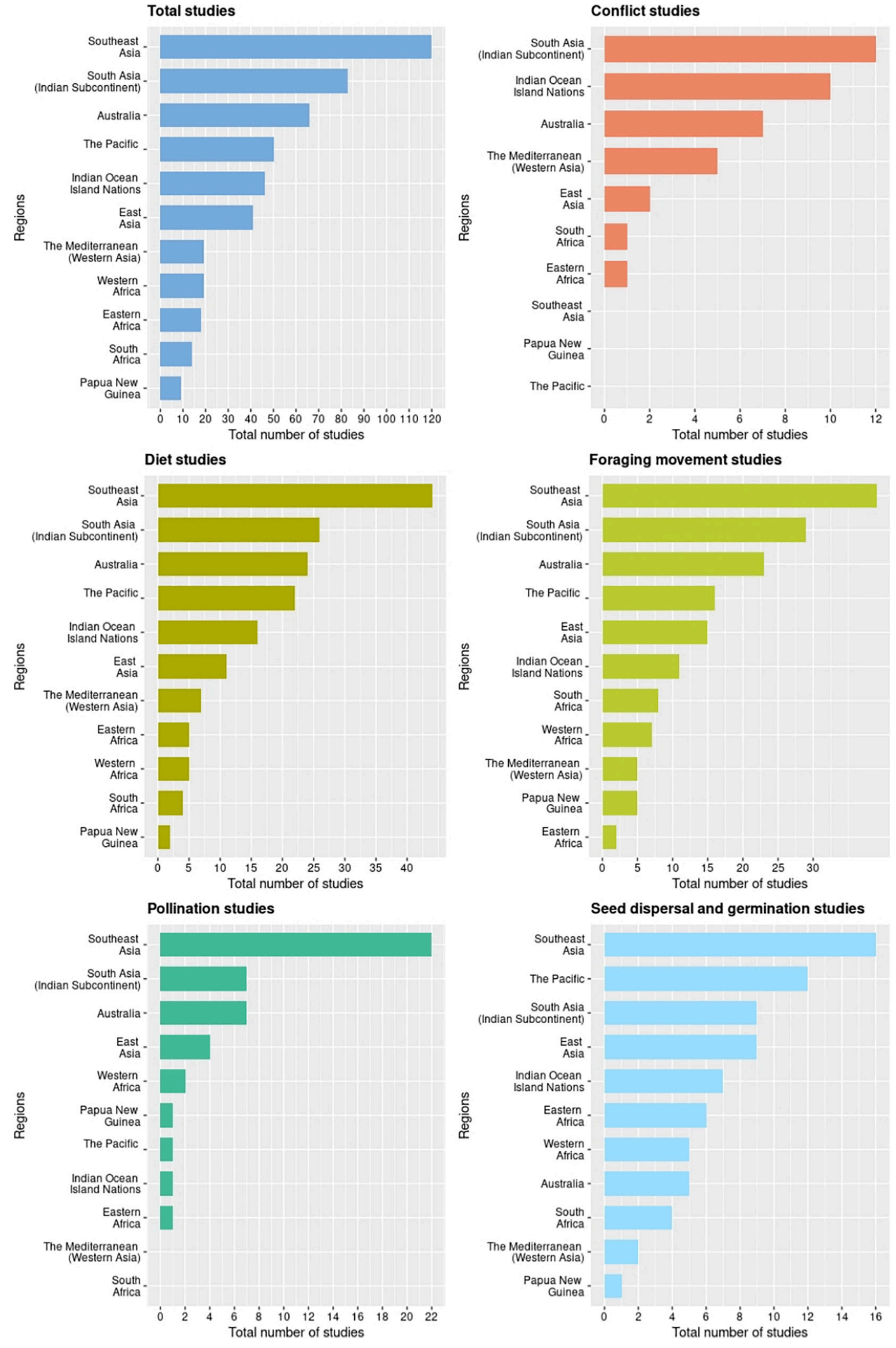

**FIGURE 1 |** Geographical distribution of research on Old World bat-plant interactions across five major themes during 1985-2020.

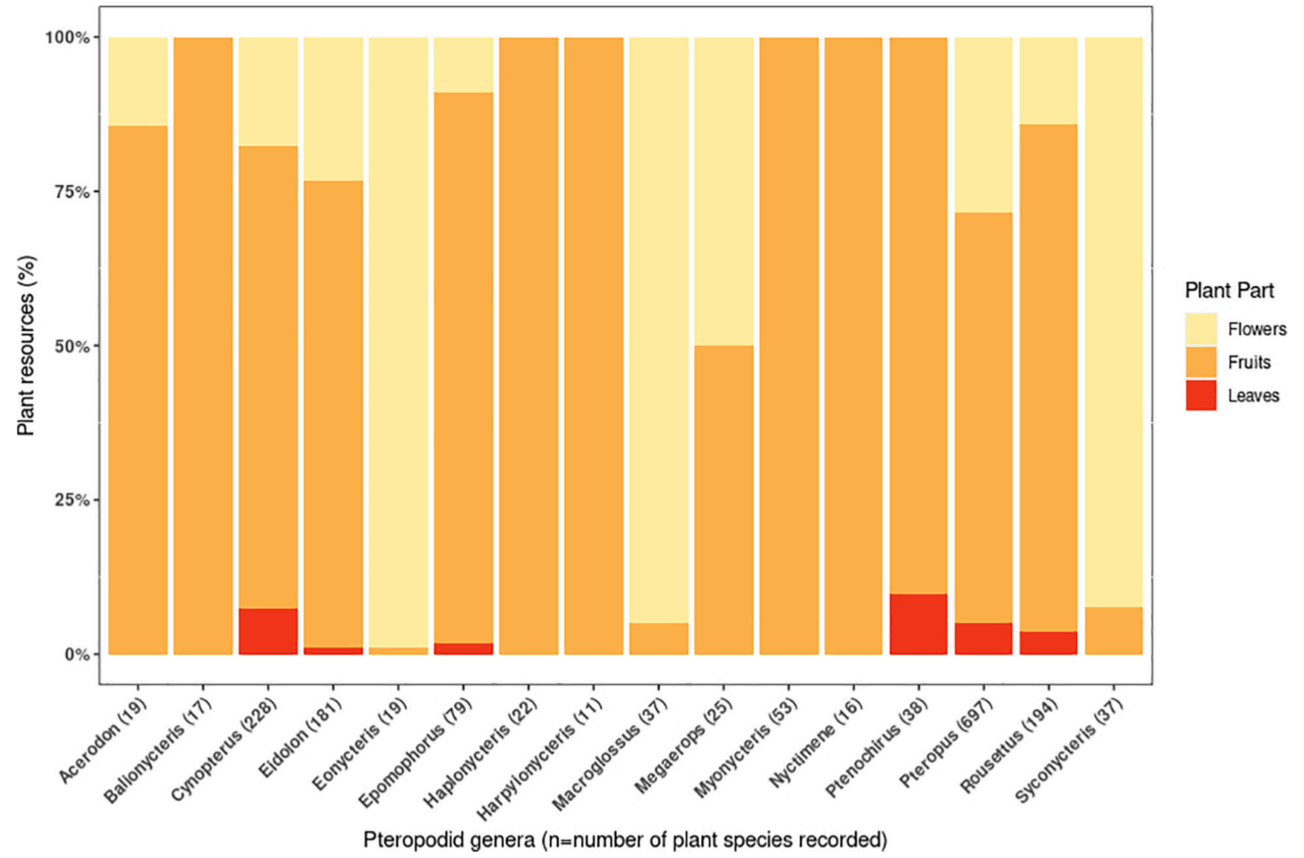

**FIGURE 2 |** Quantity of fruits, flowers and leaves recorded in the diets of pteropodid bats during 1985-2020. The quantity is represented by the proportion (percentage) of plant species consumed for each plant part, and is recorded for all studied species within 16 pteropodid genera. These genera fed on a minimum of 10 plant species recorded in the literature.

species were fruit) but had the most generalist diet, with >25% of diet as flowers and leaves.

The remaining 12 pteropodid genera (24 species) were also frugivores, covering a wide range of body weights (14-1100 g). However, the majority of pteropodid studies are biased towards detecting fruit, because plant parts are often sampled under day or night roosts, and fruits leave more recognisable remains (i.e, seeds, skin, and pulp) than pollen/nectar and leaves. For this reason, leaf consumption is considered to be more widespread than reported; because of its relatively high protein content, the "juice" of the leaves is the only part swallowed by the bats (Kunz and Diaz, 1995; Nelson et al., 2000b). Also, some pteropodid genera such as *Cynopterus, Megaerops*, and *Pteropus* have been recognised as being frugi-nectarivorous (Stewart et al., 2014; Sritongchuay and Bumrungsri, 2016; Aziz et al., 2017b; Stewart and Dudash, 2018); in one 28-month study, the most frequently occurring food item in *Pteropus rufus* faeces was *Agave sisalana* pollen, and many faecal samples were pure *Agave* pollen (Long and Racey, 2007). Further studies on frugivorous bats, employing a more holistic combination of methods over longer time periods, may reveal more diverse diets than currently recognised.

At least seven other plant parts are consumed by pteropodids, although most of these have only been recorded for the more generalist *Pteropus* spp. (**Table 1**). Twigs are the most common of these other food items, followed by seeds. Seed-eating may often be misinterpreted as fruit consumption, as several plant species with dry fruits (lacking edible pulp) were recorded as fruit consumption in studies. Seed consumption occurred mostly in the leguminous plants from the family Fabaceae. Pteropodids also consumed shoots, sap, petioles, bark and the upper stem of plants.

The plant families Arecaceae (38 genera), Anacardiaceae (18 genera), and Musaceae (all species within the genus *Musa*) provide both floral and fruit resources to multiple bat genera (**Figure 3**). Floral resources were also commonly recorded from the families Fabaceae, Malvaceae, Myrtaceae, and Bignoniaceae, with both Fabaceae and Malvaceae providing multiple resources for the highest number of pteropodid genera. Moraceae and Annonaceae provided the most common fruit resources. *Ficus* was the most commonly consumed genus, with the syconia of 114 species consumed by pteropodids. Several studies have concluded that *Ficus* dominate the diet of pteropodids (e.g., Fujita and Tuttle, 1991; Shanahan et al., 2001; Stier and Mildenstein, 2005; Oleksy et al., 2015; Aziz et al., 2017b), possibly because of the availability and abundance across seasons (Eby, 1998), but determining the accuracy of this finding has been difficult given the sampling bias in field techniques. Small-seeded *Ficus*

**TABLE 1 |** Plant parts that are a minor component in the diet of pteropodid bats.

| Plant part consumed | # plant species | Plant families (Genera) | Bat genera consuming item |
|---|---|---|---|
| Twigs | 10 | Rubiaceae (*Coffea*)<br>Araliaceae (*Schefflera*)<br>Bromeliaceae (*Ananas*)<br>Elaeocarpaceae (*Elaeocarpus*)<br>Moraceae (*Artocarpus, Ficus, Morus*)<br>Phyllanthaceae (*Bischofia*)<br>Theaceae (*Schima*)<br>Zingerbiaceae (*Alpinia*) | *Cynopterus*<br>*Pteropus* |
| Seeds | 7 | Fabaceae (*Acacia, Maniltoa, Parkia, Pithecellobium*)<br>Podocarpaceae (*Podocarpus*)<br>Arecaceae (*Cycas*)<br>Dipterocarpaceae (*Shorea*) | *Cynopterus*<br>*Pteropus* |
| Shoots | 3 | Jubulaceae (*Frullania*)<br>Metzgeriaceae (*Metzgeria*)<br>Sematophyllaceae (*Acroporium*) | *Pteropus* |
| Sap | 3 | Streliziaceae (*Ravenala*)<br>Arecaceae (*Cocos, Phoenix*) | *Pteropus* |
| Petioles | 1 | Fabaceae (*Erythrina*) | *Pteropus* |
| Bark | 1 | Moraceae (*Ficus*) | *Pteropus* |
| Upper stem | 1 | Poaceae (*Saccharum*) | *Pteropus* |

species are more likely to be found in faeces and ejecta samples than large-seeded species, which are not swallowed. Molecular techniques (e.g., Sanger and Next-Generation Sequencing) have been more recently used to investigate pteropodid diet, and this has revealed more diverse diets than determined using traditional morphological or microscope analyses (Aziz et al., 2017b; Lim et al., 2018; Chan et al., 2020).

## Foraging Landscape of Pteropodids

Of the 189 pteropodid species with habitat data recorded by the IUCN (2020), 11% were listed as dependent on primary vegetation only. Secondary habitats and agricultural areas (plantations and/or gardens) were used by 56% and 50% of species, respectively. Fifteen species (8%) are reported to use urban landscapes. Hence, the majority of pteropodid species (89%) are using various stages of disturbed habitat, and could be contributing to restoration or plant gene flow via pollination and seed dispersal. They could also serve as essential mutualists that maintain plant populations and ecosystem services in human-modified habitats (Sritongchuay et al., 2014; Oleksy et al., 2015; van Toor et al., 2019). However, this also means that the majority of pteropodids are utilising habitats that potentially bring them into conflict with humans (e.g., Aziz et al., 2016; Oleksy et al., 2018); illegal hunting is known to occur in 50% of foraging areas used by the commensal *Pteropus lylei* (Chaiyes et al., 2017).

Pteropodids use resources over a broad swathe of landscapes, with nightly foraging distances positively related to body size (Spearman Rank, $R = 0.5539$, $n = 25$ species). Movements ranged from an average of 0.1 km for the frugivorous *Cynopterus* spp. to 56 km for *Eidolon helvum*, also a frugivore ($n = 5$ studies, **Table 2** and **Supplementary Information 5**). Within this range of foraging distances travelled, the nectarivorous species generally moved shorter distances, averaging 0.25 to 5.21 km. Maximum distance moved in a night was also positively related to body size ($R = 0.5599$, $n = 22$ studies), with the longest maximum distances recorded for *Eidolon* and *Pteropus* (88 km). Within the landscapes where they forage, pteropodids use cognitive map-based navigation to forage amongst resources (*Rousettus aegyptiacus*, Harten et al., 2020; Toledo et al., 2020); *R. aegyptiacus* flew distances of up to 25 km from day roosts using a fast and straight flight track, showing loyalty to visited fruiting trees and the flight track (Tsoar, 2011). The rest of the night's foraging was spent within the vicinity of the first flight until returning to the roost before sunrise. These flight patterns have consequences for the spatial pattern of defecated seeds, with potentially very different dispersal distances achieved for seeds swallowed earlier in the night compared to those consumed later – but this aspect is currently still unstudied.

At least seven species migrate seasonally to track food resources (*Eidolon helvum, Myonycteris torquata, Nanonycteris veldkampii, Pteropus alecto, P. poliocephalus, P. scapulatus,* and *P. vampyrus*; Thomas, 1982; Richards, 1995; Richter and Cumming, 2008; Epstein et al., 2009; Moussy et al., 2013; Fleming, 2019), potentially resulting in long-distance pollen and seed movement (see below). During migration, distances of up to 370 km were recorded in a single night for *E. helvum*, with individuals travelling more than 2,500 km in total (Richter and Cumming, 2008). The fastest travel was recorded for *P. vampyrus*, moving 130 km in 2 h during migration (Epstein et al., 2009). Indirect evidence for seasonal movements related to food availability has been recorded for two of eight species studied in Malaysian forests (*Cynopterus horsfieldii, Megaerops ecaudatus*) (Hodgkison et al., 2004). Australian *Pteropus* species are highly nomadic with little uniformity among individuals (Welbergen et al., 2020), moving annually around roosts (and presumably feeding resources) across broad swathes of landscapes, with distances ranging from 1400 to 6000 km. Even for the species that do not migrate, single long-distance flights have been recorded; e.g., the 37 g *Cynopterus sphinx* and *C. horsfieldii* flew 10 km over open water to colonise Krakatau Island (Whittaker and Jones, 1994), and many island-dwelling populations move around naturally fragmented landscapes on a nightly basis (McConkey and Drake, 2007; Oleksy et al., 2019).

## Pteropodids as Pollinators

Sixteen pteropodid species from eight genera have been proven to function as pollinators (**Table 3**), based on robust scientific evidence obtained from in-depth investigations beyond mere documentation of diet, flower visitation or pollen load. Of these, the genus *Pteropus* appears to include a disproportionately high number of pollinating species within the guild ($n = 7$; 44% of all known pteropodid pollinators), related to seven different plant species, though this likely reflects the high species diversity within this pteropodid genus. The nectarivorous species *Eonycteris spelaea* alone has been proven to be a particularly important pollinator for seven different plant species, and the genus *Macroglossus* appears to be specifically important for wild bananas (*Musa* spp.) and mangrove ecosystems (Momose et al., 1998; Watzke, 2006;

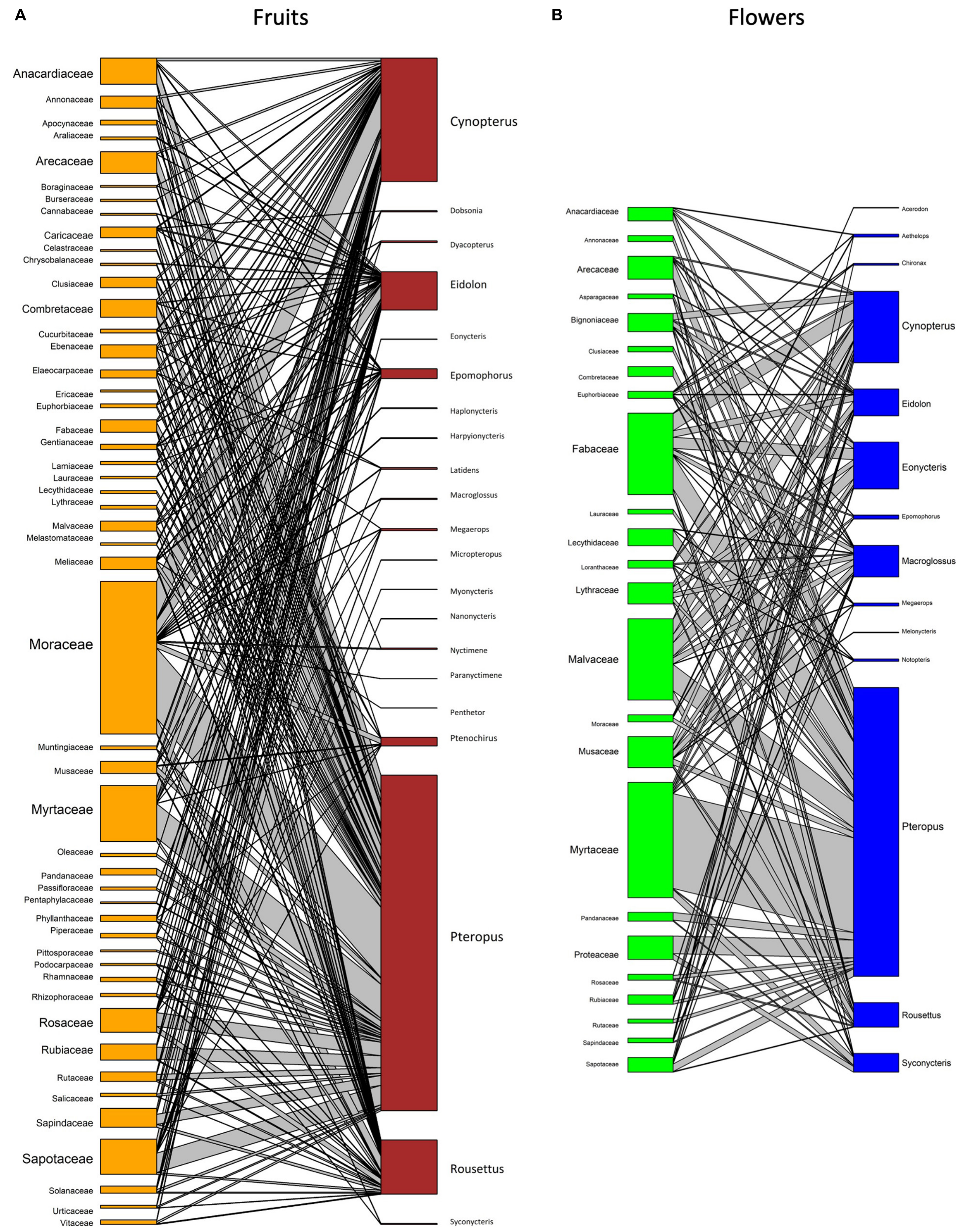

**FIGURE 3 |** Network graphs showing interactions between pteropodid bats (right) and the plant taxa (left; **(A)** flowers and **(B)** fruit) they interact with. Graphs are based on the number of species within each family consumed by pteropodid genera for five or more interactions recorded.

**TABLE 2 |** Summary of movement studies on pteropodid genera (1985–2020).

| Genera | Diet | Body weight (g) | Distance (km) | | No. of studies | Location of study |
|---|---|---|---|---|---|---|
| | | | Mean | Max | | |
| *Acerodon* | Fruit | 470-1100 | 21.0 | 87.0 | 1 | Philippines |
| *Balionycteris* | Fruit | 14 | <1 | | 1 | Malaysia |
| *Cynopterus* | Fruit | 26-58 | 1.7 | 6.0 | 6 | China, India, Malaysia |
| *Dobsonia* | Fruit | 87 | 0.6 | 1.2 | 1 | Papua New Guinea |
| *Eidolon* | Fruit | 250-300 | 56.1 | 88.0 | 5 | Burkina Faso, Ghana, Zambia |
| *Eonycteris* | Flower | 59 | 5.21 | 17.9 | 2 | Thailand |
| *Epomophorus* | Fruit | 80-110 | 1.44 | 13.9 | 3 | South Africa |
| *Macroglossus* | Flower | 16-22 | 1 | | 1 | Papua New Guinea |
| *Melonycteris* | Flower | 48 | 0.25 | 0.4 | 1 | Papua New Guinea |
| *Nyctimene* | Fruit | 30-49 | 0.5 | 1.1 | 1 | Australia |
| *Ptenochirus* | Fruit | 63-80 | 0.47 | 0.48 | 1 | Philippines |
| *Pteropus* | Fruit | 440-820 | 9.7 | 87.5 | 16 | American Samoa, Australia, Cambodia, India, Japan, India, Madagascar, Pakistan, Palau, Thailand |
| *Rousettus* | Fruit | 45-125 | 7.2 | 31.6 | 8 | China, Cyprus, Israel, Madagascar, South Africa, |
| *Syconycteris* | Flower | 18 | 1.1 | 6.8 | 2 | Australia |

*The main diet of the bat is indicated, according to whether they are nectarivores or frugivores. Mean and maximum movements in a single night are recorded in kilometres. Sources of variation in movement patterns are indicated where these have been described. Data came from 56 studies, but were focused mainly on Pteropus (n = 23).* ***Supplementary Information 5*** *has full details on foraging distances.*

Nor Zalipah et al., 2016; Stewart and Dudash, 2016, 2018; Nor Zalipah and Ahmad Fadhli, 2017; Nuevo Diego, 2018; Nuevo Diego et al., 2019), underscoring the obligate pollinator roles of the latter two genera. However, the apparently disproportionate pollinating role of *Eonycteris spelaea* compared to other species may also be a reflection of research effort, as this species is commonly found in human-dominated landscapes across most Southeast Asian countries (Francis et al., 2008), and therefore easily studied in comparison to other species. Further research would be helpful in elucidating the pollinating roles of the comparatively more threatened and rare *Pteropus, Acerodon*, and *Desmalopex*, critical facultative pollinators despite being classified as frugivores. Some of the larger *Pteropus* species have been implicated in the destruction of flowers (Soepadmo and Eow, 1977; Elmqvist et al., 1992; Nathan et al., 2009; Stephenraj et al., 2010), but this has either been incorrectly assumed (Gumal, 2001; Aziz et al., 2017c), or their positive impacts as pollinators have overridden negative effects of their feeding behaviour (Elmqvist et al., 1992; Stephenraj et al., 2010; Toyama et al., 2012). *Pteropus scapulatus* exhibits behaviour and a tongue structure that are more reminiscent of nectar-feeding bats (Birt, 2004), indicating a need to investigate the diet of all pteropodid species in more detail to understand and compare their functional roles. Documented food plants and foraging behaviour of this bat taxon provide clues that should direct future research.

Twenty-one different plant species from eight plant families are now known to be pollinated by pteropodids **Table 3**; families Fabaceae, Lythraceae, Malvaceae, Musaceae and Myrtaceae appear to have particularly important coevolutionary relationships with bats, containing multiple species that are known to be almost entirely dependent on pteropodids for effective long-distance pollen transfer (Crome and Irvine, 1986; Elmqvist et al., 1992; Law and Lean, 1999; Birt, 2004; Boulter et al., 2005; Bumrungsri et al., 2008, 2009; Bacles et al., 2009; Acharya et al., 2015; Groffen et al., 2016; Aziz et al., 2017c; Sheherazade et al., 2019; Sritongchuay et al., 2019). These plant taxa that have close associations with bats display characteristics of chiropterophily: flowers are light-coloured, large, presented on the periphery of the canopy and produce more nectar and pollen at night (Marshall, 1983). In a highly specialised case, the flowers of *Mucuna macrocarpa* in Japan open explosively when triggered by foraging *Pteropus dasymallus*, which allows pollination to occur (Toyama et al., 2012). However, just as bats can be effective dispersers of fruits that are not adapted for bat dispersal, we need improved documentation of their pollination importance for plants that are less specialised for bats.

At a community level, nectarivorous bats have been found to have higher network strength, abundance (Sritongchuay and Bumrungsri, 2016) and generalised degree (number of interactions per species divided by the number of possible interacting partners; Sritongchuay et al., 2019) than other pteropodids. However, the temporal and spatial differences that these bats show in their feeding patterns, when compared to large bats such as *Pteropus* (Aziz et al., 2017c), could suggest complementary roles in pollination. Bats are more effective pollinators than other animals for most of the documented plant species (Ratto et al., 2018; Sheherazade et al., 2019), carrying more pollen and moving across larger landscape areas compared to nectar-feeding birds (Law and Lean, 1999) or native bees (Wayo et al., 2018; Sheherazade et al., 2019). However, this finding also

**TABLE 3 |** Confirmed pteropodid pollinators and their associated bat-pollinated plant species.

| Bat Pollinator | Plant Family | Plant Species | Source/Study |
|---|---|---|---|
| *Acerodon celebensis* | Malvaceae | *Durio zibethinus* | Sheherazade et al., 2019 |
| *Cynopterus sphinx* | Fabaceae | *Mucuna championii* | Kobayashi et al., 2020 |
| | Sapotaceae | *Madhuca longifolia* var. *latifolia* | Nathan et al., 2009; Stephenraj et al., 2010 |
| *Eidolon dupreanum* | Malvaceae | *Adansonia suarezensis* | Baum, 1995 |
| *Eonycteris spelaea* | Bignoniaceae | *Oroxylum indicum* | Sritongchuay et al., 2008 |
| | Fabaceae | *Parkia speciosa* | Bumrungsri et al., 2008 |
| | | *Parkia timoriana* | Bumrungsri et al., 2008 |
| | Lythraceae | *Sonneratia alba* | Nor Zalipah et al., 2016 |
| | | *Sonneratia caseolaris* | Nor Zalipah et al., 2016 |
| | | *Sonneratia griffithii* | Nuevo Diego, 2018 |
| | Malvaceae | *Durio zibethinus* | Bumrungsri et al., 2009; Aziz et al., 2017b; Chaiyarat et al., 2019; Sheherazade et al., 2019 |
| *Macroglossus minimus* | Lythraceae | *Sonneratia caseolaris* | Watzke, 2006 |
| | | *Sonneratia griffithii* | Nuevo Diego, 2018 |
| | | *Sonneratia ovata* | Nuevo Diego et al., 2019 |
| *Macroglossus sobrinus* | Musaceae | *Musa acuminata halabanensis* | Itino et al., 1991 |
| | | *Musa itinerans* | Liu et al., 2002 |
| *Pteropus alecto* | Malvaceae | *Durio zibethinus* | Sheherazade et al., 2019 |
| *Pteropus conspicillatus* | Myrtaceae | *Syzygium sayeri* | Boulter et al., 2005 |
| *Pteropus dasymallus* | Fabaceae | *Mucuna macrocarpa* | Toyama et al., 2012 |
| *Pteropus giganteus* | Bixaceae | *Cochlospermum religiosum* | Erancheri et al., 2013 |
| | Malvaceae | *Ceiba pentandra* | Nathan et al., 2005 |
| | Sapotaceae | *Madhuca longifolia* var. *latifolia* | Nathan et al., 2009 |
| *Pteropus hypomelanus* | Malvaceae | *Durio zibethinus* | Aziz et al., 2017c |
| *Pteropus poliocephalus* | Myrtaceae | *Corymbia citriodora* | Bacles et al., 2009 |
| *Pteropus tonganus* | Malvaceae | *Ceiba pentandra* | Elmqvist et al., 1992 |
| *Rousettus leschenaultii* | Malvaceae | *Ceiba pentandra* | Nathan et al., 2005 |
| Pteropodidae (species unknown) | Fabaceae | *Parkia biglobosa* | Lassen et al., 2012 |
| Pteropodidae spp. (either *Macroglossus lagochilus* or *Syconycteris australis*, or both; indistinguishable in the field) | Myrtaceae | *Syzygium cormiflorum* | Crome and Irvine, 1986 |
| Pteropodidae spp. (*Macroglossus minimus* & *Syconycteris australis* grouped together without species-specific diet) | Myrtaceae | *Syzygium sayeri* | Boulter et al., 2005 |
| Pteropodidae (species unknown) | Malvaceae | *Adansonia digitata* | Djossa et al., 2015 |

reflects the choice of plants studied, which have been primarily crop or timber plants important to humans that were already suspected to be bat-pollinated. To understand the importance of bat pollination at the community level, we require more studies in wild ecosystems, but the cost, effort and time required to confirm pollination in these environments is likely to be challenging.

## Pteropodids as Seed Dispersers

Seed dispersal studies, including seed germination experiments, have been conducted on 41 pteropodid species from 15 genera, but only 29 pteropodid species from 9 genera have been recorded actively dispersing seeds, with documented dispersal for fruits from 311 plant species from 184 genera and 75 families. The genera with the most bat-dispersed species were *Ficus* (60 species), *Syzygium* (14 species), and *Diospyros* (8 species). Bats processed seeds gently in most cases (see below), and therefore most of the 687 species listed as fruit resources in our database (**Supplementary Information 2**) are potentially dispersed by bats. Six methods have been used to document seed dispersal by different pteropodid species (**Table 4**). Direct observations at the parent tree or of fruit being carried away have been observed for most species, followed by investigations of ejecta and faeces under day roosts.

### Fruit Selection

The primary cue pteropodids use for finding fruit and determining ripeness is odour Kshitish (Acharya et al., 1998; Luft et al., 2003; Hodgkison et al., 2007, 2013; Raghuram et al., 2009; Shafie et al., 2014), and a strong odour is among the plant traits considered indicative of bat attraction and the bat-fruit syndrome (Bollen et al., 2004; Hodgkison et al., 2013). However, pteropodids also rely, to a significant extent, on vision and have enlarged eyes and a visual cortex (Speakman, 2001). Researchers have suggested "light-coloured" fruit (and probably flowers) are an adaption for bats to find food in dark conditions (Richards, 1990), although bats consume fruit exhibiting a diversity of colours (Hodgkison et al., 2003), and at least some pteropodids are sensitive to the ultraviolet spectrum (Li et al., 2018). Other characteristics of bat-preferred fruits include fruits with high quantities of water and sugar, and low quantities of fats and proteins (Korine et al., 1998; Bollen et al., 2004; Nelson et al., 2005) and an unusual syndrome of dry fruits with a high protein content found in Mediterranean habitats (Korine et al., 1998). Fruit displays, in which the fruits are held away from the foliage and therefore more accessible to bats, are also commonly reported in bat-consumed fruits. These include fruits produced from the trunk (cauliflory), fruits available from leafless main branches (ramicarpy), or fruits on the end of long downward-pointing peduncles (flagelliflory) (Richards, 1995; Hodgkison et al., 2003; Bollen et al., 2004).

Across the studies, information was only available on fruit colour (collected for $n$ = 141 plant species) and not fruit odour, chemical composition or display. Bats dispersed seeds from fruits representing a diverse range of colours; here we categorised most species as red (31 species), green (29 species), or yellow (23 species). Fewer fruits were purple (15 species), brown (12 species), orange (11 species), black (10 species), or white (7 species), with just a few species having blue (2 species) or pink (1 species) fruits. While this colour diversity is predominantly light coloured (yellow, orange, or white = 41 species) or with no distinguishing colour against the background (dull: green or brown $n$ = 41 species), collectively, the bats show use of a broad range of fruit colours (59 species that are neither dull nor light).

Some pteropodid species are regular visitors to orchards and/or gardens, where they come into conflict with farmers by consuming fruit crops (Aziz et al., 2016). Foraging *Pteropus tonganus* in Fiji was four times more abundant in farmland than in forests, and territorial disputes over food were only documented in farmland (Luskin, 2010). This suggests a potential preference for cultivated landscapes, either because of resource distribution, resource abundance, and/or the nutritional content of the plant resources, which could disrupt natural seed dispersal processes (McConkey and Drake, 2006). However, even though bats can consume large quantities of cultivated fruits (Oleksy et al., 2018), some studies have found that pteropodids prefer to forage on native species rather than on introduced fruits (Korine et al., 1999; Nelson et al., 2000a; Mildenstein et al., 2005; Andrianaivoarivelo et al., 2012).

## Fruit Processing

Pteropodids feed by mainly swallowing fruit juices, spitting out fruit fibres and seeds as ejecta. Seeds are dispersed in ejecta, by swallowing and defecating, and by physically carrying the entire fruit; for all dispersal modes researchers report "gentle" seed treatment where seeds are dispersed undamaged. The capacity to swallow seeds is limited to the diameter of the oesophageal lumen, which can extend to 4-5 mm in the larger Australian *Pteropus* (Richards, 1995). Hence, only small seeds ($\leq$6 mm width) are dispersed through swallowing and defecation (Shilton et al., 1999; Shanahan et al., 2001; Oleksy et al., 2017). The largest seeds dispersed by defecation were 6 mm wide (*Pteropus rufus* in Madagascar; Bollen et al., 2004), and large fruit bats could disperse slightly larger seeds than small fruit bats in this way (Spearman Rank test significant at 10% level; $R$ = 0.3934, $n$ = 22 bat species); however, the fruit size of defecated seeds was unrelated to bat size ($R$ = 0.5003, $n$ = 19 bat species). Seeds dispersed by ejecta were inconsistently reported, so we did not test for a relationship with body size. Maximum widths of 12 mm were noted in ejecta, and often seeds dispersed by defecation were also dispersed in ejecta.

Seeds that are too large to be swallowed are dispersed when bats carry fruits in their mouth away from the parent plant. The largest seed dispersed by pteropodids was for *Mangifera indica* which can reach widths of 71 mm. *Mangifera* was only consumed by large bats ($\geq$250 g in body weight), indicative of the positive relationship between seed width and pteropodid body size ($R$ = 0.6994, $n$ = 14 bat species), and fruit width and pteropodid body size ($R$ = 0.8299, $n$ = 12 bat species). More important than the width of the fruit is its weight, and pteropodids can carry heavy fruits relative to their body weight. This has rarely been described, but the weight of carried fruits has ranged from 0.5 to 1.6x the body weight of the bat (Nakamoto et al., 2015; Mahandran et al., 2018). Therefore, pteropodids of more than 1 kg could potentially have the capacity to disperse fruits of more than 1.5 kg – though this requires further investigation.

The ability of pteropodids to carry heavy fruits indicates that body mass alone is a poor surrogate of these bats' dispersal abilities, as pteropodids are potential dispersers of fruits consumed by much larger animals – even elephants and rhinoceroses, which disperse mango seeds via defecation (Sridhara et al., 2016). Pteropodids can also consume large quantities of fruit relative to their own body mass; e.g., *Rousettus aegyptiacus* consumes fruit up to a maximum of 150% of its body mass (Izhaki et al., 1995). The high density at which some populations of large pteropodids can occur (Richter and Cumming, 2005; Tait et al., 2014) also implies high rates of consumption at the community level. This has rarely been quantified, but a single colony of *Eidolon helvum,* estimated at 152,000 individuals, was predicted to provide 338,000 dispersal events in a single night (van Toor et al., 2019). Even during the seasonal reduction in colony size, the authors estimated these bats moved 5,500 seeds in a night.

Population abundance is a key factor driving the seed dispersal importance of Neotropical bats (Laurindo et al., 2020), and is likely to be of similar importance for pteropodids. The importance of abundance *per se* has not been investigated in pteropodids, but sufficient population density to induce territorial disputes is essential for effective seed dispersal of large-seeded fruit by *Pteropus* species (Richards, 1995; McConkey and Drake, 2006; Mahandran et al., 2018). *Pteropus* preferentially feed on and defend small territories within fruiting trees. Seed dispersal occurs when newcomers attempting to access the tree cannot forage, because the tree is full of feeding territories already claimed by earlier arrivals – the "raiders versus residents" phenomenon (Richards, 1990). The new arrivals snatch a fruit and fly elsewhere to consume it, hence dispersing the seed from the parent tree. This behaviour has not been reported for other genera apart from *Eidolon* (Racey, pers. obs.), but small pteropodids frequently remove fruit from a parent tree for consumption at regularly used feeding roosts (Utzurrum, 1995; Deshpande and Kelkar, 2015). This feeding behaviour results in more consistent seed dispersal than that by the large 'resident feeder' pteropodids who may usually consume fruit more often at the parent plant, and are thus only effective at dispersing swallowed seeds. Eleven of these small pteropodid species with available seed dispersal information have been noted to use feeding roosts and/or have been observed carrying fruit away from the parent tree (**Table 4**).

## Seed Consumption

While researchers report consistently gentle seed processing by pteropodids, some seed-eating has been recorded in at least three *Pteropus* species (involving *Cycas* spp., Cox et al., 2003; *Podocarpus pallidus, Maniltoa grandiflora,* McConkey and Drake, 2015; *Shorea* sp. and an unknown species, Ong, 2020). "Fruit" consumption recorded for plants that have no fleshy portion are potentially also examples of seed consumption. For large seeds, such as *Cycas* spp. and *Maniltoa grandiflora,* consumption does not necessarily prevent effective seed dispersal, since seeds are not entirely consumed and could still germinate (McConkey and Drake, 2015). Seed-eating bats have also been recorded in the Neotropics, where *Chiroderma* spp. have cranio-dental morphology that promotes consumption of fig seeds (Nogueira and Peracchi, 2003; Nogueira et al., 2005); *Centurio senex* has also been recorded eating seeds with soft endocarps (Villalobos-Chaves et al., 2016). It is possible that seed-eating is more prevalent than currently appreciated in pteropodids, as an alternative strategy to folivory and insectivory, to obtain sufficient protein (Courts, 1998; Clulow and Blundell, 2011). Given the lack of apparent morphological adaptations to consume hard items, seed-eating by pteropodids would be expected to be limited to softer seeds; however, *Latidens salimalii* possibly consumes the softer content of nuts by biting through an extremely hard, thick coat (Agoramoorthy and Hsu, 2005).

## Dispersal Distances

Some pteropodid species have the capacity to disperse seeds farther than has been recorded for any other animal (**Figure 4**). The maximum dispersal distances recorded for small bats ($<$250 g) were 30 km for defecated seeds ($n$ = 6 studies) with fat-tailed kernels (Tsoar, 2011), while larger bats ($\geq$250 g) defecated seeds over distances up to 24 km (non-migratory) and 88 km (migratory) ($n$ = 8 studies) (**Supplementary Information 6**). Most seeds pass through pteropodid guts in 30–116 min, but some seeds are retained for up to 24 h (Shilton et al., 1999; Abedi-Lartey, 2016; Oleksy et al., 2017). If the longer gut retention

**TABLE 4 |** Seed dispersal study methods according to pteropodid species.

| Bat species | Observed in parent tree | Observed carrying fruit away | Ejecta/faecal samples below feeding roost | Seed traps/ejecta/faecal samples below day roost | Ejecta beneath parent tree | Ejecta/faecal samples along transects |
|---|---|---|---|---|---|---|
| *Cynopterus brachyotis* | x | x | x | x | | x |
| *Cynopterus horsfieldii* | x | x | | x | | |
| *Cynopterus minutus* | x | x | | | | |
| *Cynopterus sphinx* | x | x | x | x | | |
| *Cynopterus titthaecheilus* | x | x | | | | |
| *Eidolon helvum* | | x | | x | | |
| *Epomophorus crypturus* | x | x | | | | |
| *Epomophorus wahlbergi* | x | x | | | | |
| *Macroglossus minimus* | | | | | | x |
| *Macroglossus sobrinus* | x | x | | | | |
| *Nyctimene rabori* | | | | x | | |
| *Ptenochirus jagori* | | | | x | | x |
| *Ptenochirus minor* | | | | | | x |
| *Pteropus conspicillatus* | x | x | | x | | |
| *Pteropus dasymallus* | | x | x | | x | x |
| *Pteropus giganteus* | x | x | | x | | |
| *Pteropus mariannus* | | | | | | x |
| *Pteropus niger* | | | | | | x |
| *Pteropus poliocephalus* | | | | | x | |
| *Pteropus rufus* | x | | x | x | | |
| *Pteropus samoensis* | | x | | | | |
| *Pteropus tonganus* | x | | | x | x | x |
| *Rousettus aegypticus* | | | | | | |
| *Rousettus amplexicaudatus* | | x | | | | |
| *Rousettus leschenaultii* | | x | | x | | |
| *Rousettus madagascariensis* | x | x | | | | |

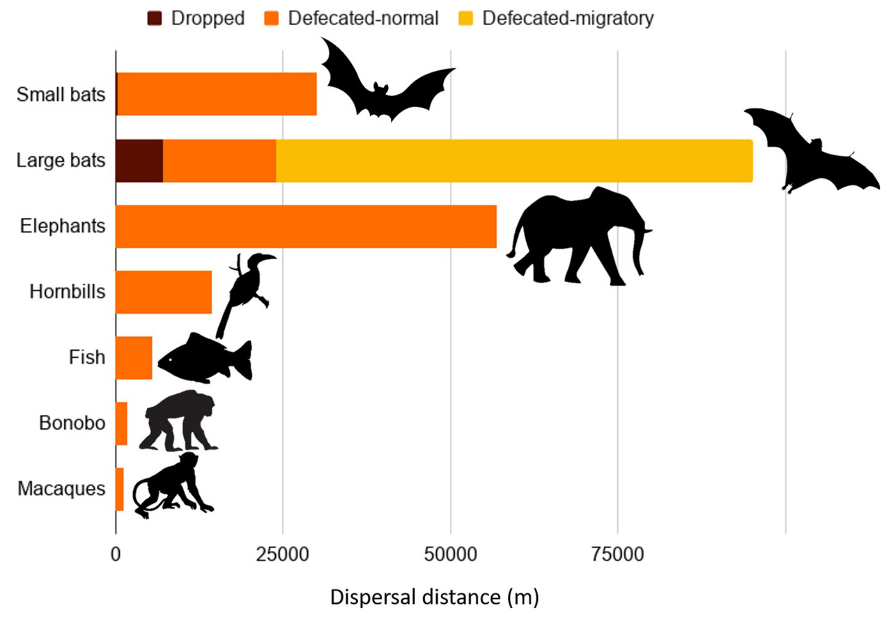

**FIGURE 4 |** Maximum seed dispersal distances for small (<250 g) and large (≥250 g) pteropodid bats and the comparison with other taxa confirmed to perform long-distance seed dispersal. References for the comparisons are given in the text. Dispersal distances for bats are given for seeds defecated without swallowing (dropped or spat), and defecated seeds during migratory and non-migratory periods.

times are used to estimate dispersal distances, bats can potentially disperse seeds up to 300 km from the parent tree (Shilton et al., 1999), at least for migratory species that traverse such long distances. These dispersal distances are within (or farther than) the maximum range reported for the longest seed dispersers recorded: elephants (6-57 km; Campos-Arceiz and Blake, 2011), fish (5.5 km; Anderson et al., 2011), and hornbills (3.5 to 14.5 km; Kitamura, 2011; **Figure 4**). In comparison, primates in the Palaeotropics have shorter dispersal distances of up to 1.3 km for macaques (McConkey, 2018) and 1.8 km for bonobos (Tsuji et al., 2010).

Seeds that are too large to be swallowed by bats are dispersed much shorter distances than described above, but documenting these patterns accurately has been difficult. Maximum distances of 400 m have been recorded for seeds spat out in ejecta or dropped by small pteropodids ($n = 9$ studies), and 7.2 km for large pteropodids ($n = 7$ studies). In the case of large pteropodids, these distances are still longer than those recorded for many other taxa (**Figure 4**), and qualifies them as long-distance seed dispersers (Cain et al., 2000). Average dispersal distances for seeds across all pteropodids and handling methods is 0-150 m, but it is difficult to determine the frequency of these distances. While dispersal distances of defecated seeds are estimated using gut retention times and movement data, seeds that are dropped or spat out are recorded through direct observations of foraging bats or locations of feeding roosts. Documenting long flights of bats carrying fruits is difficult, and hence has only been reported rarely.

Pteropodids vary in their use of feeding roosts, and this behaviour influences the likelihood of whether seeds are deposited under or away from the crowns of parent plants. Several pteropodid species have been recorded to use feeding roosts (**Table 4**) and could be carrying fruit away from parent crowns regularly, regardless of food-processing behaviour. This is most often observed in small pteropodid species (Bhat, 1994; Hodgkison et al., 2003; Deshpande and Kelkar, 2015), which move seeds 37-200 m for processing in other trees. The large-bodied *Pteropus* species process fruit within the crown, dropping 70% or more of seeds that are too large to be swallowed in this area, and yielding a likelihood of dispersal away from the plant crown that is dependent on the abundance of feeding bats (described above in "Fruit processing") (McConkey and Drake, 2006; Nakamoto et al., 2009; Mahandran et al., 2018).

## Seed Deposition and Germination

Bats defecate in flight (Tan et al., 2000), so can theoretically disperse seeds anywhere along their foraging routes. This is particularly relevant for restoration of disturbed areas, as bats only have to pass over habitat to disperse seeds into it (Sritongchuay et al., 2014; Oleksy et al., 2015). However, use of feeding roosts concentrates seed rain in more limited areas. Smaller bodied *Cynopterus* species create small homogenous seed shadows, while the larger bodied *Pteropus* create large heterogeneous patterns (Deshpande and Kelkar, 2015). While these two genera have been recorded facilitating seed dispersal in urban areas (Corlett, 2006; Vendan and Kaleeswaran, 2011; Chan et al., 2020), human activities may impact successful seedling recruitment, e.g., due to seed deposition on unsuitable substrates inhibiting germination, or seedlings crushed by footfall due to higher human traffic.

As *Pteropus* and *Acerodon* spp. are canopy feeders, this dispersal guild may be particularly important to canopy-germinating seeds of strangler figs (Laman, 1995;

Shanahan and Compton, 2001). However, the effects of dispersers on germination are notoriously variable among plant-animal combinations (Traveset, 1998). Pteropodid bats showed the same variable pattern in the 67 bat-plant interactions for which germination tests have been conducted (46 plant species, 25 bat species), but showed an overall tendency for neutral (60%) or positive effects (33%) (**Table 5**). Negative effects were recorded for just four interactions (5%), with three interactions showing a decrease in germination speed, and two interactions showing a decrease in germination rate. Seeds of *Ficus* were most commonly tested (31 interactions), demonstrating mainly positive (14 interactions), or neutral (16 interactions) effects, with one inconclusive test. Two studies compared germination of *Ficus* in ejecta and faeces, and found either positive effects for seeds in faeces, or inconclusive results. A further 41 pteropodid-plant interactions were inferred to be successful based on the presence of saplings under feeding roosts.

## Double Mutualisms: Bats as Pollinators and Seed Dispersers of the Same Species

Pteropodids act as both pollinators and seed dispersers (**Figure 5**), and are therefore potentially important "double mutualists," i.e., playing a beneficial role in two different functions (Fuster et al., 2019). Double mutualisms have been reported rarely (302 records in a review by Fuster et al., 2019), and appear to occur more commonly on islands (Olesen et al., 2018; Fuster et al., 2019). Here 138 bat-plant interactions were recorded which are potential examples of double mutualisms, involving 26 pteropodid species with 91 plant species from 36 families (**Figure 6**). The plant families Myrtaceae, Sapotaceae, Musaceae, Arecaceae and Anacardiaceae, in particular, may potentially benefit from double mutualisms involving several different pteropodid species across different countries. Many *Pteropus* species are probably double mutualists, reflecting the more generalist diet of the genus. Both bat pollination and seed dispersal have been confirmed for *Musa acuminata* (Itino et al., 1991; Tang et al., 2007b; Meng et al., 2012) and *Madhuca longifolia* (Nathan et al., 2009; Stephenraj et al., 2010; Mahandran et al., 2018), but most of these potential double mutualisms are based on fruit and flower feeding records, some of which are also confirmed seed dispersal records (**Supplementary Information 2**).

As many studies report non-destructive feeding behaviour, diet lists could provide a reasonable suggestion of function, especially for seed dispersal, but most of the potential double mutualisms we report require further investigation in order to confirm this functional role. Given the known examples of plant self-incompatibility (Bumrungsri et al., 2009), flower destruction (Gumal, 2001), and fruit consumption at parent trees (McConkey and Drake, 2006), pteropodid diet on its own cannot, and should not, be used to confirm ecological roles. While seed dispersal potential can be inferred from foraging movements, more in-depth research is needed to determine whether a pteropodid species serves as a pollinator or flower predator; e.g., *P. vampyrus* in Sarawak is reported to destructively consume whole flowers of *Madhuca motleyana* (Gumal, 2001), and *Cynopterus* spp. appear to have limited effectiveness as true pollinators despite non-destructive feeding

**TABLE 5 |** Summary of results of germination tests on seeds dispersed by pteropodids.

| Pteropodid genus | Number of plant species and the recorded effects on germination speed or rate | | | | Source/Study |
|---|---|---|---|---|---|
| | Positive | Neutral | Negative | Inconclusive | |
| *Balionycteris* | | 5 | | 2 | Hodgkison et al., 2003 |
| *Chironax* | | 1 | | | Hodgkison et al., 2003 |
| *Cynopterus* | 1 | 7 | 1 | | Shilton et al., 1999; Hodgkison et al., 2003; Tang et al., 2007a, Tang et al., 2008, 2012; Mahandran et al., 2018 |
| *Dyacopterus* | | 2 | | | Hodgkison et al., 2003 |
| *Eidolon* | 4 | 23 | | | Webala et al., 2014 |
| *Epomophorus* | 3 | 4 | 1 | | Djossa et al., 2008; Voigt et al., 2011; Jordaan et al., 2012; Helbig-Bonitz et al., 2013 |
| *Micropteropus* | 1 | | | | Djossa et al., 2008 |
| *Myonycteris* | 1 | | | | Djossa et al., 2008 |
| *Nanonycteris* | 1 | | | | Djossa et al., 2008 |
| *Nyctimene* | 1 | | | | Utzurrum and Heideman, 1991 |
| *Ptenochirus* | 1 | | | | Utzurrum and Heideman, 1991 |
| *Pteropus* | 7 | 29 | | | Entwistle and Corp, 1997; Bollen and van Elsacker, 2002; Goveas et al., 2006; Vendan and Kaleeswaran, 2011; Scanlon et al., 2014; Chen et al., 2017; Oleksy et al., 2017 |
| *Rousettus* | | 9 | 2 | | Izhaki et al., 1995; Tang et al., 2007a; Mahandran et al., 2018; Andrianaivoarivelo et al., 2011 |
| Unidentified | 1 | | | 1 | Utzurrum, 1995; Djossa et al., 2008 |
| Total | 21 | 79 | 4 | 3 | |

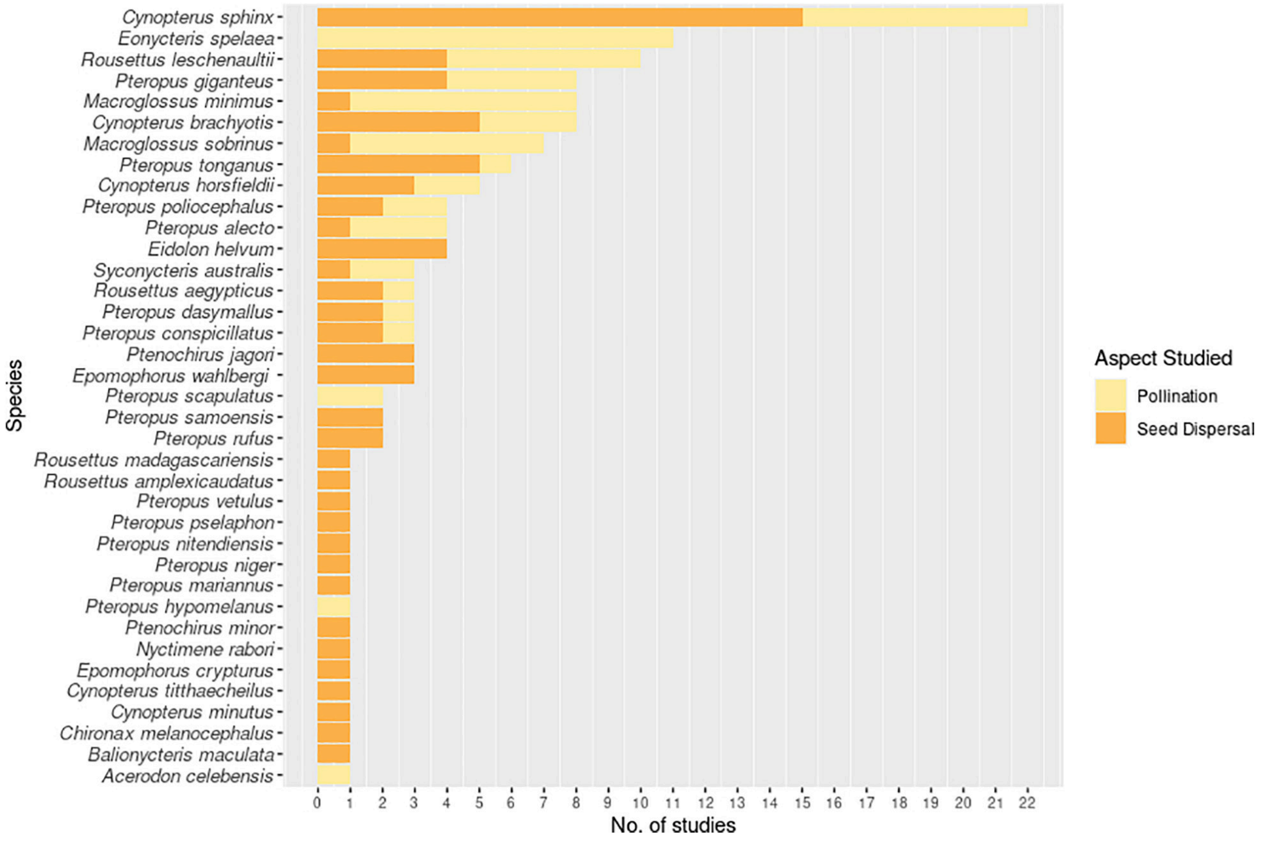

**FIGURE 5 |** Number of studies investigating the roles of specific pteropodid species as pollinators or seed dispersers during 1985-2020.

(Stewart and Dudash, 2017). However, flower consumption does not necessarily prevent successful pollination from happening for an individual plant, as long as some gynoecia remain intact and have a chance to receive conspecific pollen in the process (Elmqvist et al., 1992; Nathan et al., 2009; Stephenraj et al., 2010; Toyama et al., 2012).

It is also important to note that some plant species that could potentially benefit from both pollination and seed dispersal by bats (e.g., *Agave americana, Diospyros kaki, Eriobotrya japonica, Mangifera indica*, *Manilkara zapota, Musa acuminata, Prunus armeniaca, P. persica, Psidium guajava,* and many *Syzigium* species – although, pollination for all except *M. acuminata* is currently still unconfirmed) are commercially important trees, commonly planted in gardens, orchards and plantations – and as such, bat consumption of these fruits can be perceived as a form of ecosystem disservice (Zhang et al., 2007; Shackleton et al., 2016).

## Intra-Specific Variation in Ecological Function

Variation among individuals is important for maintaining diverse seed dispersal and pollination roles at the population level (Zwolak, 2018; Schupp et al., 2019). To our knowledge, intra-specific or intra-population variation in pteropodids has only been described for movement distances, although the independent movement trajectories that individuals from a single roost display (e.g., Tsoar, 2011; Harten et al., 2020; Toledo et al., 2020; Welbergen et al., 2020) could mean considerable variation in foraging choices as well (e.g., Scholesing et al., 2020). Future studies might reveal broad variation in food choice, seed dispersal, and pollination among individuals. Of the 160 studies on pteropodid foraging movements (**Supplementary Information 5**), intra-population variation in movement distances was described for five genera, and for three of these (*Cynopterus, Pteropus*, and *Rousettus*) female bats moved further than males. Sexually immature *Melonycteris* moved further than adults, and seasonal and habitat differences in ranging distances were found for *Eidolon helvum* and *Rousettus aegyptiacus*.

## Function of Pteropodids at the Community Level

The role that pteropodids fulfil within the wider pollinator and seed disperser communities is poorly understood. Twenty-six studies have investigated either pollination or seed dispersal roles of bats across multiple plant species, and compared these roles with other animals in the community. While feeding assemblages have been documented for individual plant species, particularly to document pollination of cultivated plants, we cannot interpret the broader community roles from these

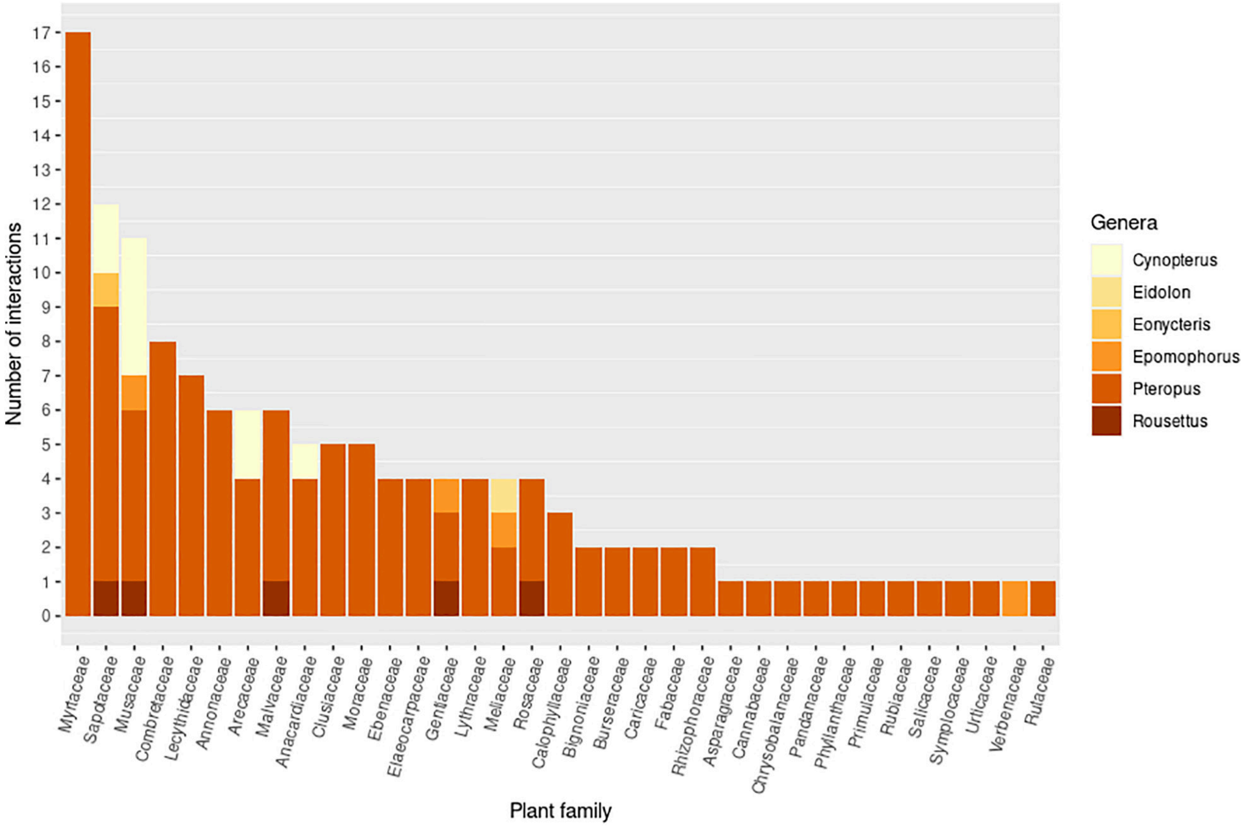

**FIGURE 6 |** Pteropodid-plant interactions that are potentially double mutualisms, in which a pteropodid species might act as both pollinator and seed disperser for the same plant species. Double mutualisms were assessed at the species level for both plants and bats. The graph shows the number of species-species interactions within each plant family and pteropodid genus. Only families with more than one interaction are shown. Plants in an additional 13 families were recorded for overall pteropodid diet, but only one interaction was recorded for each, and thus not included here.

studies. In the Tongan archipelago, *Pteropus tonganus* was the only effective seed disperser of 57% of the plant species that it consumed, an importance resulting from the prehistoric extinctions of other large dispersers (McConkey and Drake, 2015). The importance of *Pteropus* on islands is also reflected in their large diet breadth. *Pteropus tonganus* and *P. samoensis* interacted with 59% of the forest tree species on Samoa, and about 25% of the woody plant species in Mauritius have their seeds dispersed by *P. niger* (Florens et al., 2017), while *P. ornatus, P. tonganus*, and *P. vetulus* were found to be far more efficient seed dispersers compared to introduced rats on New Caledonia (Duron et al., 2017). In the Philippines, birds dispersed a higher diversity and number of seeds into successional forests (Ingle, 2003; Gonzales et al., 2009), and there was substantial overlap in fruit consumption between birds and bats especially during some seasons (Gonzales et al., 2009). Given the depauperate frugivore and pollinator communities on islands, pteropodid bats are likely to play dominant, keystone roles within these systems wherever their populations have not been decimated by hunting or pest control (Cox et al., 1991). The high *Pteropus* diversity across islands and probable origin of the genus from the islands of Wallacea (Tsang et al., 2020) suggest a long evolutionary history in island ecosystems, which could be reflected in the ecological roles they perform there.

Within continental habitats, the importance of bat pollination and seed dispersal have been studied at the community level in Australia and Thailand (e.g., Birt, 2004; Sritongchuay et al., 2019), but no community-wide studies have been conducted in most regions. In a dipterocarp forest in Peninsular Malaysia, *Pteropus vampyrus* occurred in a different module of a seed dispersal network compared to smaller bats, indicating they tended to interact with a different subset of plant species (Ong, 2020). The large bats shared a module with other large mammals, including elephants and frugivorous primates, while the smaller bats occurred in the same module as squirrels and rats. *Pteropus vampyrus* was found to be among the 10 most important seed dispersers in the community, with smaller bats also playing an important community role. A study focused on bat-used fruit resources in a Malaysian rainforest found that 56% of the resources used by bats were also consumed by primates, civets or squirrels, but the ecological roles were not evaluated (Hodgkison et al., 2003).

In Peninsular Malaysia and Thailand, small pteropodids are important *Sonneratia* pollinators in mangroves (Nor Zalipah et al., 2016; Stewart and Dudash, 2016, 2017; Nuevo Diego, 2018;

Nuevo Diego et al., 2019), with *Pteropus* spp. likely playing a similar role possibly supplemented by seed dispersal (Gumal, 2001; Weber et al., 2015; Wee et al., 2017). Since mangrove health can be crucial for maintaining healthy functioning coral reefs (Mumby, 2006; Yates et al., 2014; Loh et al., 2018; Friess et al., 2020), this suggests that the ecological importance of pteropodids extends beyond terrestrial and even coastal/estuarine ecosystems to potentially influence the health of marine ecosystems too. To fully understand the importance of pteropodid bats at the community level, we need broad studies across a greater breadth of ecosystems. Because pteropodids are important for both pollination and seed dispersal, studies focusing on a single function will underestimate their importance to ecosystems, and hence ambitious studies are needed that measure pollination and seed dispersal jointly.

Population abundance is an important predictor of functional importance for a broad range of animals including bats (Bauer and Hoye, 2014; Winfree et al., 2014; Simmons et al., 2019; Laurindo et al., 2020). Even animals that rarely disperse seeds can be important contributors to community-wide seed dispersal if they occur in very abundant populations, as has been documented for ducks (Soons et al., 2016) and proposed for the now extinct passenger pigeon (Webb, 1986). Hence, the sheer abundance of some pteropodid populations (Roberts et al., 2012; van Toor et al., 2019) combined with their effectiveness as pollinators and seed dispersers suggest vital functional roles within the ecosystems where they occur. The fact that many of these abundant pteropodid species are also migratory, thereby promoting long-distance dispersal of pollen and seeds (Bauer and Hoye, 2014), creates an even greater urgency in understanding the community-level roles of pteropodids.

## Assessing the Ecosystem Services for Humans Provided by Pteropodids

Although the majority of studies on the ecological roles of pteropodids have focused on seed dispersal, attempts to document the benefits these bats bring to humans have more frequently focused on pollination, likely because such benefits are more direct and can be more easily quantified in economic terms. The majority of pollination studies have documented the ecosystem services provided by bats for major fruit crops in Asia such as durian (*Durio zibethinus*; Bumrungsri et al., 2009; Acharya et al., 2015; Aziz et al., 2017c; Sheherazade et al., 2019), petai (*Parkia* spp.; Bumrungsri et al., 2008), midnight horror (*Oroxylum indicum*; Sritongchuay et al., 2008), and madhuca (*Madhuca longifolia*; Nathan et al., 2009; Stephenraj et al., 2010), with some Australian research focusing on important timber species (e.g., Bacles et al., 2009). The durian pollination services from pteropodids in Sulawesi, Indonesia alone have been valued at $117 ha/fruiting season or USD 450,000 for a single village (Sheherazade et al., 2019). The only study to place a financial value on seed dispersal was for *Eidolon helvum* and its role in reforestation in Ghana. van Toor et al. (2019) estimated that bats contributed a total annual gross revenue of $11,939 to $858,068 per bat colony depending on season and area. In an unusual case, the fruit foraging behaviour of *Cynopterus* and *Pteropus* in mixed-fruit agricultural landscapes in India was perceived by farmers to be beneficial (Deshpande and Kelkar, 2015); the bats aggregate cashew (*Anacardium occidentale*) and *Areca* palm nuts in accessible places, which reduces the labour required for collection. In addition, their pollination service was recognised for bananas (*Musa* spp.) and kapok (*Ceiba pentandra*), so that overall, farmers perceived that bats brought more benefits than the losses through crop raiding.

Pteropodids have also been implicated in potential ecosystem disservices to humans through their consumption of agricultural fruits (Zhang et al., 2007; Aziz et al., 2016) and the dispersal of exotic plants (Corlett, 2005; von Döhren and Haase, 2015; Vaz et al., 2017; Chan et al., 2020). As such, future approaches to assessing, quantifying and valuing the ecological roles of pteropodids should attempt to more be more holistic in including both services and disservices (Friess et al., 2020).

## Threats to the Ecological Roles of Pteropodids

Of the 201 pteropodid species to date, 189 have been assessed by the IUCN (2020), such that 37% are currently threatened (7 species are "Critically Endangered," 27 species are "Endangered," 36 species are "Vulnerable") and 48% are of "Least Concern;" 9% of species are "Data Deficient" (**Figure 7**). However, caution must be exercised when using the assigned Red List status as sole indicator of species extinction risk. The status of many pteropodids are poorly known particularly in more localised contexts, and delays in communicating research result in outdated assessments. Furthermore, pteropodids found on islands pose special challenges when applying the IUCN's habitat-based criteria of extent of occurrence (EOO) and area of occurrence (AOO) to assess status (Mildenstein, 2020). Finally, while "common" pteropodids are studied more than "threatened" species at a rate of 5:1 (Mildenstein, 2012), "common" bat species, particularly those deemed as low conservation priorities based on their widespread distribution at a regional level, can be misinterpreted as not threatened at a country level. This can sometimes conflict with national-level species assessments that have not been captured or reflected by the IUCN Red List in a timely manner; e.g., *Pteropus hypomelanus* and *P. vampyrus* are both classified as "Endangered" in Peninsular Malaysia (PERHILITAN, 2017), but are both only listed as "Near Threatened" by the IUCN Red List (Bates et al., 2008; Tsang, 2020).

Another problem with looking at IUCN species assessments in isolation is that such assessments do not reflect the functional importance of many common and abundant species (**Figure 5**) at an ecosystem or landscape level (Redford et al., 2013; Baker et al., 2018). Since pteropodids are crucial for introducing seed rain into cleared areas and maintaining plant gene flow amongst forest fragments (Sritongchuay et al., 2014; Oleksy et al., 2015; Lim et al., 2018; van Toor et al., 2019; Chan et al., 2020), their disappearance could potentially disrupt reforestation and regeneration processes (Castillo-Figueroa, 2020). Modifications

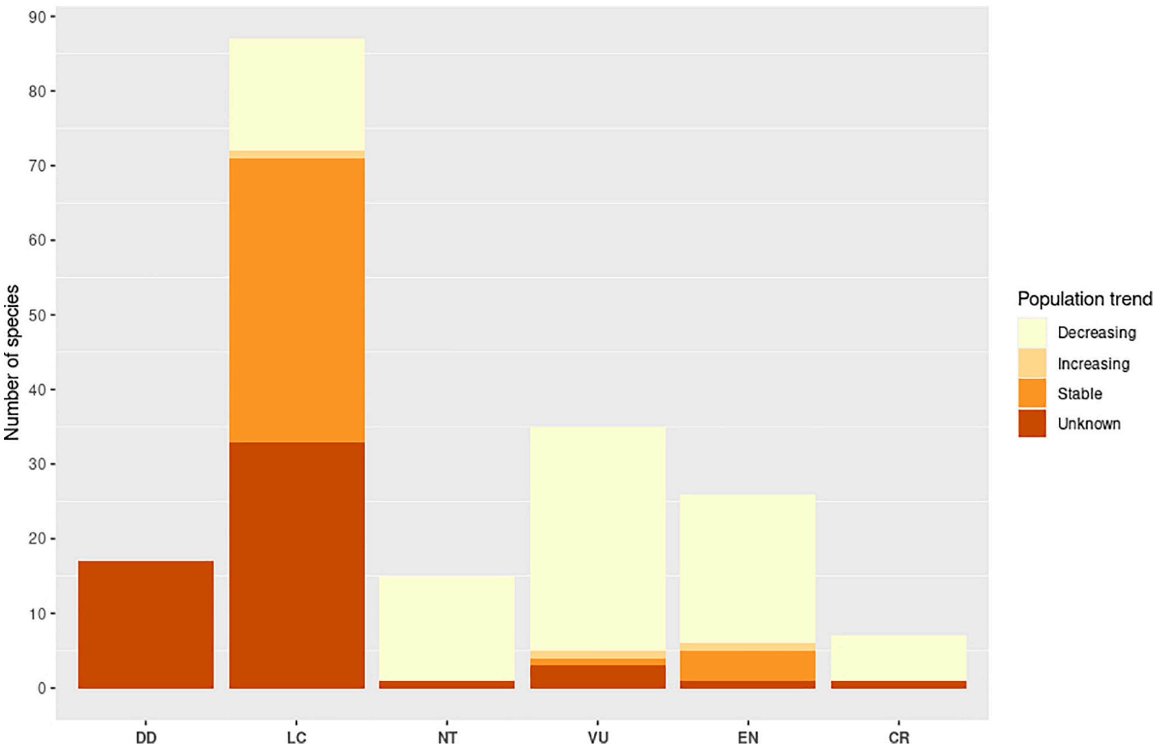

**FIGURE 7 |** IUCN Red List status and associated population trends of all assessed pteropodids (2020).

of the frugivore community (through logging or hunting of birds and terrestrial mammals) in the Neotropics have already been shown to reduce seed removal at the community level, leading to reduced plant recruitment and sapling density (Boissier et al., 2020). Similar effects can be expected for the Palaeotropics (Osuri et al., 2020), and it is crucial for such assessments to include pteropodids.

Comprehensive reviews have identified a number of major threats to pteropodids (Mickleburgh et al., 1992; Jones et al., 2009; O'Shea et al., 2016; Frick et al., 2019). These include direct and indirect anthropogenically caused mortality. Logging, agriculture (commercial and private or community-based), and hunting were reported by the Red List to present major threats to pteropodids (**Supplementary Information 7**), while fire and pollution have emerged as more recent threats. The following specific drivers are likely to cause major disruptions to pteropodid-plant interactions and their associated ecosystem functions.

## Hunting and Harvesting for Consumption and Trade

Bat hunting is widespread across the Old World, affecting about 50% of pteropodid species across 24 pteropodid genera, and particularly prevalent on the large pteropodids in Southeast Asia, and to a lesser extent Africa (Struebig et al., 2007; Epstein et al., 2009; Mildenstein et al., 2016), having already caused the extinction of several *Pteropus* species on islands (Jones et al., 2009; Mildenstein et al., 2016). Bats are hunted for a variety of different reasons, including for bushmeat consumption (either for subsistence or as a perceived delicacy), medicinal value, trade, recreation, and for the decorative and currency values of their teeth (Mildenstein et al., 2016; Lavery and Fasi, 2019). However, hunting for consumption remains the most widespread reason. While bat meat may serve as a necessary source of protein for certain impoverished communities (Mildenstein et al., 2016), elsewhere it is prized as a high-value and high-status meat costing more than poultry (Mohd-Azlan, pers. comm.; Jenkins and Racey, 2008) when it is sold on the black market (e.g., *Pteropus mariannus* is sold by poachers for as much as 150 USD on Guam; Mildenstein, unpubl. data).

Hunting causes changes to bat foraging and roosting behaviour, as well as population declines (Mildenstein et al., 2016). As the ecosystem roles of *Pteropus* become disrupted when their population abundance is too low, their functional extinction in the Asia-Pacific region – where their ecological importance in many countries is still poorly understood – can become a concern long before actual species or population extinction (McConkey and Drake, 2006). Similarly, *Eidolon helvum* travels the longest distance of any known mammal in Africa (van Toor et al., 2019), and its annual transboundary migration likely makes it the most important long-distance disperser of seeds and pollen on the continent (Richter and Cumming, 2008) – yet it is intensively hunted whenever it is found in abundance (Mildenstein et al., 2016; O'Shea et al., 2016). Although most bat hunting and trade is local rather than international, hunting pressure selectively targets the large pteropodids (Mildenstein et al., 2016) that function as the most crucial and often sole long-distance dispersers (Elmqvist et al., 1992; Fleming and Racey, 2009; McConkey and Drake, 2015), which should be a cause for serious concern (Stoner et al., 2007).

These examples underscore how conservation action is imperative even for abundant and/or common species due to their disproportionately important roles in maintaining ecosystem health (Redford et al., 2013; Florens et al., 2017; Baker et al., 2018; Laurindo et al., 2020). The majority of assessed pteropodid species are considered Least Concern by the IUCN Red List, with conservation efforts skewed towards highly threatened species. Yet this approach overlooks common and less threatened species that are often more vulnerable to hunting due to their hyperabundant populations (e.g., *Rousettus amplexicaudatus* in the Philippines), which may lead to a "Passenger Pigeon Fiasco" effect whereby a common and widespread species went extinct over time due to continuous overhunting and the lack of effective statutory conservation attention (Tanalgo and Hughes, 2019).

### Killings and Persecution Driven by Conflict and Negative Perceptions

Negative public perceptions of bats have been documented as remnant and long-standing socio-cultural values of Western, Eurocentric cultures that increasingly permeate international media reporting on bats (Thiriet, 2010; Lunney and Moon, 2011; Kingston, 2016). However, specific and localised negative perceptions of bats can also arise through conflict interactions such as crop-raiding and shared living space, often exacerbated by low awareness of bats' ecological importance (Larsen et al., 2002; Kung et al., 2015; Aziz et al., 2016, 2017a; O'Shea et al., 2016). In particular, intentional killings of bats for crop protection purposes can result in multiple species being intensively culled at very high numbers (O'Shea et al., 2016), with the national cullings of *Pteropus niger* on Mauritius being the most recent extreme example (Florens and Baider, 2019). Such massive population reductions are especially damaging at the ecological level, because it directly affects the role of such a keystone island species in maintaining native forests (Florens et al., 2017). Ironically, persecution and culling of pteropodids can also negatively impact the very commercial crops that rely on these bats for successful pollination and fruit production, such as durian (Aziz et al., 2016).

These issues are further compounded by misguided fear over speculated disease risks, which are often sensationalised and exaggerated by the media (Thiriet, 2010; Schneeberger and Voigt, 2016; López-Baucells et al., 2018; Tuttle, 2018). The current COVID-19 pandemic is the latest and worst example of this problem, with premature speculations in the media misleadingly associating the disease with bats, i.e., assuming or implying all bats to be a direct source of human infection (as opposed to one insectivorous genus merely being a possible evolutionary origin of the virus currently causing the disease), stoking even more public backlash (Rocha et al., 2020; Tuttle, 2020; Zhao, 2020). This has even resulted in violent and cruel attempts to cull or eradicate bats in a completely misguided effort to control the disease, despite the fact that the animal host for the SARS-CoV-2 virus is still unknown (Bittel, 2020; Lu et al., 2021).

### Habitat Loss and Disturbance

Habitat loss and disturbance affect both roosting areas and food sources for pteropodids. Deforestation is especially a concern in the tropics (Jones et al., 2009; Meyer et al., 2016), and a decreasing abundance of native food plants can drive pteropodids to feed more on introduced exotics in human-dominated areas (Luskin, 2010; Mildenstein, 2012; Aziz et al., 2016; Lim et al., 2018). This could lead to ecosystem disservices (Zhang et al., 2007; von Döhren and Haase, 2015; Vaz et al., 2017) such as the propagation of invasive plants (e.g., Voigt et al., 2011; Jordaan et al., 2012), and also crop-raiding of commercially cultivated fruits (Aziz et al., 2016), but even simple interventions to address habitat degradation, such as invasive alien plant control in native forests, can improve foraging habitat for pteropodids and therefore potentially reduce crop raiding (Krivek et al., 2020).

Land-use change in surrounding areas is also known to alter pollination networks in mixed-fruit orchards, affecting fruit production (Sritongchuay et al., 2019). Moreover, cave-roosting pteropodid pollinators such as *Eonycteris* and *Rousettus* are particularly vulnerable to limestone quarrying activities by the cement and marble industries, land-clearing around caves, and disturbance by human visitors (Clements et al., 2006). The lack of suitable cave roosts can thus have a direct detrimental impact on pollination services, affecting economically significant fruit industries (Sritongchuay et al., 2016).

Habitat modification, fragmentation and urbanisation are already known to affect pollination and seed dispersal by phyllostomid bats (Meyer et al., 2016; Regolin et al., 2020); e.g., in the Neotropics, frugivorous bats avoid areas with too much light, and feed less (on both fruit and nectar) in these areas (Lewanzik and Voigt, 2014). However, the impact of such processes on the ecological roles of pteropodids is still poorly understood. In particular, more studies are needed to document the full seed dispersal cycle, from seed deposition all the way through to plant recruitment, and how this is affected by habitat alteration (Meyer et al., 2016).

### Invasive Species

Invasive species are a significant threat to island-dwelling pteropodids, but the impacts of invasions are not well studied (Welch and Leppanen, 2017). Non-native cats, dogs, rats, ants and snakes prey on pteropodids (e.g., Vincenot et al., 2017a; Oedin et al., 2021), but how this threatens pteropodids' population stability and behaviour is mostly unknown. On Guam, predation by the invasive brown tree snake (*Boiga irregularis*) is thought to have contributed to the extinction of the endemic *Pteropus tokudae*, and partially to declines in *P. mariannus* (Wiles, 1987; Mildenstein, 2020). The snake has also caused the extirpation of most forest bird species on Guam (Savidge, 1987), which, in turn, has disrupted plant recruitment (Rogers et al., 2017). How this reduction in fruit bat population size and behaviour has affected the island's ecosystem, in terms of loss of ecological roles, has not been investigated. Invasive yellow crazy ants (*Anoplolepis gracillipes*) on Christmas Island disrupt the activity budgets of *Pteropus melanotus* (Dorrestein et al., 2019), but impacts on foraging and movement behaviour have not been documented.

Invasive species also have indirect effects on pteropodids when they alter habitats either through overgrazing (such as by deer and goats), competition for food resources (e.g. by macaques; Reinegger et al., 2021), or through the spread of non-native plants (Welch and Leppanen, 2017). The effect this has on foraging movement and ecological roles of pteropodids will depend on whether they avoid invaded areas (Krivek et al., 2020) or are attracted to them (Luskin, 2010), and there have not been enough studies to quantify responses.

### Climate Change

Climate change can threaten pteropodids through increased frequency and intensity of extreme weather events such as cyclones and intense heat/droughts (Welbergen et al., 2008; Jones et al., 2009). Cyclones are known to have an impact on endemic *Pteropus* spp. on Indian Ocean and Pacific islands, drastically reducing populations (e.g., 80-90% for some island *Pteropus*) and their food sources, and leaving bats vulnerable to increased hunting pressure from humans, conflict due to greater foraging in anthropogenic landscapes, or predation from domestic animals (Craig et al., 1994; Grant et al., 1997; McConkey et al., 2004; Esselstyn et al., 2006; O'Shea et al., 2016; Scanlon et al., 2018). Again, the negative impacts on island ecosystems are likely to be disproportionately high, particularly since the ecological roles of island *Pteropus* cannot be replaced by other animals (McConkey and Drake, 2015; Duron et al., 2017).

Australian *Pteropus* are known to regularly die en masse from extreme heat stress due to ambient temperatures exceeding 42°C (Welbergen et al., 2008; Jones et al., 2009; Daly, 2020), and the most recent bushfires associated with such an extreme weather condition (van Oldenborgh et al., 2020) will likely have far-reaching and long-lasting impacts on the Myrtaceae-dominated forests that rely heavily on pteropodid pollination (Birt, 2004; Boulter et al., 2005; Bacles et al., 2009; Reuters, 2019). Climate change could also lead to increased and intensified precipitation that would depress foraging activity, and perhaps even cause pups to starve or be abandoned, whilst sea level rise could inundate coastal roost sites (Jones et al., 2009).

Temperature changes could alter the timing of flowering and fruit development which could interfere with pollination and seed dispersal relationships (Sherry et al., 2007), particularly for migratory species that follow resource pulses (see Foraging Landscapes section). Pteropodids locate flowers and fruits using olfaction primarily, but the volatile organic compounds (VOCs) that they use as a guide could be altered by climate change (Yuan et al., 2009). However, while impacts of climate change are already being predicted for Neotropical pollination (e.g., Zamora-Gutiérrez et al., 2021) and seed dispersal (e.g., bird dispersal of a palm; Sales et al., 2021), potential effects on the ecological roles of pteropodids have not yet been studied.

## Knowledge Gaps and Future Research Directions

Although research efforts on pteropodid-plant interactions have increased markedly over the last three decades (**Figure 8**), the gaps in our knowledge of their ecological roles are large (**Supplementary Information 8**). We propose eleven key priorities for future studies and other efforts to bolster our understanding of bat-plant interactions in the region (**Table 6**). Of the 201 pteropodid bat species to date, only 37% ($n$ = 75) have been studied, with the majority of research focused on a few common species. Documenting the role of common or widely distributed species that could be playing major ecological roles (e.g., *Pteropus vampyrus,* Gumal, 2001; *Eidolon helvum*, van Toor et al., 2019) is important, but we have not yet sufficiently understood or recognised the importance of abundance per se, in order to ensure that populations of highly abundant species are maintained at appropriate levels. However, it is also vital to understand the roles of rare species that have dwindling populations. These population declines are often a direct result of human persecution (Aziz et al., 2016; Mildenstein et al., 2016; O'Shea et al., 2016), and strong arguments are required to bolster support for species conservation – especially for those deemed "common" and "abundant" (Redford et al., 2013; Baker et al., 2018).

Most of the countries with highly diverse pteropodid assemblages have had few studies, hindering our understanding of how these diverse bat communities are structured with respect to their ecological roles. The highest diversity of pteropodids is found in Indonesia (77 species), Papua New Guinea (36 species), and India (13 species) (**Figure 9**), but only India features in the five countries that have had more than 20 studies published on pteropodids, while African bats have been particularly poorly studied. Forested and cultivated habitats have received the most research effort, yet pteropodids inhabit other habitats, such as caves and urban areas, where more research attention is required.

A paucity of studies on the ecological roles of pteropodids has hindered a broader understanding of their importance. The majority of studies we report were focused only on diet, followed by foraging movement. Relatively few studies have directly investigated the roles played by pteropodids in pollination and seed dispersal (**Figure 1**). Pteropodids in Africa, the Mediterranean and Papua New Guinea are especially poorly studied in these aspects. Also, even with pteropodid species for which some documentation of diet exists, more comprehensive and detailed dietary records are still needed.

A research focus on pollination of cultivated plants is an important conservation need for countering persecution and negative opinions against bats. Yet robust empirical evidence is still lacking for confirming the role of bats in pollinating more commercially important plants, even though some of these plants have been erroneously cited as examples of bat pollination services (e.g., *Artocarpus, Palaquium*; Lee et al., 2002). Bat diet, flower visitation and pollen load identification/quantification, on their own, are insufficient for determining pollination success. In order to confidently determine pollinator effectiveness and pollination services of individual pteropodid species, investigations must employ appropriate exclusion experiments, identify relevant floral biology traits (including timings of anther dehiscence and stigma receptivity), document bat feeding behaviour, assess successful pollen transfer, and/or analyse the effect of bat visits on mature fruit set; without the use of these methods, any conclusions about the pollinating

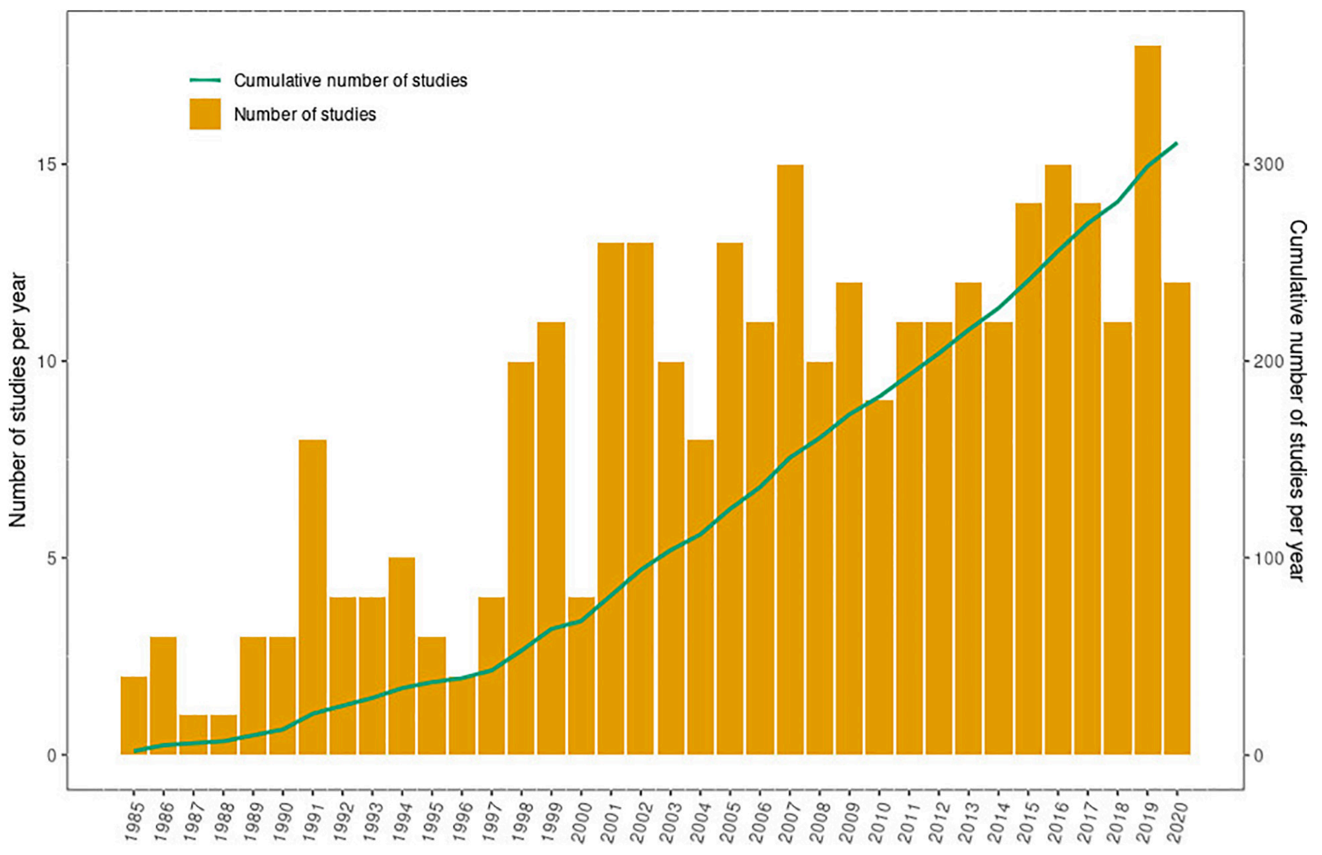

**FIGURE 8 |** Research effort on Old World bat-plant interactions during 1985-2020.

**TABLE 6 |** Directions for future research directions and other efforts to bolster progress on understanding pteropodid-plant interactions.

| Future directions | Description | Type |
|---|---|---|
| Understudied species and in understudied regions and habitats | Studies in mega-diverse countries (e.g., Indonesia and Papua New Guinea), understudied geographical territories and habitat types (e.g., caves, urban areas, mangroves, peat swamps). | Research |
| Value of abundance | Importance of abundance *per se* to the maintenance of bat-plant interactions. Understanding how reductions in highly abundant bats could impact ecological roles. | Research |
| Pollination and seed dispersal roles | Pollination studies for more commercially important and wild plant species; studies across a broader range of bat species; confirming the role of bats as double mutualists; assessment of the role pteropodids play in restoration. | Research |
| Community-wide studies | Broader studies encompassing more animal and plant species within communities to more accurately evaluate the importance of bats. Where possible studies should jointly evaluate both pollination and seed dispersal. | Research |
| Economic evaluation of roles | Quantify the positive contributions of bats via pollination, seed dispersal, habitat restoration and other services. Evaluate positive roles in the context of negative roles, such as crop raiding. | Research |
| Impact of disturbances and population declines on ecological roles | Determine how changes in population abundance, landscape structure, resource abundance, pollution, climate and other disturbances alter ecological roles | Research |
| Open data-sharing | Increase research visibility, up-to-date open data-sharing online, and promote responsible and equitable resource exchange amongst scientists, countries, and regions. | Other |
| Partnerships | Promote equitable and transparent partnerships among scientists, organisations, and conservation practitioners inside and outside the Old World tropics to effectively achieve the set priorities. | Other |
| Protocols on bat-plant interaction studies | Establish a standardised and easy-to-follow protocol or guide on bat-plant interaction studies. Also, increase capacity-building and training (study design, field surveys, data analysis, science communication) for early-career researchers in the Global South. | Other |
| Public awareness | Intensify and improve the translation of technical research to be more accessible to the mass public to promote and gain support for species and habitat conservation efforts. | Other |

**FIGURE 9 |** Proportion of studied pteropodid species per country during 1985–2020 (map excludes countries with no pteropodid occurrence).

roles of bats are premature and potentially inaccurate, and therefore cannot be made.

There remains a paucity of data on pteropodid pollination of wild plants. This knowledge deficit must be addressed to better appreciate the role of bats at the community level, and to understand their roles as double mutualists for a variety of ecosystems. Indeed, bat pollination could well be occurring for many plant species that are not currently known or suspected to be chiropterophilous, especially since some *Pteropus* species forage diurnally (Pierson et al., 1996; Richmond et al., 1998; Lavery et al., 2020; Aziz, pers. obs.). Broader studies are required to compare bats with other mutualists (Ong, 2020; Ingle, 2003; McConkey and Drake, 2015); where feasible, such studies should integrate the combined roles of pollination and seed dispersal to more accurately determine the importance of pteropodids. Studies quantifying the ecosystem services and economic contributions of the large-bodied pteropodids most intensively threatened by hunting and conflict (e.g., *Pteropus* spp. and *Eidolon* spp.) are a particularly critical and urgent need.

The frequent conflict between pteropodids and people requires economic assessments of ecosystem services and disservices to fairly and accurately represent the losses and gains that pteropodids generate. This can be done by incorporating cost-benefit analyses into assessments of such bat-plant interactions involving commercial fruit growers. Conflict was the least studied topic in our review (12% of studies) (**Figure 1**), only conducted in 14 countries, and only three of these (Japan, Kenya and Mauritius) also have studies on the ecological roles of the bats. An exception to this imbalance is Mauritius, where supposed losses of cultivated fruit to foraging *Pteropus niger* has resulted in two culls by the Government of Mauritius, causing the deaths of over 90,000 bats (Vincenot et al., 2017b). Researchers here have noted the ineffectiveness of culls (Florens and Baider, 2019), calculated minimal fruit losses to the bats (Oleksy et al., 2018), documented the ecological roles of the species (Nyhagen et al., 2005; Florens et al., 2017), and attempted to identify appropriate mitigation methods to reduce economic loss (Oleksy et al., 2018; Krivek et al., 2020). Such efforts are urgently needed but still lacking in many regions, such as Southeast Asia, East Asia, West Africa, South Africa, and Papua New Guinea. Negative perceptions of bats might be countered by more studies on the roles pteropodids play in pollinating important plants, or restoring habitats via seed dispersal. This restoration role is well recognised in Neotropical regions but represented by less than five studies for pteropodids (Sritongchuay et al., 2014; Oleksy et al., 2015, 2017; van Toor et al., 2019), even though 89% of pteropodid species are tolerant of anthropogenic disturbance (IUCN, 2020).

The final major research gap is our limited understanding of how pteropodids respond to disturbances and threats, and the impacts these have on ecological roles. Disturbance and fragmentation alter pathways used by Neotropical bats (Meyer et al., 2016; Ferreira et al., 2017; Regolin et al., 2020) but is largely unstudied in pteropodids, even though it has consequences for resources used, pollination success, and seed deposition (McConkey and O'Farrill, 2016). Although pteropodids frequently feed on cultivated fruits and flowers, this is influenced by the quality of the native habitat (Krivek et al., 2020) and food abundance (Luskin, 2010), as many pteropodids appear to prefer wild fruits to cultivated ones (Korine et al., 1999; Nelson et al., 2000a; Andrianaivoarivelo et al., 2012). A more in-depth understanding of resource selection can improve our understanding of pteropodid-plant interactions and conflict with humans. Finally, population abundance has been shown to influence seed dispersal capacity for some *Pteropus* species (McConkey and Drake, 2006), and testing for this in more species, as well as how it impacts pollination, is required.

More open, equitable collaborations and data-sharing (e.g., the Bat Eco-Interactions Database[1]) among researchers and other professionals working on pteropodids would enable a more comprehensive documentation of the benefits pteropodids bring to ecosystems and humans. Such collaborations could be used to establish standardised protocols for documenting bat-plant interactions, and to develop training opportunities particularly for Global South researchers. Finally, the translation of research findings into a format accessible to the general public through popular science communication channels is particularly essential for overcoming negative public perceptions of bats, especially in places where awareness and appreciation of bats remains low.

## CONCLUSION

Pteropodid bats play vital roles in seed dispersal and pollination, and are implicated as double mutualists for a diverse range of plant taxa. Island species are especially important for ecosystem functioning, but continental species have rarely been studied at a community level despite having the capacity for moving large numbers of seeds over the longest distances recorded for any animal. Well over half the world's pteropodid species remain unstudied in terms of their ecological roles, and many may experience multiple threats in various dimensions and scales. Even species that have been studied remain severely understudied in many aspects, often limited to just one study or single aspect. There is also a need to move away from species-driven research, especially research efforts and funding that focus solely on species diversity or endemism, and instead conduct research highlighting the importance of pteropodid-plant interactions at a landscape level, and their importance for healthy ecosystem functioning, even when common or less threatened species are involved.

We hope the results and findings highlighted by this review will encourage more studies on pteropodid-plant interactions to bolster the knowledge necessary for understanding the conservation values associated with this important animal group. We urge more efforts to be directed towards areas where pteropodid-plant interactions are poorly understood or explored. A comprehensive understanding of pteropodid ecological roles and their implications for human well-being is necessary to initiate effective conservation actions for an animal group that remains one of the least charismatic, and is consistently overlooked in research and conservation efforts.

## AUTHOR CONTRIBUTIONS

SA, KM, KT, TS, TM, and PR contributed to the conception and design of the study. SA, KM, KT, M-RL, CN-D, VC-L, and PR performed the literature search and/or organised the database. SA, KM, KT, TS, M-RL, JY, and CN-D produced the figures and/or tables. SA wrote the first draft of the manuscript. SA, KM, KT, JY, TM, CN-D, and PR wrote sections of the manuscript. All authors reviewed and/or analysed the literature and contributed to manuscript revision, read, and approved the submitted version.

## FUNDING

This research was made possible thanks to funding provided by the Embassy of France in Kuala Lumpur, Muséum National d'Histoire Naturelle, University of Nottingham Malaysia Campus, the Rufford Foundation (grants 17325-1, 25376-2, and 29639-B), Bat Conservation International, the University of Southampton, Rainforest Trust, The Habitat Foundation, the United States Fish and Wildlife Service (USFWS; grant F17AP00829), and numerous anonymous donors. This publication was made possible thanks to funding from the University of Exeter to cover the Article Processing Charge.

## ACKNOWLEDGMENTS

We are deeply grateful to the Southeast Asian Bat Conservation Research Unit (SEABCRU; USA NSF Award #1051363) for bringing the authors together, and the Philippines Biodiversity Conservation Fund (PBCF), Mabuwaya Foundation, Prince of Songkla University and the Secretariat of the 18th International Bat Research Conference (IBRC, 2019) for helping to facilitate further meetings and discussions. We are indebted to Reuben Clements for assisting with the literature search and analyses. We would also like to thank Khatijah Haji Hussin, Sara Bumrungsri, Tigga Kingston, Ahimsa Campos-Arceiz, Pierre-Michel Forget, Lisa Marie Paguntalan, Marcus Chua, and Tom Hughes for contributing input, advice, or information. We gratefully acknowledge Ricardo Rocha and Merlin Tuttle for providing constructive feedback to help improve the manuscript. We dedicate this manuscript to the memory of Greg C. Richards and Lim Boo Liat.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.641411/full#supplementary-material

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[1] https://batbase.org/

**Supplementary Information 1 |** Methods and workflow for literature review.

**Supplementary Information 2 |** Pteropodid-Plant Interactions Database (1985–2020).

**Supplementary Information 3 |** Regional summaries of research on pteropodid-plant interactions during 1985–2020.

**Supplementary Information 4 |** Feeding signs of *Pteropus* bats on fruit.

**Supplementary Information 5 |** Foraging distances of pteropodids.

**Supplementary Information 6 |** Seed dispersal distances of pteropodids.

**Supplementary Information 7 |** Threats to pteropodid species as assessed by the IUCN (2020).

**Supplementary Information 8 |** IUCN Red-Listed pteropodid species with corresponding numbers of species-specific studies documenting aspects of bat-plant interactions during 1985–2020.

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
in Ecology and Evolution

ORIGINAL RESEARCH
published: 16 August 2021
doi: 10.3389/fevo.2021.698885

# Within-Species Trait Variation Can Lead to Size Limitations in Seed Dispersal of Small-Fruited Plants

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**Specialty section:**
This article was submitted to Population, Community, and Ecosystem Dynamics, a section of the journal Frontiers in Ecology and Evolution

**Received:** 22 April 2021
**Accepted:** 20 July 2021
**Published:** 16 August 2021

**Citation:**
Rehling F, Jaroszewicz B, Braasch LV, Albrecht J, Jordano P, Schlautmann J, Farwig N and Schabo DG (2021) Within-Species Trait Variation Can Lead to Size Limitations in Seed Dispersal of Small-Fruited Plants. Front. Ecol. Evol. 9:698885. doi: 10.3389/fevo.2021.698885

The inability of small-gaped animals to consume very large fruits may limit seed dispersal of the respective plants. This has often been shown for large-fruited plant species that remain poorly dispersed when large-gaped animal species are lost due to anthropogenic pressure. Little is known about whether gape-size limitations similarly influence seed dispersal of small-fruited plant species that can show a large variation in fruit size within species. In this study, fruit sizes of 15 plant species were compared with the gape sizes of their 41 animal dispersers in the temperate, old-growth Białowieża Forest, Poland. The effect of gape-size limitations on fruit consumption was assessed at the plant species level, and for a subset of nine plant species, also at the individual level, and subindividual level (i.e., fruits of the same plant individual). In addition, for the species subset, fruit-seed trait relationships were investigated to determine whether a restricted access of small-gaped animals to large fruits results in the dispersal of fewer or smaller seeds per fruit. Fruit sizes widely varied among plant species (74.2%), considerably at the subindividual level (17.1%), and to the smallest extent among plant individuals (8.7%). Key disperser species should be able to consume fruits of all plant species and all individuals (except those of the largest-fruited plant species), even if they are able to consume only 28-55% of available fruits. Fruit and seed traits were positively correlated in eight out of nine plant species, indicating that gape size limitations will result in 49% fewer (in one) or 16–21% smaller seeds (in three plant species) dispersed per fruit by small-gaped than by large-gaped main dispersers, respectively. Our results show that a large subindividual variation in fruit size is characteristic for small-fruited plant species, and increases their connectedness with frugivores at the level of plants species and individuals. Simultaneously, however, the large variation in fruit size leads to gape-size limitations that may induce selective pressures on fruit size if large-gaped dispersers become extinct. This study emphasizes the mechanisms by which gape-size limitation at the species, individual and subindividual level shape plant-frugivore interactions and the co-evolution of small-fruited plants.

**Keywords: gape-size limitation, phenotypic variation, seed mass and number, seed dispersal mutualism, trait matching, fruit selection, frugivores, intraindividual variability (IIV)**

# INTRODUCTION

The fleshy fruits of plants are an important food source for many animals (Snow and Snow, 1988; Jordano, 2014; Albrecht et al., 2018b; Quintero et al., 2020; González-Varo et al., 2021). In exchange for the provided pulp, animals disperse the seeds of the fruits, which is critical to the recruitment of plant populations (Howe and Smallwood, 1982). Seed dispersal is typically carried out by multiple species of animals; conversely, those animals feed on the fruits of multiple species of plants (Zamora, 2000; Blüthgen et al., 2007). Nevertheless, the morphological, physiological, and behavioral traits of plants and animals have co-evolved such that certain plant-animal interactions are favored over others (Gautier-Hion et al., 1985; Jordano, 1987; Albrecht et al., 2015, 2018b).

Co-evolved traits include the sizes of the fruits and the gapes of their animal dispersers (Moermond and Denslow, 1985; Wheelwright, 1985; Jordano, 1995a; Eklöf et al., 2013; Albrecht et al., 2018a,b; Schleuning et al., 2020). Because animals can poorly feed on fruits that are larger than their gape (Levey, 1987; Rey et al., 1997), the diversity of dispersing animals decreases with increasing fruit size, such that large-fruited plant species are dispersed only by a few large-gaped animals (Janzen and Martin, 1982; Guimarães et al., 2008). The reliance of a plant species on large-gaped animals for seed dispersal may result in strong population declines, once their main dispersers become functionally lost. This has been observed especially in the tropics (e.g., Galetti et al., 2013; Kurten, 2013; Correa et al., 2015; Lim et al., 2020) and on islands (e.g., Pérez-Méndez et al., 2016; Brodie, 2017; Case and Tarwater, 2020), where, following anthropogenic pressure, the inability of simplified, down-sized animal communities to consume large fruits limits the regeneration of the respective plants (Terborgh et al., 2008; Brodie et al., 2009). Despite these strong examples, the diameters of the vast majority of fruits and gapes are < 1.2 cm (Wheelwright, 1985; Wenny et al., 2016), and the extent to which gape-size limitations determine the interactions between small-fruited plants and small-gaped seed dispersers is unclear.

Within-species levels of trait variation may lead to gape-size limitations that do not take place at the species level (as observed in the large-fruited plant species), but subtler at the individual or subindividual level. Fruits of plants can vary in their size among different plant individuals (interindividual variation) due to genetic differences, differences in plant size or the environment (Foster, 1990; Wheelwright, 1993; González-Varo and Traveset, 2016). Simultaneously, fruit size also varies within individuals (subindividual variation), because flowers receive a different amount of pollen during pollination, or fruits differ in their vertical position, or light conditions (Jackson and Sharples, 1971; Lloyd, 1984; Dogterom et al., 2000; Herrera, 2009, 2017). Thus, for example, in the common myrtle *Myrtus communis*, while all of its main dispersers are able to feed on its fruits, the actual disperser assemblage differs between plant individuals bearing fruits of different sizes, such that some individuals have potentially only two and others up to five main dispersers (González-Varo and Traveset, 2016). In the olive tree *Olea europaea*, the large fruits of cultivated plants can be consumed by only one disperser whereas the small fruits of wild individuals are consumed by all four main dispersers (Rey et al., 1997). On top of the variation in plants, substantial interindividual variation occurs in the gape width of seed-dispersing animals, due either to ontogenetic differences or due to sexual dimorphism (González-Varo and Traveset, 2016; Zwolak, 2018).

The resulting community-wide trait variation in fruit and gape sizes not only determine the interactions among plants and animals at different ecological levels (species, individual, subindividual level), they may also play a role in the success and effectiveness of seed dispersal (Schupp et al., 2010). Within plant species, fruit size was shown to positively correlate with the number or mass of dispersed seeds per fruit (e.g., Sallabanks, 1993; Alcántara and Rey, 2003; Hernández, 2009). Seed size, in turn, mediates other dispersal-related processes, such as the dispersal ability of seeds, the seed's susceptibility to natural enemies, and the performance of the seedlings after germination (Leishman et al., 2000; Muller-Landau, 2010; Fricke et al., 2019). If the animal's gape size constrains its fruit choice, then large-gaped animals will be able to disperse more or larger seeds per fruit than small-gaped animals, because they are able to consume larger fruits (Alcántara and Rey, 2003; Hernández, 2009; Herrera, 2009; Galetti et al., 2013; González-Varo et al., 2014; Carvalho et al., 2021). Thus, the within-species trait variation in fruit size could potentially have direct and indirect consequences for both the quantity and the quality of seed dispersal, even in plant species with small fruits.

In this study, we describe the extent to which gape-size limitations structure species interactions between small-fruited plants and their associated frugivores, and how gape-size limitations influence the mass or number of dispersed seeds by small-gaped frugivores. We combined data on fruit removal (Albrecht et al., 2013), seed deposition (Schlautmann et al., 2021), fruit and seed traits, and gape widths (Herrera, 1984; Jordano, 1984a) of a diverse plant-frugivore community of the lowland temperate forests in Białowieża Forest (Eastern Poland). First, we characterized the community-wide trait variation in both the fruit diameter of plants and the gape widths of seed dispersers at the species level. In addition, we characterized the within-species trait variation for a subset of nine plant species and their six main dispersers. Second, we tested and quantified to which extent the different levels of trait variation of fruit diameter and gape width affect the interactions between plants and their seed dispersers at the species level (all species) and within species at the individual and subindividual level (subset only). For the species subset, we further tested, third, if fruit size is positively related to the key determinants of post-dispersal seedling establishment, i.e., the number and mean mass of seeds per fruit. Finally, we described the consequences of gape-size limitations for seed dispersal by testing whether fruit diameter and gape width affected the number and mass of dispersed seeds.

# MATERIALS AND METHODS

## Study Area and Species

The study was conducted in the Białowieża Forest, which covers an area of 1,506 km$^2$ and spans the borders of Poland and Belarus. The Polish part of the forest (∼625 km$^2$) is divided into the Białowieża National Park (∼105 km$^2$) and state forests. The 48-km$^2$ Białowieża National Park has been continuously protected for almost 500 years, first as a royal hunting ground and since 1921 strictly as a national park. It is therefore the best-preserved lowland forest in Europe (Samojlik et al., 2019; Jaroszewicz et al., 2019). By contrast, commercial logging has been allowed in > 80% of Polish state forests since the First World War (Mikusiński et al., 2018; Jaroszewicz et al., 2019).

Up to 20% of the Białowieża Forest is dominated by alder (Jaroszewicz et al., 2019), and is home to a diverse community of at least 15 woody, fleshy-fruited plant species (**Supplementary Table 1**, see also Albrecht et al., 2015). For the study of within-species trait variation and fruit-seed trait relationships of small-fruited plant species, we focused on the nine most abundant plant species in the middle layer and understory of the forest: *Euonymus europaeus* (European spindle), *Frangula alnus* (alder buckthorn), *Prunus padus* (bird cherry), *Rhamnus cathartica* (European buckthorn), *Ribes nigrum* (black currant), *Ribes spicatum* (downy currant), *Sambucus nigra* (elder), *Sorbus aucuparia* (rowan), and *Viburnum opulus* (guelder rose). These species belong to five plant families and are either trees ($n$ = 6 species) or shrubs ($n$ = 3). They produce red ($n$ = 4) or black ($n$ = 5) fruits and their fruiting season starts in June (*P. padus* and *R. spicatum*) and ends in October (*E. europaeus*). The seeds of these plants are dispersed by many different animal species (**Supplementary Table 2**), including at least 10 mammalian and 31 avian frugivores (Albrecht et al., 2013; Jaroszewicz et al., 2013; Schlautmann et al., 2021). However, the contribution of these species to the total seed dispersal of the plant community in the Białowieża Forest is highly heterogeneous, as only five bird species, i.e., *Erithacus rubecula* (European robin), *Sylvia atricapilla* (Eurasian blackcap), *S. borin* (garden warbler), *Turdus merula* (common blackbird), *T. philomelos* (song thrush) and one mammal, the European pine marten *Martes martes*, account for 97.0% of the fruit removal interactions and 98.6% of the seed rain (Schlautmann et al., 2021). In the following, these six species are referred to as the main seed dispersers in the studied community.

## Fruit and Seed Traits of Plants at the Species, Individual and Subindividual Levels

To describe the community-wide trait variation in fruit diameter of small-fruited plants, we collected data for 15 woody, fleshy-fruited plant species at the species level, and for a subset of nine plant species also at the individual and subindividual levels. The data on the fruit diameters at species level were based on the fruit measurements performed in this study (see next section) and on the measurements reported in Albrecht et al. (2018b) for the remaining six plant species (**Supplementary Table 1**). For *Rubus ideaus* (raspberry), a fruit-size diameter of 0.34 cm (Robbins and Moore, 1991) was assumed because animals feed on single drupelets of the polydrupe.

For the subset of nine plant species, fruit samples from (7–)12–15 individual adult plants per species (mean ± SD: 12.8 ± 2.5) were collected in the Białowieża Forest, for a total of 115 adult plant individuals (**Supplementary Table 1**). Ripe fruits were sampled between June and September 2018, aligned with the fruiting phenologies of the species. The collected fruits were stored in a freezer at −4°C. Between 4 and 22 fruits per plant individual (8.8 ± 2.8) were depulped for use in the analysis, resulting in 99–151 fruits per plant species (112.4 ± 22.0, **Supplementary Table 1**). The diameters of the frozen fruits were recorded to the nearest 0.01 cm (mean length and width of the fruit; referred to hereafter as fruit diameter). Intact seeds were extracted and then dried at room temperature for 2 days. The number of seeds per fruit and the mean dry mass of the seeds per fruit, defined as the total mass of seeds divided by the number of seeds per fruit, were determined. Fruits of *R. cathartica* often contained aborted seeds (mass < 7 mg) that seldom germinated (data not shown). Those seeds were excluded from estimates of the number and mass of seeds per fruit. In addition, because some fruits of *S. aucuparia* were infested by larvae of *Argyresthia conjugella* and seed-dispersing species tend to avoid eating infested fruits (Manzur and Courtney, 1984), only the non-infested fruits of *S. aucuparia* were considered.

## Gape Width of Animals at the Species and Individual Levels

Data from three sources (Herrera, 1984; Jordano, 1984a, specimen collection, unpublished) were used to determine the gape width for 30 of the 41 studied disperser species from the Białowieża Forest at the species level. Herrera (1984) and Jordano (1984a) measured the gape width of mist-netted birds from Southern Spain (1978–1982) and Northern Spain (1980–1983), respectively. In the Appendix of the report by Herrera (1984), only the mean values were listed whereas in the dataset provided by Jordano (1984a) the gape widths of up to 20 individuals per bird species were recorded. Because gape widths can differ between animals of different populations and depending on the observer (see Supplement of González-Varo and Traveset, 2016), in this study the mean values of Herrera (1984) and Jordano (1984a) were averaged when data from both sources were available. In the absence of information on the gape width of living individuals, the values were based on measurements of up to four mounted specimens within the animal collection of Philipps-Universität Marburg (**Supplementary Table 2**). However, the gape of mounted animals is stiff, such that the mean gape width was consistently smaller ($F$ = 14.08, $p < 0.001$) than reported by Herrera (1984) and Jordano (1984a). In all three data sources, the gape width of birds was measured at the internal commissures of the mouth using a caliper and recorded to the closest 0.01 cm. For *Carpodacus erythrinus* (common rosefinch), no data were available and the gape width was therefore predicted based on bird species with a similar body mass and diet as reported by Herrera (1984) (**Supplementary Figure 1**). For mammalian dispersers, information on gape width was rare and all mammalian species ($n = 10$) were therefore expected to have a gape width of ≥2 cm.

Because the gape width of bird species was averaged from measurements reported in different studies, interindividual differences in the gape width of the main dispersers were simulated by sampling 50 individuals with the species-specific mean gape width and the variation of gape width reported by Jordano (1984a), SD in *E. rubecula* = 0.036 cm, *S. atricapilla* = 0.033 cm, *S. borin* = 0.037 cm, *T. philomelos* = 0.037 cm, *T. merula* = 0.101 cm. Small-gaped, intermediate-gaped, and large-gaped individuals within species were defined accordingly, based on the 10% quantile, the mean, and the 90% quantile of the gape width of simulated individuals (**Supplementary Table 3**).

## Statistical Analyses

### Trait Variation in Fruit Diameter and Gape Width at the Species, Individual and Subindividual Level

We summarized the community-wide trait variation in the fruit diameter of plants and the gape width of frugivores at the species level (**Figure 1**). Frugivores were defined as all animal species recorded eating fruits of any of the studied plant species at least once, either during fruit removal observations in 2011/2012 (Albrecht et al., 2013) or based on the seeds found in the scat of animals collected in 2016–2018 (Schlautmann et al., 2021). Because we did not have direct measures of the gape widths of mammals, a cut-off was set at 2 cm. The black woodpecker *Dryocopus martius* (the only bird with a gape size > 2 cm) was grouped with mammals, thus yielding a group of large-gaped animals whose choice of fruits was not limited by gape width (**Figure 1** and **Supplementary Tables 1, 2**).

We tested if the gape width of seed disperser species was related to the maximum and mean diameters of the consumed fruits at the species level. The maximum diameter of the consumed fruits was defined as the mean fruit diameter of the largest-fruited plant species fed upon by a given seed disperser species, based either on observations of fruit removal (Albrecht et al., 2013) or on seed deposition (Schlautmann et al., 2021). The mean diameter of fruits removed by a given seed disperser species was calculated by weighting the fruit diameter of each plant species by the disperser-specific fruit consumption rate. The mean fruit diameter was determined solely on the basis of fruit-removal observations (Albrecht et al., 2013), not on seed depositions, because the mean fruit consumption rate cannot be adequately calculated from the number of seeds in scats. This is because birds usually deposit only a few seeds at most (not all seeds of one or more fruits) at the same place, and this behavior might depend on the plant species. Similarly, the number of seeds in scats might have been confounded by within-species differences in fruit choice among animal species. The effect of gape width on an animal's choice of fruits at the species level was evaluated using linear models in which the maximum and mean diameters of the fruits removed by each seed disperser species served as the response variable and the gape width of the respective seed disperser species as the continuous explanatory variable. In the analyses of maximum and mean fruit diameters, mammals were not included due to the missing values for gape width ($n = 4$). The nine avian seed disperser species that were observed less than six times were also excluded because the sample size was too low to consider their fruit choices as representative. Thus, the maximum and mean diameters of consumed fruits were analyzed for 17 seed disperser species. To test if the number of disperser species of a plant species decreases with the increasing diameter of fruits at the species level, generalized linear effect models were used with the number of disperser species as the response variable and fruit diameter as the fixed explanatory variable. The models included a logit link and a Poisson error distribution (analysis of deviance, Quinn and Keough, 2002).

For a subset of the nine most abundant plant species and their six main dispersers (see "Study area and species"), we quantified the extent to which gape-size limitations could potentially affect fruit removal and fruit consumption within species. To do so, we calculated the proportion of fruits from each plant species that could be swallowed (i.e., falling within the size interval of the gape widths) by the six main seed disperser species (proportion of accessible fruits for consumption, i.e., animal perspective; or proportion of fruits that can be dispersed by different animals, respectively, i.e., plant perspective; see **Supplementary Table 3**). In addition, we quantitatively compared the different levels of trait variation in fruit diameter of the nine plant species. The coefficient of variation (CV), defined as the sample variability (standard deviation) divided by the mean of the sample, and variance partitioning were used to compare the levels of trait variation in fruit diameter and the mean seed mass in plants among species, among individuals, and within individuals (subindividual). The CV was chosen because it provides a measure of trait variability from the individual perspective and is thus well-suited for comparisons of individuals within species. As a second measure, we used variance partitioning as it is able to provide a measure of trait variability from the community perspective and was thus well-suited for comparing the variability within and among species. In this study, it was used to separate the total community variability in fruit diameter and mean seed mass per fruit into the underlying contributions of species, individual, and subindividual variation. As both the CV and variance partitioning have different mathematical drawbacks and describe different aspects of trait variation, they were used in combination (Herrera, 2009). The variance component (VC) models were fitted using an ANOVA-type (type I sums of squares) estimation for unbalanced mixed models, with the fruits nested in plant individuals nested in plant species as random factors (Searle et al., 1992). Significant differences between factors were based on 95% confidence intervals (95%CIs) using Satterthwaite's correction. Differences in the subindividual variation in fruit diameter between plant species were tested using the subindividual CV of individuals as replicates and by constructing linear models with plant species as the fixed factor. A potential relationship between the individual and subindividual CVs among plant species was investigated by averaging the subindividual CV per species and using a Spearman correlation.

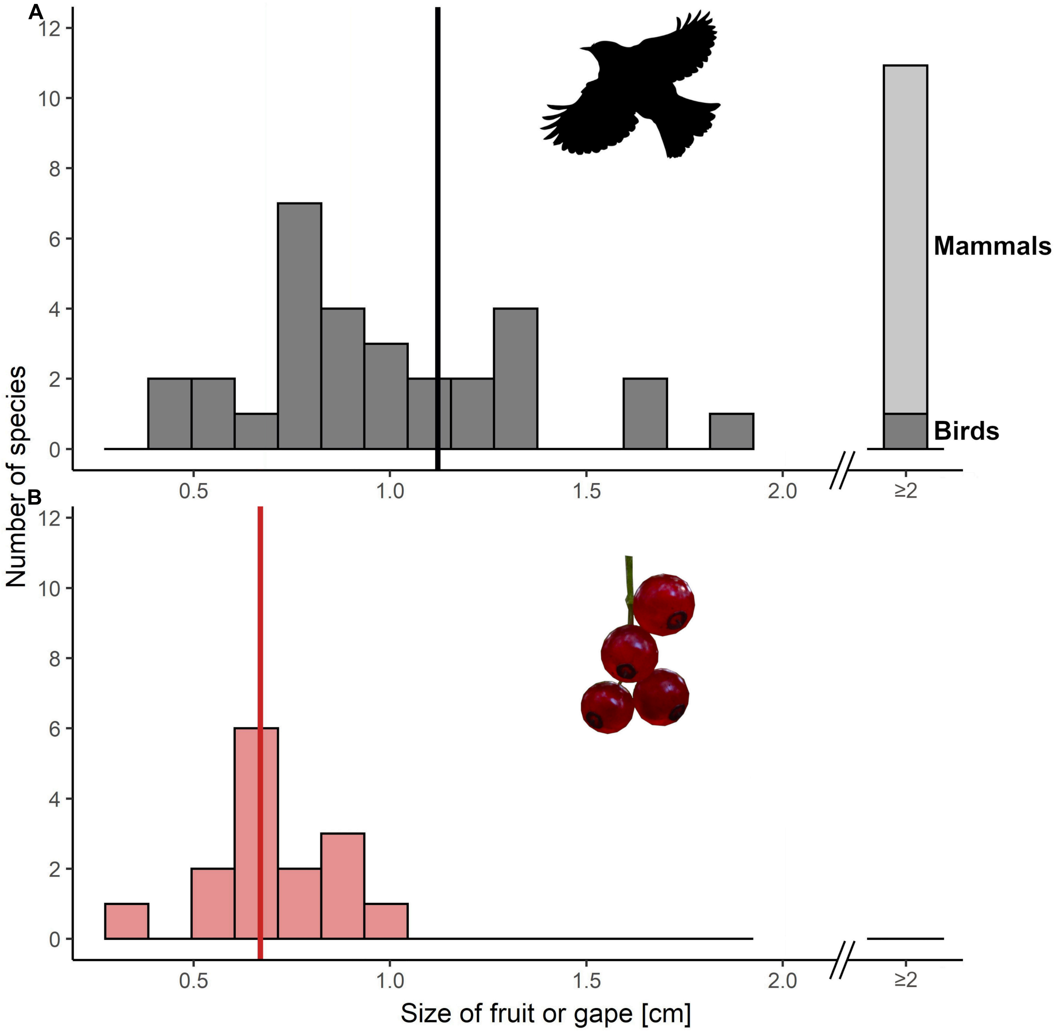

**FIGURE 1 |** Distribution of **(A)** the gape widths of animal dispersers and **(B)** the fruit diameter of associated plants at species level in temperate forests in Białowieża Forest, Poland. This is a summary of the local plant-frugivore community, whose contributing species were identified either based on fruit removal observations (Albrecht et al., 2013) or based on seeds in scats of animals (Schlautmann et al., 2021). Vertical lines illustrate the median value of a distribution.

## Fruit-Seed Trait Relationships

For the subset of the nine most abundant plant species, the relationship between fruit diameter and the seed traits of the plant species was assessed using (generalized) linear mixed models. In these models, the number or the mean mass of seeds per fruit served as the response variable, the fruit diameter, plant species and their interaction as the continuous explanatory variables, and the individual plant (from which the fruits had been collected) as a random factor. However, these models performed poorly which may have been due to the following reasons: first, the number of seeds per fruit was morphologically constrained in five of the nine plant species (*E. europaeus, F. alnus, P. padus, S. nigra*, and *V. opulus*) and hardly varied among fruits. Second, a linear mixed model with a normal error distribution performed best in the analyses of the number of seeds of *R. cathartica* and *S. aucuparia*, but a Poisson error distribution performed better in the models of *R. nigrum* and *R. spicatum*. Third, the number of seeds, the mean seed mass, and fruit size strongly differed between plant species, and the absence of overlap in the ranges of the values of the different plant species cast doubt on the accuracy of the model outcome. Thus, each of the nine plant species was tested separately and the probability values for multiple comparisons were adjusted using a Bonferroni correction to avoid a type I error.

## Consequences of Gape-Size Limitations for Dispersed Seeds

For the subset of the nine most abundant plant species and the six main dispersers, a possible effect of the gape width of the seed disperser species on the number or mean mass of seeds per fruit was analyzed. The number or mean mass of seeds per fruit was bootstrapped by randomly sampling 50 fruits of the studied plant species with 1,000 replacements. For each of the bootstrap replicates, the maximum fruit diameter that could be sampled was limited according to the gape width of the animal species or individual. Pairwise mean value comparisons of the bootstrapped data were performed to test whether differences in the gape width of seed disperser species was the sole explanation for the differences in the number or mass of dispersed seeds, i.e., the probability that the mean value of the dispersed seeds of small-gaped seed dispersers was larger than that of large-gaped seed dispersers (based on one-tailed $p$-values). This was achieved by grouping the large-gaped main seed disperser species (*M. martes, T. merula*, *T. philomelos*), because they were not limited in their fruit choices, and adjusting the probability values for multiple comparisons between the main seed disperser species using a Bonferroni correction, to avoid a type I error. Because the results were slightly variable among iterations (i.e., random seeds), we present the mean effect sizes and mean probability values of 50 iterations of the pairwise mean value comparisons of the bootstrapped data.

All statistical analyses were conducted using the R program version 4.0.3 (R Core Team, 2020). Variance component analyses were performed using the R-package VCA version 1.4.3 (Schuetzenmeister and Dufey, 2020). Generalized linear mixed models were constructed using the package lme4 version 1.1-23 (Bates et al., 2015). Significance values for the effect of fixed factors were obtained using Wald-$\chi^2$-tests (type II sums of squares) in the package car version 3.0-9 (Fox and Weisberg, 2019).

# RESULTS

## Trait Variation in Fruit Diameter and Gape Width at the Species, Individual and Subindividual Level

In Białowieża Forest, 15 fleshy-fruited plant species are dispersed by 41 animal species (10 mammal and 31 bird species, **Figure 1**; Albrecht et al., 2013; Schlautmann et al., 2021). At the species level, the mean fruit diameter ranged from 0.51 cm in *S. nigra* to 0.96 cm in *V. opulus* and was therefore smaller (median = 0.67 cm) than the gape width of the associated seed disperser assemblage (median = 1.12 cm).

From the community perspective, trait variation in fruit diameter was largely explained by differences among plant species ($VC_{species}$ = 1.87, 95%CI: 0.84–6.99). However, more of the total variation in the fruit diameter within a plant species was explained by the subindividual than by the interindividual variation ($VC_{subindividual}$ = 0.43, 95%CI: 0.39–0.47 and $VC_{individual}$ = 0.22, 95%CI: 0.16–0.31, respectively, **Table 1**). The subindividual variation in fruit diameter differed significantly between plant species [$F_{(8,106)} = 11.8, p < 0.001$] and was smallest in *V. opulus* and largest in *R. spicatum* (**Figure 2** and **Supplementary Figure 2**). The interindividual and subindividual variation in fruit diameter were not significantly rank-correlated across species ($n = 9$, Spearman's $\rho = 0.45$, $p = 0.230$).

The gape width of seed dispersers also varied considerably among species (**Figure 1**) and among individuals of the same species (**Figure 2**). When the community-wide trait variation in gape width and fruit diameter was considered, all plant species produced fruits small enough to be swallowed by all of the main seed dispersers in the studied plant-frugivore community (*E. rubecula, M. martes, S. atricapilla, S. borin, T. merula, T. philomelos*). In five of the nine studied plant species, the main seed disperser could potentially feed on > 90% of the total available fruits (based on the mean gape width of seed disperser species, **Figures 2, 3** and **Supplementary Table 3**). In three plant species (*F. alnus*, *R. nigrum, S. aucuparia*), the main small-gaped seed dispersers (*E. rubecula*, *S. atricapilla*, *S. borin*) could feed only on 28–55% of the available fruits, but could interact with most individuals in the population (66–100%, based on the mean gape width of the studied species, **Figure 3**). The fruits of *V. opulus* seemed to be barely accessible to small-gaped main dispersers (*E. rubecula*, *S. atricapilla*, *S. borin*) whereas the three main large-gaped seed dispersers (*M. martes*, *T. philomelos*, and *T. merula*) were potentially not size-limited in their fruit choices, neither among nor within plant species (**Figures 1–3** and **Supplementary Table 3**).

Fruit size did not affect the number of seed disperser species feeding on the fruits ($\chi^2 = 0.07$, $p = 0.794$, **Figure 4A**). However, the maximum [$F_{(1,15)} = 4.69, p = 0.047$] and mean [$F_{(1,15)} = 5.50, p = 0.033$] diameters of the consumed fruits increased with the increasing gape width of the seed disperser species (**Figure 4B**). Only in 16.4% of the interactions did seed dispersers consume the fruits of plant species that were on average larger than their mean gape width (**Figure 4B**).

**TABLE 1 |** Analyses of variance components of fruit diameter and mean seed mass per fruit across nine fleshy-fruited plant species in Białowieża Forest, Poland.

| | VC | % Var | CV [%] | |
|---|---|---|---|---|
| | | **Fruit diameter** | | |
| Community | 2.51 | 100.00 | 22.16 | |
| Species | 1.87 | 74.17 | 19.08 | a |
| Individual | 0.22 | 8.71 | 6.54 | c |
| Subindividual | 0.43 | 17.11 | 9.16 | b |
| | | **Mean seed mass** | | |
| Community | 833.53 | 100.00 | 103.76 | |
| Species | 765.50 | 91.84 | 99.44 | a |
| Individual | 31.30 | 3.76 | 20.11 | b |
| Subindividual | 36.74 | 4.41 | 21.78 | b |

*VC, ANOVA-type estimation of variance components (Searle et al., 1992).*
*% Var, Proportion of total community-level variance in plant traits explained by different ecological scales: variation among species (species), variation among individuals of the same species (individual), variation within individuals of the same species (subindividual).*
*CV [%], Coefficient of variation of different ecological scales.*
*Lower cases (a, b, c) indicate significant differences between VC, %Var and CV [%] of the different ecological scales based on 95% confidence levels using "Satterthwaite" approach (Schuetzenmeister and Dufey, 2020).*

## Fruit-Seed Trait Relationships

The number of seeds per fruit was biologically constrained in five of the nine studied plant species (1–3 seeds per fruit). For example, *P. padus* and *V. opulus* have drupaceous fruits, always

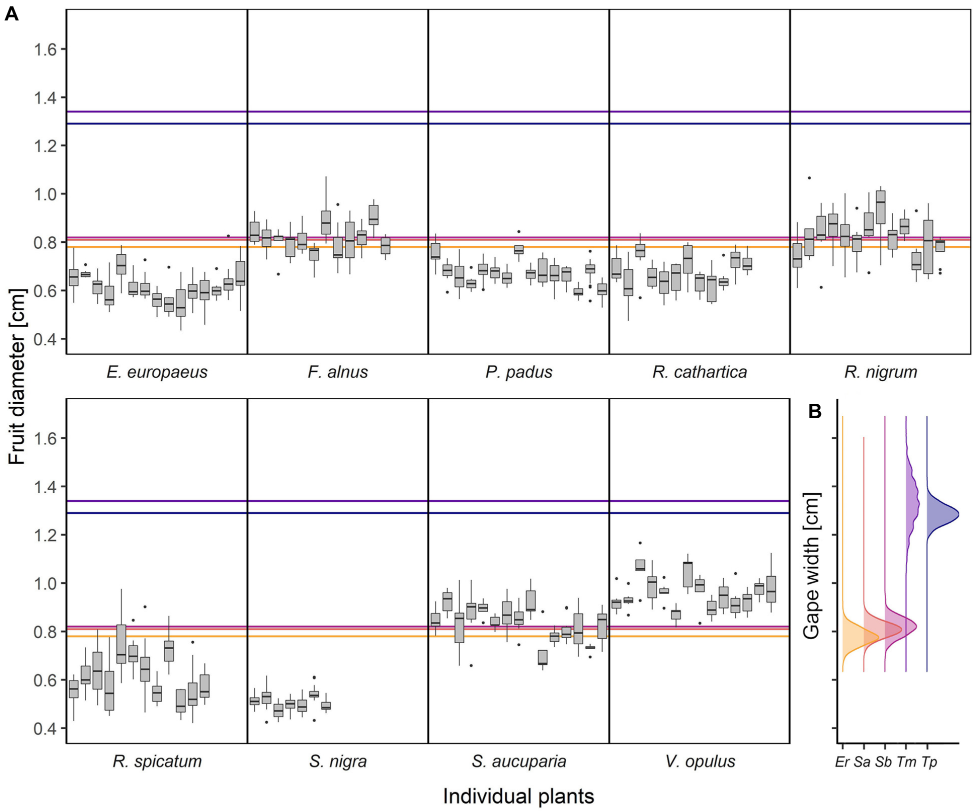

**FIGURE 2 | (A)** Boxplots showing sub- and interindividual variation in fruit diameter across nine fleshy-fruited plant species in Białowieża Forest, Poland. **(B)** Density plot of gape width of the five most important frugivore species (Er, *Erithacus rubecula*; Sa, *Sylvia atricapilla*; Sb, *Sylvia borin*; Tm, *Turdus merula*; Tp, *Turdus philomelos*). Together with *M. martes* (gape width > 2 cm), these disperser species account for 97.0% of fruit removal interactions and 98.6% of the seed rain in the Białowieża Forest, Poland. The color of horizontal lines in **(A)** depict the mean gape width of disperser species in **(B)**, respectively.

with one seed per fruit. Fruit diameter correlated positively with the number of seeds per fruit only in three plant species (**Figure 5**): *R. cathartica* (Wald-$\chi^2$ = 10.61, $p = 0.004$), *R. nigrum* (Wald-$\chi^2$ = 199.97, $p < 0.001$) and *R. spicatum* (Wald-$\chi^2$ = 75.15, $p < 0.001$). The variation in the mean seed mass per fruit was mostly explained by the differences between species, with only small contributions by individual and subindividual differences (**Table 1**). The mean seed mass per fruit correlated positively with fruit diameter in five of the nine studied plant species (**Figure 6**): *F. alnus* (Wald-$\chi^2$ = 57.53, $p < 0.001$), *P. padus* (Wald-$\chi^2$ = 14.83, $p = 0.001$), *S. nigra* (Wald-$\chi^2$ = 7.06, $p = 0.071$), *S. aucuparia* (Wald-$\chi^2$ = 17.55, $p = 0.010$) and *V. opulus* (Wald-$\chi^2$ = 26.63, $p < 0.001$).

## Consequences of Gape-Size Limitations for Dispersed Seeds

Tests for pair-wise differences between the main seed disperser species with respect to the number or mass of dispersed seeds per fruit showed significant differences for four of the nine plant species (**Figures 5**, **6**). For *R. nigrum*, the small-gaped *E. rubecula* tended to disperse, on average, seeds from fruits with fewer seeds per fruit than did *S. borin* ($p = 0.033$) or the three main large-gaped seed dispersers *M. martes*, *T. merula*, *T. philomelos* ($p < 0.001$). *S. atricapilla* and *S. borin*, in turn, dispersed seeds from fruits with fewer seeds than did the three main large-gaped seed dispersers (both $p \leq 0.002$, **Figure 5D**). The mean mass of the dispersed seeds did barely differ between the main small-gaped seed dispersers (*E. rubecula, S. atricapilla, S. borin*). However, in all but one comparison, *E. rubecula, S. atricapilla* and *S. borin* dispersed smaller seeds than the main large-gaped seed dispersers for three plant species, namely, *F. alnus*, *S. aucuparia* and *V. opulus* ($p < 0.001$ for two, $p < 0.05$ for one, and $p < 0.1$ for three species comparisons, respectively). Only in *F. alnus*, *S. borin* did not disperse seeds smaller than those dispersed by the large-gaped dispersers ($p = 0.148$), but differences between the main seed dispersers (even between the small-gaped seed dispersers) became significant ($p < 0.001$), when we strongly increased the number of samples for bootstrapping (e.g., 500 instead of 50 fruits).

## DISCUSSION

The importance of within-species trait variation in structuring species interactions such as seed dispersal has long been noted (Wheelwright, 1985; Jordano, 1995b), but only recently ecologists have started to investigate the eco-evolutionary consequences

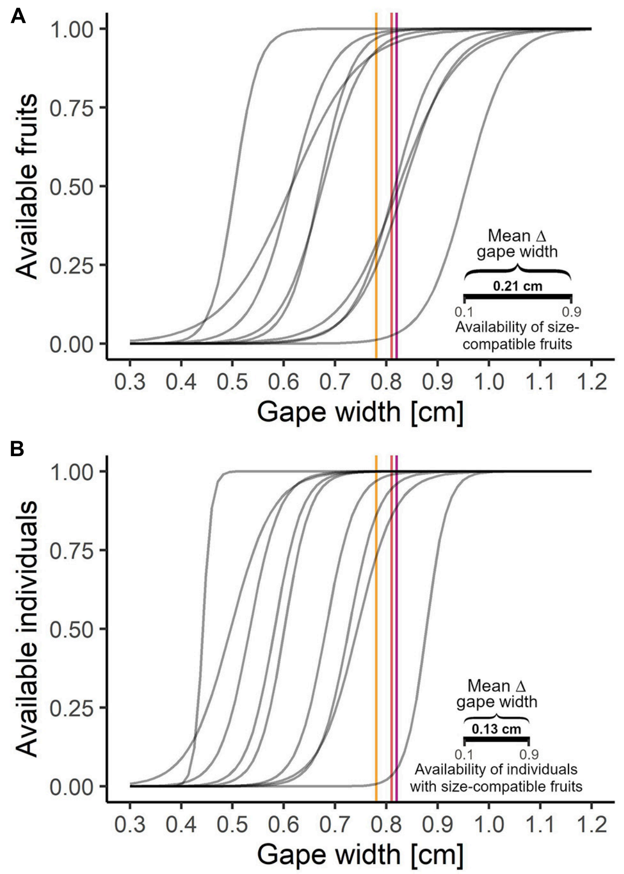

**FIGURE 3 |** Availability of **(A)** fruits or **(B)** individuals with size-compatible fruits as a function of the gape width of frugivorous animals. Each gray solid line displays one plant species. The black horizontal line illustrates the mean difference in the gape width of animals that allows animals to feed on either 10 or 90% of **(A)** fruits, or **(B)** plant individuals, respectively. The colored vertical lines depict the mean gape width of the small-gaped disperser *E. rubecula* (0.78 cm), *S. atricapilla* (0.82 cm) and *S. borin* (0.83 cm), respectively.

(Herrera, 2009; Bolnick et al., 2011; González-Varo and Traveset, 2016; Des Roches et al., 2018; Schupp et al., 2019; Snell et al., 2019). Our study showed that the community-wide trait variation in fruit diameter of plants and the gape width of frugivores is large among species, but also considerable within species. Every plant species produced fruits of a size that could be swallowed by the six seed disperser species previously shown to be quantitatively important for seed dispersal in this community (Albrecht et al., 2013). Only the fruits of *V. opulus* were too large to be consumed and dispersed by all main seed disperser species. However, these fruits are usually eaten several months after their appearance, when they have become smaller due to desiccation (Hernández, 2009). The dried, smaller fruits provide a food source in winter that is accessible even to small-gaped dispersers (Hernández, 2009). Consequently, mismatches in size between plants and their disperser are rare in temperate forests at the species level (González-Varo and Traveset, 2016), which might render the associated seed dispersal processes relatively robust against anthropogenic pressures (Albrecht et al., 2013, 2014; Farwig et al., 2017; Emer et al., 2019).

By contrast, at the subindividual plant level, the fruit choices of small-gaped seed dispersers were limited for the plants *F. alnus*, *R. nigrum*, and *S. aucuparia*, with the main small-gaped seed dispersers being able to feed only on 31–55% of the accessible fruits. Such size mismatches between dispersers and fruits may in part explain why 47–78% of the fruits in previously described populations of *F. alnus* remained undispersed (Hampe, 2008; Szewczyk et al., 2019). However, because the fruit size of each plant species varied more strongly within than between individuals, the main seed dispersers were still able to feed on the fruits of individual plants (except those of large-fruited *V. opulus*). On average, plants were able to interact with animals, whose gape width was 0.082 cm smaller, only due to subindividual trait variation in fruit size. These tiny differences in size could correspond to a potential increase of 0–7 disperser species (mean = 2.1) at the level of plant individuals. Herrera (2009) found that, in terms of the total variation in fruit size, subindividual variation was larger than variation among individuals in 20 of 25 fleshy-fruited plant species. Together, these findings indicate that the large subindividual trait variation is characteristic for fleshy-fruited plant species, and allows for a broader range of interaction partners than would be expected based on the mean fruit sizes of plant species in plant-frugivore communities.

The number of disperser species did not decline with increasing fruit diameter, although species with larger gape widths preferentially fed on larger fruits. Large-gaped frugivores were usually heavier (**Supplementary Information 1.2**) and their feeding on energy-rich resources would maximize their energy intake (Albrecht et al., 2018a,b; Quintero et al., 2020). An increase in preferred fruit size with increasing gape width has been observed in other plant-frugivore communities as well, especially in tropical ecosystems, where at the species level there is a much larger trait variation in both fruit diameter and gape width (Wheelwright, 1985; Jordano, 1987; Lambert, 1989; Noma and Yumoto, 1997; Moran and Catterall, 2010; Burns, 2013; Chen and Moles, 2015; Dehling et al., 2016; Bender et al., 2018). Even within the same plant species, dispersers not limited by gape width were shown to preferentially feed on larger fruits (Sallabanks, 1993; Sobral et al., 2010).

In previous studies, birds were observed to peck rather than to swallow fruits that were larger than their gape (Levey, 1987; Rey et al., 1997; Hernández, 2008; Rey, 2011), but the birds were either kept under captive conditions and forced to feed on the fruits (Levey, 1987; Rey et al., 1997) or large fruits were the only food resource in the close vicinity (Hernández, 2008; Rey, 2011). In our study, there was no evidence of pecking with increasing fruit diameter, as the pecking of fruits was very rarely observed during fruit removal (<2% of all interactions). In addition, pecking may reflect responses other than gape-size limitations, such as difficulty in approaching less accessible fruits or exploratory probing (**Supplementary Figure 3**). These observations suggest that fruit pecking is not frequently used to overcome gape-size limitations, at least in most seed dispersers and as long as multiple food sources are available. A recent

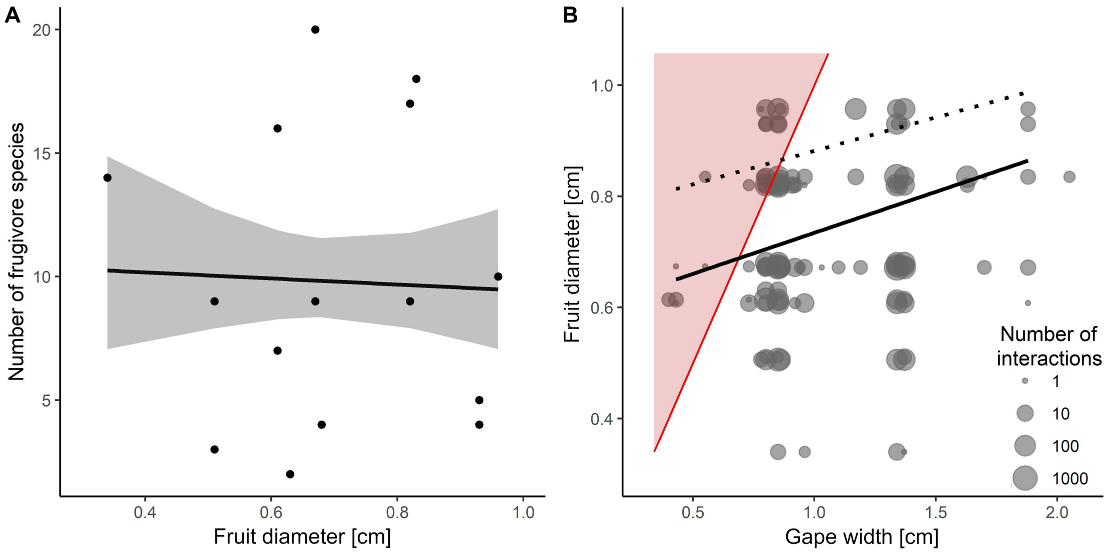

**FIGURE 4 | (A)** The effect of fruit diameter on the number of disperser species per plant species. The gray-shaded area indicates 95% confidence intervals. **(B)** The maximum and mean diameter of fruits of plant species dispersed by animals with different gape widths during fruit removal observations (Albrecht et al., 2013; Schlautmann et al., 2021) in Białowieża Forest, Poland. The red-shaded area displays morphological "forbidden links" between plants and animals based on mean trait-values.

study likewise showed that the diet and body conditions of the small-gaped Sardinian warbler *Curruca melanocephala* could be primarily predicted by the local density of accessible fruits, not by the total fruit density (González-Varo et al., 2021).

The finding that no plant species only produced fruits larger than the gapes of *E. rubecula*, *S. atricpilla*, and *S. borin* suggests more effective seed dispersal by the six main seed dispersers than by the three large-gaped seed dispersers (*T. merula*, *T. philomelos*, and *M. martes*) alone. For fruit plants, interactions with many seed disperser species may increase the overall quantity of dispersed seeds, the probability of plant recruitment, and therefore parental fitness (Herrera, 1984; Schupp et al., 2010). In addition, the reliance on a large diversity of seed disperser species provides a bet-hedging strategy of plant individuals to guarantee constant seed dispersal even during years when the population sizes of single disperser species are low (Herrera, 1998; Blüthgen et al., 2016). This suggests that subindividual variation in plant traits can influence the fecundity of plant species and may thus be under selection (Herrera, 2009, 2017), especially in animal-dispersed plants (Jordano, 1995b; Sobral et al., 2013, 2019). However, rather than increases or decreases in subindividual trait variation *per se*, natural selection might affect the variation in fruit diameter among plant individuals to promote interactions with key seed dispersers.

Our study showed that fruit diameter correlated positively with the number or the mean mass of seeds in eight of the nine studied plant species. This suggests that a positive relationship between fruit diameter and seed traits is common in fleshy-fruited plants, in line with the findings of many single-species studies (e.g., Herrera, 1988; Sallabanks, 1993; Herrera et al., 1994; Jordano, 1995b; Alcántara and Rey, 2003; Hernández, 2009; Rodríguez-Pérez and Traveset, 2010; González-Castro et al., 2019; Traveset et al., 2019; Carvalho et al., 2021). Potential mismatches between gapes and fruits can, therefore, decrease the mass and the number of dispersed seeds in small-gaped dispersers, as this was shown for four of the nine studied plant species. Even small differences in the gape width among species, such as those between *E. rubecula* (gape width = 0.78 cm) and *S. atricapilla* (0.82 cm) or *S. borin* (0.83 cm), might be large enough to induce selective pressures on fruits traits on the long-term.

A similar pattern was found in an *in-situ* study conducted in the Mediterranean areas, where for *Olea europea* the fruit choice by dispersers was limited by their gape size (Rey et al., 1997). The positive correlation between fruit size and seed size (Alcántara and Rey, 2003) also explains the smaller seeds dispersed by *S. atricapilla* than by large-gaped *T. philomelos* (González-Varo et al., 2014). For the spurge olive *Cneuorum tricoccon*, larger seeds were found in the scat of *M. martes* than in that of small-gaped lizards (Traveset et al., 2019). Even in aggregated fruits such as *Rubus* spp., avian frugivores may select for seeds of different sizes by selecting fruits of different sizes (e.g., Jordano, 1984b). Seed size can, in turn, affect the post-dispersal regeneration of plants. Larger seeds are usually less susceptible to soil pathogens and produce larger seedlings, especially in adverse environments, but they may also be poorly dispersed (Murray et al., 1993; Leishman et al., 2000; Fricke et al., 2019; González-Castro et al., 2019). This suggests that gape-size limitations play a pivotal role in seed dispersal and influence the effectiveness of seed disperser species by constraining the efficiency of fruit handling (Schupp et al., 2010), even in small-fruited plants. This mechanism provides an explanation how the co-evolution of large seeds and animal seed dispersal started from small seeds 80 million years ago

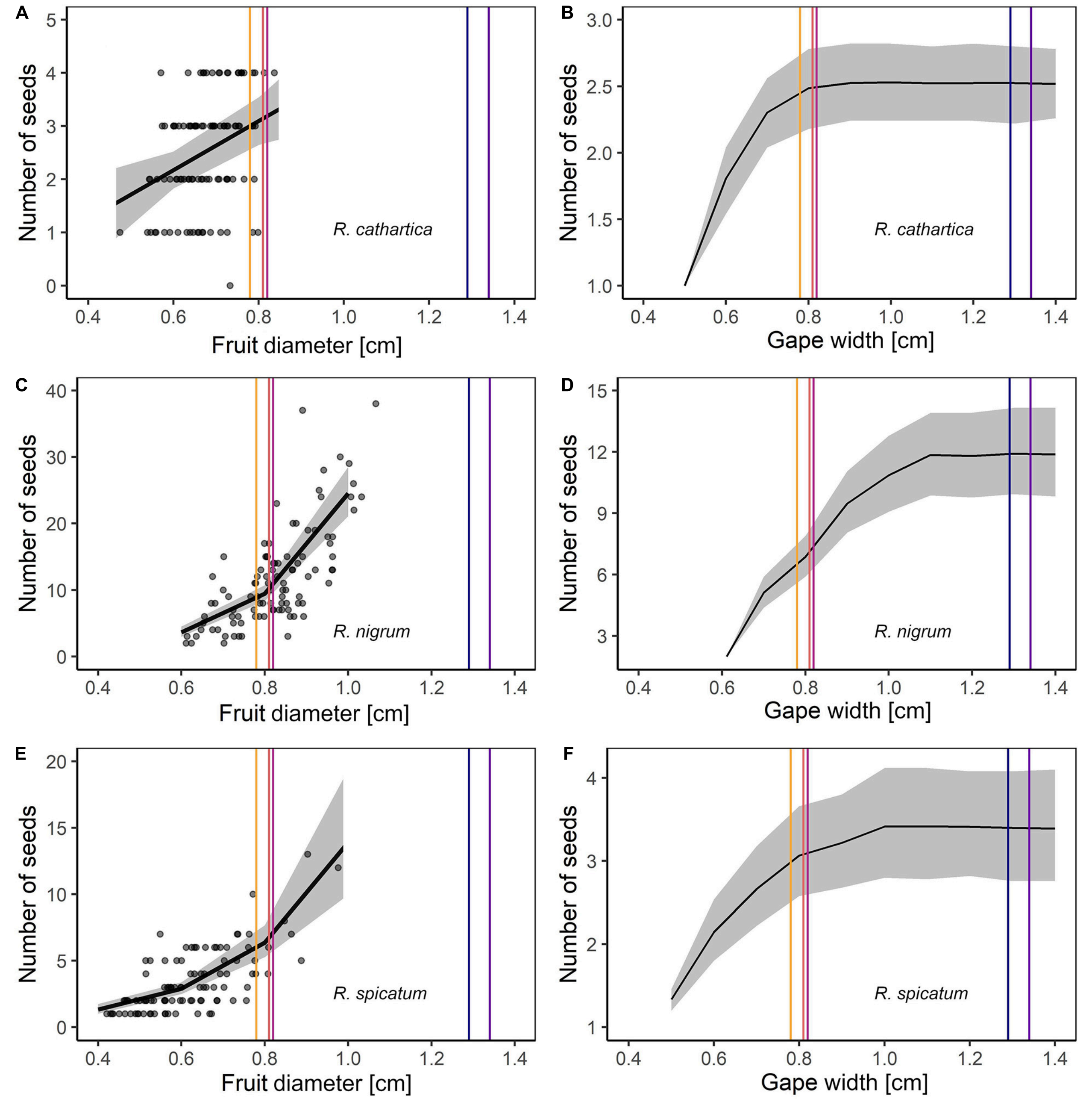

**FIGURE 5 | (A,C,E)** The number of seeds per fruit in relation to fruit diameter and **(B,D,F)** the predicted number of seeds per fruit taken up by animals depending on their gape width in *Rhamnus cathartica* **(A,B)**, *Ribes nigrum* **(C,D)** and *Ribes spicatum* **(E,F)**. The number of seeds per fruit was not related to the diameter of fruits in the other study species in Białowieża Forest, Poland. Shaded areas indicate the 95% confidence intervals. The colored vertical lines display the mean gape width of the most important animal dispersers of the plant species (but *M. martes* with a gape width > 2 cm). Please see **Figure 2** for more information.

(Eriksson, 2016). However, as the effect of seed size on plant performance during and after seed dispersal may differ between plant species and disperser species, the effects of gape-size limitations on the efficiency of seed dispersal may not always be straightforward (Fricke et al., 2019; Schupp et al., 2019). The consequences of the patterns identified in this work may be a promising avenue for future research (e.g., Fricke et al., 2019; Carvalho et al., 2021).

## CONCLUSION

Comparisons of community-wide patterns of the sizes of fruits and gapes in a temperate plant-frugivore association revealed that, even for small fruits (<1 cm diameter), gape-size limitations affect fruit removal and seed dispersal interactions, mostly at the subindividual level and only rarely at the plant species

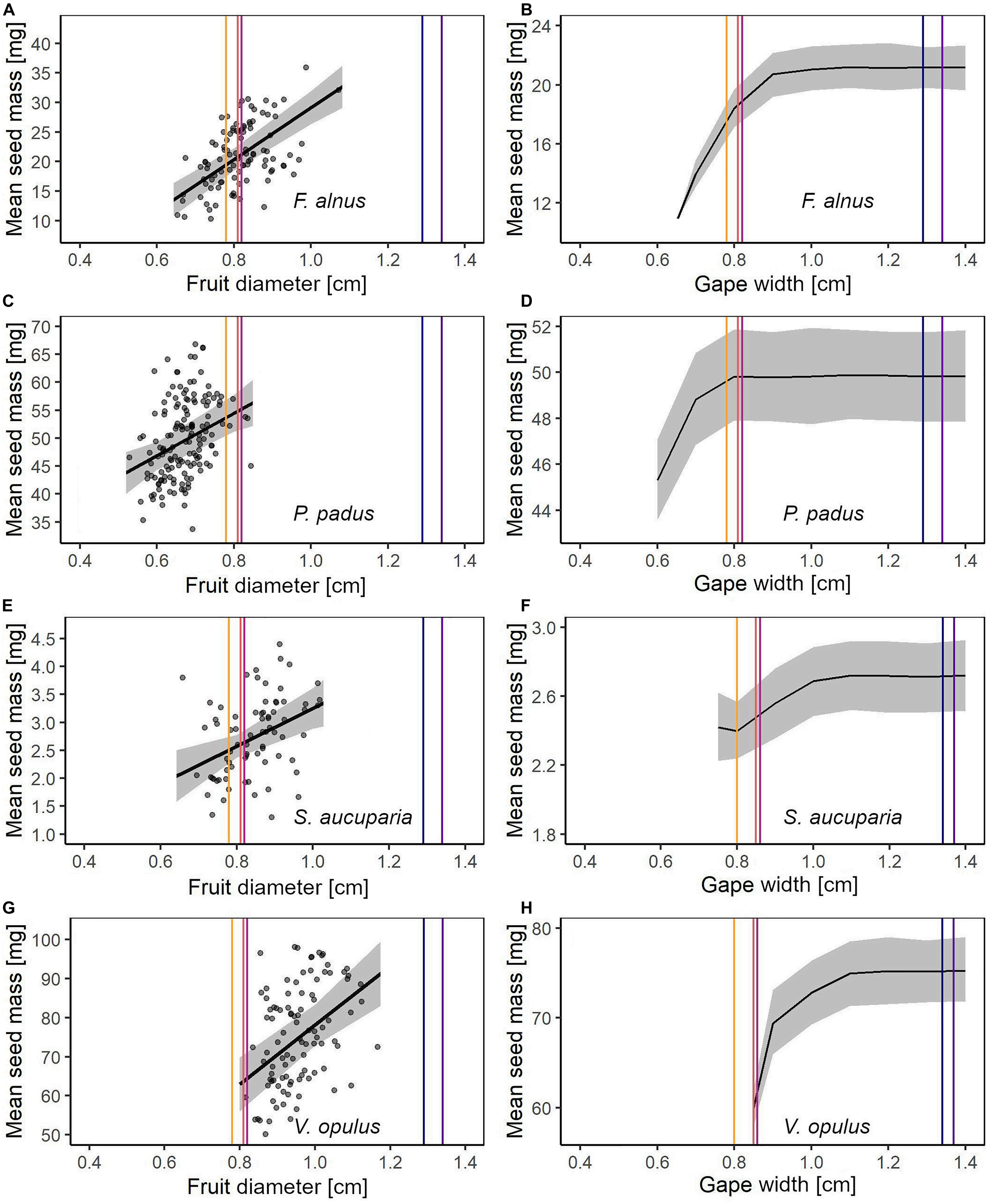

**FIGURE 6 |** The mean mass of seeds per fruit in relation to fruit diameter **(A,C,E,G)** and the mean mass of seeds per fruit taken up by animal dispersers depending on their gape width **(B,D,F,H)** in *F. alnus* **(A,B)**, *P. padus* **(C,D)**, *S. aucuparia* **(E,F)** and *V. opulus* **(G,H)**. In *S. nigra*, mean mass of seeds per fruit was related to fruit diameter, but the largest fruit was 0.617 cm and, thus, too small to induce gape-size limitations in the main disperser. Shaded areas indicate the 95% confidence intervals. The colored vertical lines display the mean gape width of the most important animal dispersers of the plant species (but *M. martes* with a gape width > 2 cm). Please see **Figure 2** for more information.

level. The large subindividual trait variation in fruit size ensures a high connectedness of the small-fruited plants with small-gaped animal dispersers, and possibly makes fruit removal of these plants tolerant to the loss of frugivores. Simultaneously, however, positive fruit-seed trait relationships are common in fleshy-fruited plants species, such that gape-size limitations lead to altered dispersal pattern in the number or size of seeds, when only the large-gaped dispersers become extinct. This study highlights the importance of the different levels of trait variation and gape-size limitation in plant-frugivore communities for structuring species interactions and the co-evolution of seed size and animal seed dispersal.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, or are available online from Dryad Digital Repository https://doi.org/10.5061/dryad.0vt4b8gzk (Rehling et al., 2021).

## ETHICS STATEMENT

Ethical review and approval was not required for the animal study because this study relied on previously published data and indirect methods for assessing the behavior of animals.

## AUTHOR CONTRIBUTIONS

FR, NF, and DS conceived and designed the experiments. FR and LB collected and measured fruits and seeds, and performed a pilot germination study. BJ administrated field work. JA and JS provided data on species interactions in Białowieża Forest. PJ provided data on within-species variation in gape widths of the main seed dispersers. FR analyzed the data and wrote the first draft of the manuscript. All authors contributed substantially to revisions thereafter and gave final approval for publication.

## FUNDING

This project was funded by the German Research Foundation (FA925/10-1, 10-2; BE 6041/1-1, SCHA 2085/1-2).

## ACKNOWLEDGMENTS

We greatly thank the administration of the Białowieża National Park, the forest administrations of Białowieża, Hajnówka and Browsk, and Polish authorities [Ministry of Environment, GDOS (Polish General Directorate of Environment Conservation, Warsaw) and RDOS (Regional Directorate of Environment Conservation, Białystok)] for the permission to research in Białowieża Forest. The research was conducted in cooperation with the International Centre for Research on Forest Ecosystems of the University of Warsaw. We thank the two reviewers DL and MO for their constructive comments on earlier versions of the manuscript.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.698885/full#supplementary-material

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
in Ecology and Evolution

ORIGINAL RESEARCH
published: 16 September 2021
doi: 10.3389/fevo.2021.655441

# Simplified Communities of Seed-Dispersers Limit the Composition and Flow of Seeds in Edge Habitats

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**Edited by:**
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**Specialty section:**
*This article was submitted to*
*Population, Community,*
*and Ecosystem Dynamics,*
*a section of the journal*
*Frontiers in Ecology and Evolution*

**Received:** *18 January 2021*
**Accepted:** *26 August 2021*
**Published:** *16 September 2021*

**Citation:**
*Razafindratsima OH, Raoelinjanakolona NN, Heriniaina RR, Nantenaina RH, Ratolojanahary TH and Dunham AE (2021) Simplified Communities of Seed-Dispersers Limit the Composition and Flow of Seeds in Edge Habitats. Front. Ecol. Evol. 9:655441. doi: 10.3389/fevo.2021.655441*

Edge effects, driven by human modification of landscapes, can have critical impacts on ecological processes such as species interactions, with cascading impacts on biodiversity as a whole. Characterizing how edges affect vital biotic interactions such as seed dispersal by frugivores is important for better understanding potential mechanisms that drive species coexistence and diversity within a plant community. Here, we investigated how differences between frugivore communities at the forest edge and interior habitats of a diverse tropical rainforest relate to patterns of animal-mediated seed dispersal and early seedling recruitment. We found that the lemur communities across the forest edge-interior gradient in this system showed the highest species richness and variability in body sizes at intermediate distances; the community of birds showed the opposite pattern for species richness. Three large-bodied frugivores, known to be effective dispersers of large seeds, tended to avoid the forest edge. As result, the forest edges received a lower rate of animal-mediated seed dispersal compared to the interior habitats. In addition, we also found that the seeds that were actively dispersed by animals in forest edge habitats were smaller in size than seeds dispersed in the forest interior. This pattern was found despite a similarity in seed size of seasonally fruiting adult trees and shrubs between the two habitats. Despite these differences in dispersal patterns, we did not observe any differences in the rates of seedling recruitment or seed-size distribution of successful recruit species. Our results suggest that a small number of frugivores may act as a potential biotic filter, acting on seed size, for the arrival of certain plant species to edge habitats, but other factors may be more important for driving recruitment patterns, at least in the short term. Further research is needed to better understand the potential long-term impacts of altered dispersal regimes relative to other environmental factors on the successional dynamics of edge communities. Our findings are important for understanding potential ecological drivers of tree community changes in forest edges and have implications for conservation management and restoration of large-seeded tree species in disturbed habitats.

**Keywords: edge effects, habitat fragmentation, species interaction, seed dispersal, tropical forest, primate**

## INTRODUCTION

Human-induced habitat loss, fragmentation, and degradation of forested habitats around the world have resulted in 70% of the world's forested area existing within 1 km of a forest edge (Haddad et al., 2015). While there has been a long history of studies examining the influence of edge habitat on forest ecosystems (Ries et al., 2004; Lindenmayer and Fischer, 2007; Ruffell and Didham, 2016), how ecological processes respond to edges remains poorly explored. Understanding processes that affect forest regeneration, such as seed dispersal, is critical for our ability to predict the impacts of forest fragmentation on forest structure and composition (Magrach et al., 2014). It also has implications for designing conservation and management practices for maintaining biodiversity (Camara-Cabrales and Kelty, 2009).

In forest edges, abiotic factors such as reduced soil moisture or increased sunlight exposure can act as an environmental filter by preventing or favoring the establishment of certain plant species (Kraft et al., 2015). Along with these abiotic factors, changes in ecological processes and species interactions such as seed dispersal by animals (zoochory) can also act as a potential filter for plant communities by limiting or increasing seed supply, which will affect the initial template for regeneration and may ultimately affect the patterns of species occurrence and diversity in a local community (George and Bazzaz, 1999; Myers and Harms, 2009; Albert et al., 2015). Differences in abundance and composition of frugivores may differ due to avoidance or attraction to habitat or resource characteristics present on the forest edge (Johns and Skorupa, 1987; Gray et al., 2007; Gomes et al., 2008).

Edge effects (i.e., the impacts of creating edges of forest habitat as a result of fragmentation) have altered the diversity and density of animal communities across many systems. In a global analysis of 1,673 vertebrate species, the abundances of 85% of species were affected positively or negatively by forest edges (Pfeifer et al., 2017). These effects result from behavioral and physiological tolerances to the environmental conditions, increased hunting, predation pressures, and differences in available resources (Murcia, 1995; Lenz et al., 2014; Haddad et al., 2015; Pfeifer et al., 2017). For example, edge-related variations in the quality of lemur food trees and the pressures associated with predation avoidance have been found to influence lemur density and distribution in a Malagasy dry forest (Lehman et al., 2006a,b). These edge-driven changes in the floral and faunal communities may affect the dynamics of species interactions. For example, the different environmental conditions at the edge may lead to avoidance of or attraction to the area by certain important seed-dispersing frugivores, which is likely to impact seed dispersal services and alter the composition of seeds moving toward the forest edge with implications for forest regeneration patterns.

Differences in the composition of frugivores in edge habitat could, therefore, influence the distribution of important plant functional traits, such as the size of plant propagules that are more likely to arrive in the disturbed community (Cordeiro and Howe, 2001; Michalski et al., 2007; Santos et al., 2008; Albert et al., 2015). Thus, in addition to habitat characteristics on the forest edge, altered seed dispersal patterns may contribute, in the long-term, to tree communities that are taxonomically less diverse and have lower phylogenetic and functional diversity than those in interior habitats (Santos et al., 2010; Haddad et al., 2015; Razafindratsima et al., 2018a). While much work has focused on how plant communities differ in forest edge habitat, more empirical work is needed to better understand and link how altered ecological processes such as animal-mediated seed dispersal may contribute to these differences (Murcia, 1995; Farwig et al., 2017; Bovo et al., 2018; Pires et al., 2018; Rehm et al., 2018; González-Castro et al., 2019).

Characterizing how edge effects alter the composition of disperser assemblages and how this may reflect on patterns of seed-dispersal and recruitment may provide important insights into the mechanisms structuring plant communities in forest edges and provide a better understanding of successional processes in disturbed habitats. To address this, we investigated how a frugivore community differed across a gradient from forest edge to the interior in a diverse tropical rainforest in Madagascar. We then examined how these differences may relate to patterns of animal-mediated seed dispersal and early seedling recruitment in forest edge and interior habitats.

We tested the hypothesis that larger frugivores, which often prefer more pristine habitat in many tropical systems (Emer et al., 2018; Messina et al., 2021), would avoid forest edges, reducing overall frugivore diversity, and that smaller-bodied frugivores would be more abundant in edge habitat because of habitat preferences and/or competitive release (Pfeifer et al., 2017; Püttker et al., 2019). If larger-bodied frugivores are less common near edge habitat, we predicted that this would be reflected by an overall smaller size of seeds dispersed by frugivores in edge habitat. We also predicted that differences in the rates or patterns of seed dispersal between habitat types would be reflected in subsequent recruitment patterns.

## MATERIALS AND METHODS

### Study Site and Systems

We carried out this project in a forest managed by local communities in the Andasibe region, within the rainforest Ihofa.

This forest is part of the Mantadia-Zahamena corridor, located in eastern Madagascar and transversed by the Ihofa River. Its protection is partly overseen by community-based management, but intense anthropogenic pressures (e.g., unmanaged shifting agricultural practices, logging, and hunting) persist. During our study period (January 2017–2018), the field site experienced an average temperature of 19.2°C (range: 10.8–34.7°C) and an average monthly rainfall of 76.97 mm (range: 6–333 mm) (Razafindratsima, unpbl.). The forest boundaries or edges, where we set up the transects and plots described below, are characterized by soft edges with a small expanse of successional fields separating the forest from the adjacent small-scale agricultural fields. These fields result from shifting agricultural practice, in which part of the forest is clear-cut, burnt, and converted for cultivation. According to local communities, the edges in this study were created between 1990 and 2012; thus, many of the current adult trees at the forest edge may have been remnants from the edge creation. Such a landscape matrix is similar to other forested environments found along Madagascar's eastern biome (Razafindratsima et al., 2018a). Fruiting in the site during our study period occurs year-round with a peak in the number of species fruiting and in the intensity of fruiting in June (**Supplementary Appendix 1**).

## Frugivore Communities

To characterize the frugivore community of the area, we conducted animal surveys along five linear transects running 3 km from forest edge to the interior, for a total of 253 days of sampling. These transects were at least 1 km apart from each other. We surveyed each transect once a week from January to December 2017, for a total of 49–54 sampling days per transect (**Supplementary Table 1**). Each survey took place either during the day (starting at 05:00 h) or at night (starting at 17:30 h). It took between 2 and 9 h (for an average of 3 h and 40 min) to complete each survey. Longer sampling time was a result of the difficulty in hiking the steep terrain during or after heavy rains. We conducted our weekly day and night surveys for each transect on two consecutive dates (i.e., 2 days in a row) but alternated between edge and interior where we started the survey of each transect. For example, on a given week, we first started the day survey at the forest edge and then did the night survey starting at the forest interior for the same transect, and these patterns were then alternated for our repeated weekly surveys. For the night surveys, we used LED Flashlights (Maglite ST3D016) to help with the visibility on the trail; whenever we encountered an animal, we used a headlamp with Red LED Light to help identify the species. Walking along the trail at a slow pace and pausing every few steps, a team of 2–3 people looked in every direction for any animal (on trees, on the ground, and in cavities if present). When encountering a vertebrate, we recorded the following standard survey data: time and location of sighting along the transect, identity and number of animals detected, perpendicular distance to the animal sighted from the transect (visual estimation), and animal behavior (Buckland et al., 2010; Brook et al., 2019). If the animal was observed feeding on plants, we recorded the food item (e.g., leaves, fruits, and flowers) and the plant species.

During these surveys, we made 2,096 animal sightings in total, including 60 species of birds, 14 species of lemurs, two species of carnivores, and two species of rodents (details in **Supplementary Table 2**). We focused the analyses in this article on birds and lemurs because these are the known major taxonomic groups serving as primary seed dispersal agents in Malagasy ecosystems among these encountered taxa (Razafindratsima, 2014; Razafindratsima et al., in press). While the carnivoran species *Galidia elegans* and the rodent species *Nesomys rufus* and *Eliurus* sp. also consume fruits and/or seeds in other systems (Nowak, 2005; Garbutt, 2007; Razafindratsima, 2017; Razafindratsima et al., in press), we did not include them as part of the frugivore community in this study because we observed each species only once during the 1-year-long transect surveys and their ability to disperse seeds is unknown. It is also important to note that fruit bats also play an important role in seed dispersal services in Madagascar (Racey et al., 2010; Andrianaivoarivelo et al., 2012; Razafindratsima et al., in press). They might also be present in our study site given their current geographic distribution (Racey, 2016; Andrianaivoarivelo et al., 2019, 2020). However, we did not encounter any fruit bats during our transect monitoring. The only bat species we saw passing by in a few instances was *Miniopterus manavi*, an insectivorous species (Rakotoarivelo et al., 2007). The other vertebrate species were either seed predators or are not known to consume or disperse fruits/seeds (Razafindratsima et al., in press).

We assigned each bird/lemur species as being frugivorous if the species is known to consume fruits/seeds and present seed dispersal behavior. Data on these behaviors were based on frugivory observations during the transect sampling, direct and camera-trap observations of animal visitors feeding in fruiting trees in the area (Raoelinjanakolona, unpbl. data), and on data from the literature (Razafindratsima et al., 2018c,d; Razafindratsima et al., in press). Thus, we had 21 species of birds and 11 species of lemurs categorized as frugivores in this study (**Supplementary Table 2**).

To describe the frugivore community, we determined species richness, encounter rates (number of individuals per sampling effort), and distribution of body masses for encountered frugivores for each 100-m increment along the edge-interior transects (i.e., 0–100 m, 101–200 m, etc.). We used quadratic polynomial regression models to investigate how these metrics varied as a function of distance from forest edge for each taxonomic group. Polynomial regressions were used because ecological edge effects are unlikely to be linear with distance from the edge (Murcia, 1995; Lehman et al., 2006a,b) and because it was clear that linear regressions were a poor fit to the data. We ran these models in R (R Core Team, 2020).

We also examined the patterns of encounters for individual species that we expected to be especially important dispersers for large-seeded plant species in the community. We focused specifically on fruit-eating pigeons (*Alectroenas madagascariensis* and *Treron australis*) and the large-bodied frugivorous lemurs, *Eulemur fulvus, E. rubriventer,* and *Varecia variegata,* because they are known to play an important role as seed dispersers in Madagascar's forested ecosystems (Bollen et al., 2004; Razafindratsima and Dunham, 2015;

Razafindratsima et al., in press). While the large-sized folivorous lemur species *Indri indri* and *Propithecus diadema* also consume fruits and, thus, were considered as part of the larger frugivore community, they are known to masticate and consume large seeds and are likely not important dispersers for large-seeded plants (Dew and Wright, 1998; Powzyk and Mowry, 2003; Semprebon et al., 2004).

We investigated how the frugivore communities differed across the edge-interior gradient in terms of diversity in body mass by characterizing the functional diversity of each community in the 100-m increments. To do that, we calculated the Petchey and Gaston's (2002) functional diversity index, *FD*, a dendrogram-based metric that estimates the dispersal of a community of species in a trait space. We used the R-package *pincate* (Kembel et al., 2010) to calculate *FD* values. We also performed quadratic polynomial regressions to determine how the values of *FD* vary as a function of distance from forest edge for each taxonomic group.

## Adult Tree and Shrub Communities

To better understand how adult tree and shrub communities found in edge and interior forest habitats may be associated with patterns of dispersal and recruitment, we established ten botanical plots of 40 × 40 m. We set up one plot at the start and end of each transect (five plots in forest edge and five in interior habitats). Within each plot, we identified all trees/shrubs greater than 10 cm in diameter at breast height (DBH, set at 1.30 m from the ground). We also tagged these individuals using a numbered aluminum tag nailed to each tree. Local Malagasy field technicians with extensive knowledge of the local flora helped identify the trees to their vernacular names. We determined the scientific names using a database of vernacular species names in the area previously established with expert Malagasy botanists (Razafindratsima, unpbl.). If they could not identify the species in the field, we collected samples of leaves and any reproductive materials present (flowers and fruits) for identification by specialists at the Malagasy National Herbarium at the Parc Botanique et Zoologique de Tsimbazaza (PBZT). Using this dataset, we measured the species richness and density of the adult trees/shrubs in forest edge and interior habitats. We assigned the dispersal mode of each identified plant species as zoochoric or abiotic, based on frugivory and seed dispersal data found in the literature and reported in databases (Razafindratsima et al., 2017; Razafindratsima and Dunham, 2019; Albert-Daviaud et al., 2021), from direct and camera-trapping observations of animal consuming fruits (Raoelinjanakolona, unpbl. data; Nantenaina, unpbl. data), from the observations of seeds found in feces in our seed traps, and from observations of seed and fruit traits. We gathered information on the seed length of each species from these same sources as available. We focused only on seed length as a measure of seed size because it made our data comparable to other studies in Madagascar and other tropical systems (Osuri et al., 2016; Razafindratsima et al., 2018b) and seed length and diameter were strongly correlated in our system (**Supplementary Figure 1**; $N = 79$, $R^2 = 0.89$, $p < 0.001$).

We analyzed how the habitat edge and interior differed in terms of adult plant species composition, richness, density, and the proportions of zoochoric plant species and individuals. To compare species composition between habitat types, we used a non-parametric permutational multivariate analysis (PERMANOVA) with the R-package *vegan* (Oksanen et al., 2007) based on Bray-Curtis similarity metrics with 9999 permutations (McArdle and Anderson, 2001). We also examined how the richness, density, and proportions of the species that were actively dispersed by animals during this study (found in seed traps as described below) differed between the two habitat types using linear mixed-effects (LME) regression models with the R-package *nlme* (Pinheiro et al., 2017). For each LME, we considered each metric as a dependent variable (richness, density, and proportions), habitat type as fixed effect (edge vs. interior) and transect as random effect. We incorporated latitude and longitude into the model to account for potential spatial autocorrelation.

## Seed Dispersal Rates

We sampled animal-mediated seed rain using seed traps, a widely used method in estimating seed rain in other studies (e.g., Razafindratsima and Dunham, 2016; Rogers et al., 2017), from January to December 2017. Each trap was made of a fine-mesh net attached to a flexible wood ring and hung on trees at 1.50 m high to reduce predation by ground-dwelling rodents that may predate and/or remove the seeds. This set-up is adequate for this study because the birds and lemurs in this system either fly or are arboreal; thus, they are likely to defecate, regurgitate, or drop most of the seeds they consume or handle from above. We used a total of 90 seed traps, placed at regularly spaced points in the square grid plot described above (9 traps × 5 plots × 2 habitats). We checked each trap at least once a week, during which we identified, counted, and measured the length of all seeds found in the trap. The timing of trap monitoring varied over time due to some logistical constraints (for example, heavy rain may have prevented the team from getting into the site). To account for these differences, we incorporated the number of days between monitoring into the calculation of seed dispersal rates, which was then defined as the number of animal-dispersed seeds into each trap per $m^2$ per day. Any trap that did not have seeds or fruits in it at a given monitoring day was assigned a value of zero. When needed for accurate identification, and when possible, we compared the seed from the trap with seeds from plants that were fruiting during the vegetation surveys. Additionally, we also relied on the expert knowledge of the local research technicians familiar with the local flora to identify the species based on specific seed characteristics. We focused our analyses of seed dispersal rates on seeds that appeared to have been dispersed by animals (active dispersal), i.e., depulped seed (pericarp removed) still in feces or with some fecal material attached to it, or it was a depulped seed from a species with an animal-dispersal syndrome. We compared how the mean rates of active seed dispersal differed between the two habitat types by performing an LME regression model, in which we considered the habitat type as a fixed effect (edge vs. interior) and transect as a random effect. An initial visualization of the data through boxplot showed some outliers; thus, we used the interquartile range (IQR) criterion (Vinutha et al., 2018) to identify and exclude these outliers in the model. We also incorporated in the model the exact location of each trap within the plot grid to account for potential spatial autocorrelations. We examined how the patterns of distribution frequency of seed length varied between the two habitat types by comparing the median of seed length in the two habitat types using a two-sample Wilcoxon test.

In addition to this active dispersal of seeds by animals, we also recorded the dispersal of seeds that were still contained within fruits, and we assumed they were dispersed by abiotic means regardless of dispersal syndrome (defined here as passive dispersal). We considered these dispersal events as passive because they may have reached the traps without assistance from animal dispersers (e.g., falling from nearby adults when ripe, being knocked down by wind). We performed the same statistical analyses as with the active seed dispersal for the passively dispersed seeds.

## Seedling Communities

We examined community structure and early-stage recruitment of seedlings through observations in plots of 10 by 10 m that we established at a random location within each of the aforementioned botanical plots. Each plot was left open but delimited with inconspicuous material to easily locate it for later monitoring. We identified and counted all seedlings that were between 2 cm and 100 cm tall within each plot; we also tagged each individual using a Tyvek water-resistant wristband marked with a waterproof marker. We set up these plots in February–May 2017 and monitored them in November–December 2018 to check whether each tagged seedling was still alive (approximately 21 months). During the monitoring, we recorded and tagged all newly established seedlings that had reached 2 cm in height.

Similar to the analysis of the adult plant community, we also analyzed how the habitat edge and interior differed in terms of seedling species composition, richness, and density. To determine differences in species composition, we used a non-parametric permutational multivariate analysis (PERMANOVA) with the R-package *vegan* (Oksanen et al., 2007) based on Bray-Curtis similarity metrics with 9999 permutations (McArdle and Anderson, 2001). We performed linear mixed-effects (LME) regression models to examine statistical differences in seedling species richness and density between the two habitat types. For each LME, we considered habitat type as a fixed effect (edge vs. interior) and transect as a random effect. We also incorporated in the model the latitude and longitude where the plots were located to account for potential spatial autocorrelation.

We examined the linkages between the seedling and adult communities by comparing species richness at both stages with a PERMANOVA and by investigating how the percentages of dispersed seedlings (those without adults of the same species occurring in the same plot) differed between edge and interior habitats using an LME (with habitat type as a fixed effect, transect as random effect and longitude and latitude incorporated to account for potential spatial autocorrelation).

We calculated a recruitment rate for each plot, as follows: $RR = (N_i - D + E)/N_i$ in which $\boldsymbol{N_i}$ is the initial number of seedlings, $\boldsymbol{D}$ corresponds to the number of seedlings that died, and $\boldsymbol{E}$ represents the number of seedlings that emerged after the initial counting. We analyzed how seedling recruitment differed between the two habitat types by performing an LME, using two types of datasets: (1) only the species found in the seed traps and (2) all the species in the seedling plot. The first one allows us to examine the link between animal-mediated seed dispersal rates and seedling recruitment. The second one considers passive dispersal, given that seeds within fruits may still be able to germinate. In either case, we consider seed species as a random factor because different species may have different recruitment probabilities. We also examined the association between dispersal rates and seedling recruitment using a Pearson correlation test. To test if recruitment of species that we observed being actively dispersed in our study was biased in terms of seed size in either habitat, we ran a generalized linear model in R (R Core Team, 2020), with seed length of the recruited species as the dependent variable and habitat type as the factor.

# RESULTS

## Frugivore Community

Species richness values of birds and lemurs across the edge to interior gradient were not explained by linear relationships, but showed marginally significant or significant fit, respectively, to polynomial (quadratic) regression models. Bird richness showed a weak “U” shaped relationship across the gradient (**Figure 1A**; $R^2 = 0.04$, $F_{2,147} = 3.12$, $p = 0.05$) while lemurs displayed a humped shaped pattern of species richness with higher richness at intermediate distances (**Figure 1A**; $R^2 = 0.12$, $F_{2,147} = 9.54$, $p < 0.001$). The mean encounter rates (number of individuals encountered per sampling efforts) of birds and lemurs showed no relationship to distance to edge habitat (**Figure 1B**; Birds: $R^2 = 0.01$, $F_{2,147} = 0.15$, $p = 0.86$; Lemurs: $R^2 = 0.03$, $F_{2,147} = 2.45$, $p = 0.08$).

There were also no significant patterns (**Figure 1C**) regarding the size distribution of fruit-eating birds ($R^2 = 0.03$, $F_{2,147} = 2.38$, $p = 0.09$) or lemurs ($R^2 = 0.01$, $F_{2,147} = 0.14$, $p = 0.87$) along the edge-interior gradient. Among these fruit-eating species, 11 bird species and 4 lemur species were observed within 100 m of the forest edge; however, all of them were also found in the interior habitats (**Supplementary Table 2**). None of the fruit-eating bird species appeared to be edge-specialists, as they were all observed in the interior forest up to 3,000 m from the edge. With the exception of four species (*Eurystomus glaucurus, Coua caerulea, C. reynaudii,* and *Coracopsis vasa*), the bird species found near the edge were small-sized (<100 g). The site’s two frugivorous pigeons, *A. madagascariensis* and *T. australis,* may avoid edge habitat as they were absent from forest edges and were only observed at a minimum distance of 475 and 1,529 m from the edge, respectively (**Figure 1C** and **Supplementary Table 2**). None of the encountered lemur species in our study appeared to be edge-specialists; however, one of the three large-bodied (>1,500 g), seed-dispersing lemur species, *V. variegata,* was only encountered in the interior, at ≥1,829 m (**Supplementary Figure 2**). The other two large-bodied, seed-dispersing lemur species, *E. fulvus* and *E. rubriventer,* were observed both near the edge and in the interior habitats.

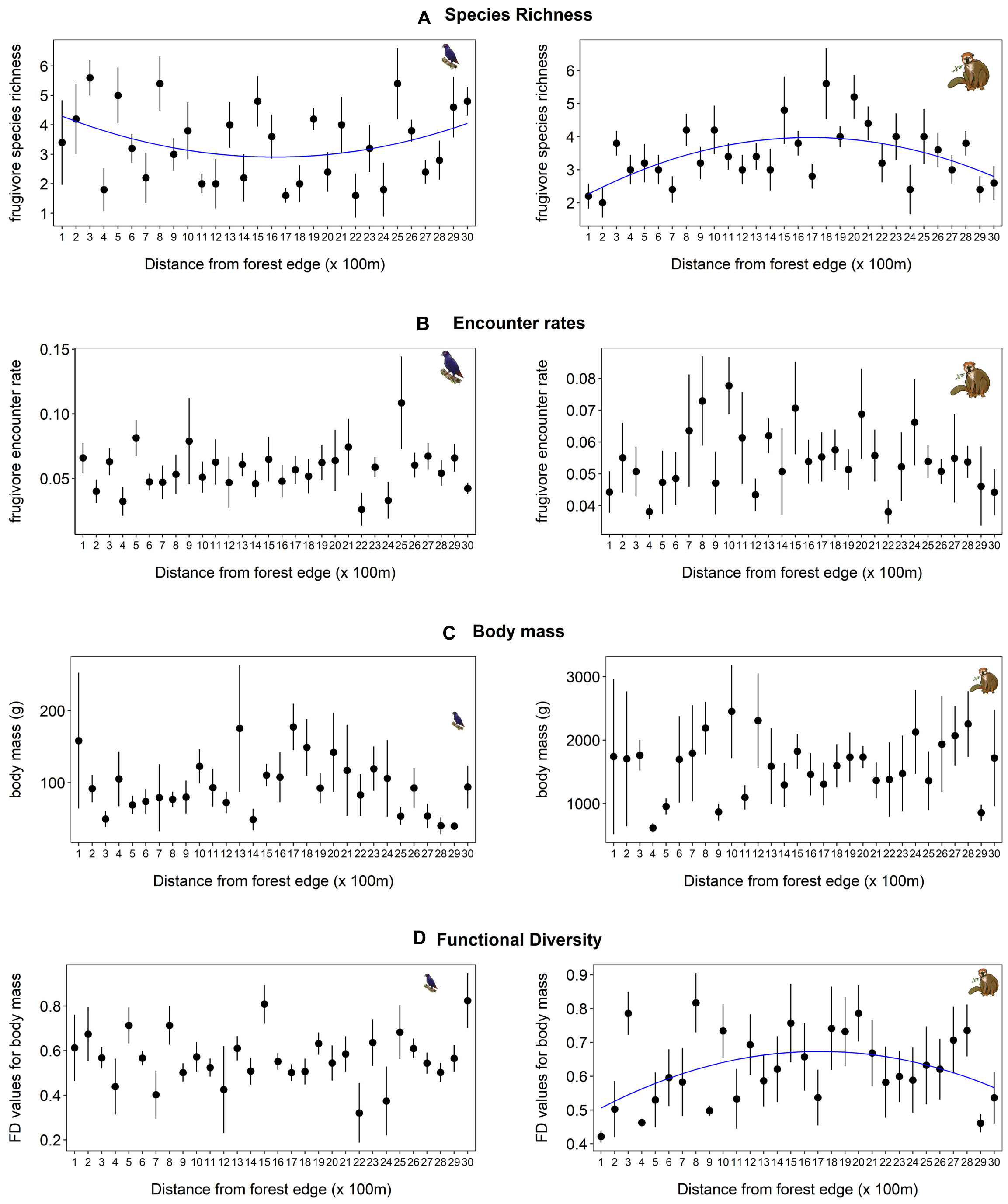

**FIGURE 1 |** Species richness **(A)**, encounter rates **(B)**, body mass **(C)**, and functional diversity measures of size variation **(D)** of frugivorous birds (left) and lemurs (right) along an edge-interior gradient in transects of 3,000 m. Circles represent mean values across transects, whiskers indicate standard deviations. The blue line corresponds to the fit of a significant quadratic polynomial regression. Taxa illustrations by Finaritra Randimbiarison.

Our most common lemur encounters at the edge habitat were small-sized omnivorous and folivorous lemurs (*Microcebus lehilahytsara* and *Avahi laniger*) and the large-sized folivorous species, *Indri indri* (**Supplementary Table 2**).

Functional diversity of size did not show a clear pattern across the habitat gradient for the bird community (**Figure 1D**; $R^2 = 0.042$, $F_{2,27} = 0.594$, $p = 0.559$). However, for frugivorous lemurs, functional diversity of body sizes demonstrated a weak hump-shaped curve with the highest values at intermediate distances between habitats, mirroring the pattern of species richness, though the significance of the pattern was only marginal (**Figure 1D**; $R^2 = 0.194$, $F_{2,27} = 3.265$, $p = 0.053$).

## Adult Tree/Shrub Richness and Density

We sampled tree/shrub communities on the forest edge and interior habitats for comparison. In total, we surveyed 1,305 individual trees and shrubs greater than 10 cm in diameter (601 in forest edge and 704 in interior) of 140 species. The plant species making up the assemblages in the forest edge and interior habitats showed no significant difference in species composition ($F_{1,8} = 0.85$, $p = 0.65$). In addition, the two habitat types had similar size distributions of adult trees (diameter and height; **Supplementary Figure 3**). Further, they did not differ in terms of species richness ($t_4 = -0.24$; $p = 0.82$; **Supplementary Figure 4A**) or density ($t_4 = 1.49$, $p = 0.21$; **Supplementary Figure 4B**).

We found that on average, across plots, a higher percentage of plant species in the interior were zoochoric (84.93% at the edge vs. 89.52% interior); these differences were small but statistically significant ($t_4 = 5.02$; $p < 0.01$). However, there was no significant difference in the percentage of zoochoric plant individuals between the edge and interior plots ($t_4 = 2.20$; $p = 0.09$; 86.60% zoochoric individuals in the edge vs. 92.28% in the interior). Additionally, we found that the density of the adult plant individuals of the species that were actively dispersed by animals during this study did not differ between the two habitat types ($t_4 = 0.82$; $P = 0.45$). These communities of plants also had similar patterns of seed sizes between the edge and interior habitats (**Supplementary Figure 5**; $t_{48} = -0.46$, $p = 0.65$).

## Seed Dispersal Rates

The mean rates of active seed dispersal, based on the count of animal-mediated seed rain into the traps, were significantly different between habitat types, with a higher rate reaching the forest interior than the forest edge ($t_{77} = 2.14$, $p = 0.04$; **Figure 2A**). The frequency distribution of the length of seeds dispersed by animals appears to be different, with a higher proportion of small seeds dispersed in the forest edge than the interior habitats, which had a higher proportion of medium-sized seeds (**Figure 2B**). The median length of the seeds in the edge habitats was shorter than that of seeds in the interior habitats ($p < 0.0001$). Nine out of 13 identified species of seeds collected in the seed traps that were actively dispersed in the forest edge were absent from the seed rain observed in the forest interior. In comparison, 15 out of the 19 identified seed species in the interior seed rain were not present in the forest edge (**Supplementary Table 3**).

Seeds dispersed passively (i.e., whole fruits found within the seed rain), accounted for 86.20% of the total collected propagules in the seed traps. Dispersal rates of passively dispersed seeds in the edge and the interior forest did not differ significantly (average dispersal rates in the edge: 0.07 fruits per $m^2$ per day, interior: 0.08 fruits per $m^2$ per day; $t_{30} = 0.41$, $p = 0.68$). Regarding the distribution of the size of the fruits in the traps, both edge and interior habitats appear to have a high proportion of small-sized fruits, but the interior forest also received a higher proportion of medium-sized fruits than the forest edges (**Supplementary Figure 6**). However, these differences were not statistically significant ($p = 0.81$).

## Seedling Richness, Density, and Recruitment Dynamics

The forest edge and interior had significantly different species compositions of seedlings ($F_{1,8} = 1.75$, $p = 0.04$). However, the seedling communities in these two habitat types did not differ significantly in terms of species richness ($t_4 = 1.00$; $p = 0.37$; **Figure 3A**) or density ($t_4 = 0.19$, $p = 0.86$; **Figure 3B**).

Overall, we found significantly different species compositions between the seedling and adult stages ($F_{1,18} = 6.08$, $p < 0.001$); Some species found at the seedling stage were not present at the adult stage in the same habitat type but were potentially actively dispersed (**Supplementary Table 5**). At the edge habitats, we estimated that an average of 60.99% of seedling species, did not have the same species of adults in the plot where they occurred; whereas in the forest interior, it was the case for 67.76% of the seedling species. These differences, however, were not significantly different ($t_4 = 1.66$, $p = 0.17$).

When we looked only at species that were also found in seed traps, we did not find a significant difference in seedling recruitment ($t_{136} = -0.15$, $p = 0.88$; **Figure 3C**) between the two habitat types. There was also no significant difference found when all the seedlings in the plot were considered in the analysis, i.e., both passive and active seed rain (seedling recruitment: $t_{159} = 0.52$, $p = 0.60$; **Supplementary Figure 7**). We found that the rates of seedling recruitment were not associated with the rates of seed dispersal, neither for actively dispersed seed species found in the traps ($t_8 = -0.13$, $p = 0.29$) nor when all the seedling species were considered ($t_8 = -0.09$, $p = 0.93$). We also found no bias in seedling recruitment of animal-dispersed species between the two habitat types in terms of seed size of the plant species recruiting over the 21 months of study ($t_{102} = -0.542$, $p = 0.589$).

## DISCUSSION

Edge habitats are pervasive around the world as deforestation and fragmentation of forested ecosystems continue to intensify through human activities (Murcia, 1995; Haddad et al., 2015). Understanding how forest edges affect ecosystem processes such as seed dispersal is critical for better understanding potential mechanisms driving plant community differences in edge habitats (Magrach et al., 2014). In our study of Madagascar's eastern rainforest, one of the most biodiverse and endangered forest ecosystems in the world, we found, as predicted, that the seeds reaching edge habitat through dispersal by frugivores were smaller in size than those dispersed in the interior despite no difference in seed size distributions of the adult plant communities. While fruit-eating primate and bird species were not, on average, smaller near the edge habitat, one lemur and two bird species that are known to be effective dispersers of large seeds were not observed near the forest edge. Such findings suggest that a small number of frugivores may act as a potential biotic filter for incoming dispersal of plant propagules. While seedling recruitment overall was not affected in terms of rates or seed-size distribution of successful recruits, long-term suppression of dispersal of some species could ultimately affect community regeneration patterns. Results suggest that active management

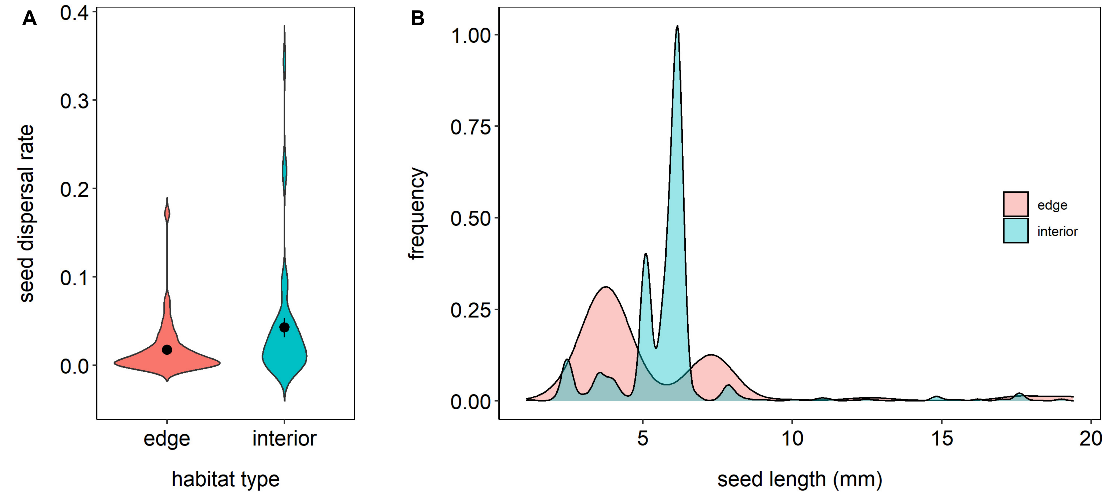

**FIGURE 2 |** Rates of animal-mediated seed dispersal **(A)** and size distribution of the animal-dispersed seeds **(B)** in forest edge and interior habitats.

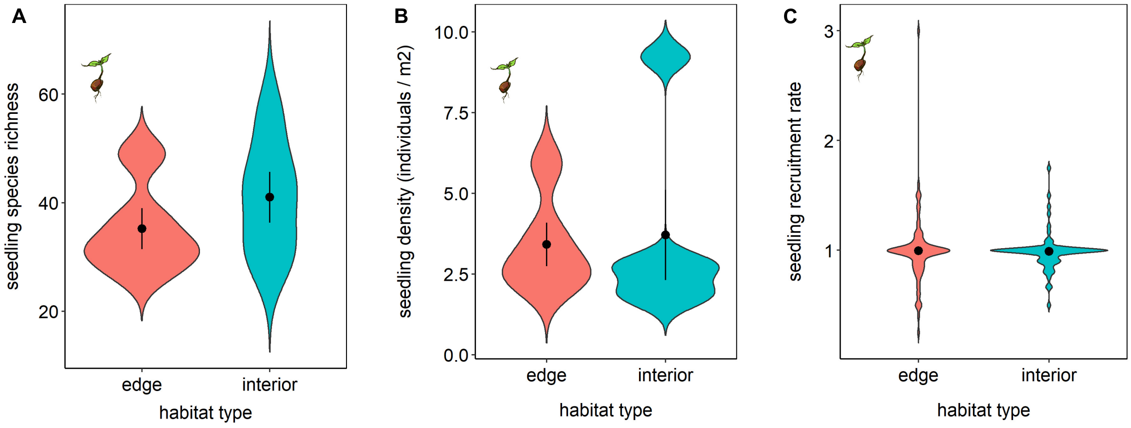

**FIGURE 3 |** Species richness **(A)** and density **(B)** of seedlings as well as rates of seedling recruitment after 21 months **(C)**, for the zoochoric species that were captured in seed traps, in forest edge and interior habitats.

might be necessary to maintain, restore and manage rare large-seeded tree species in fragmented habitats. These findings also highlight the complexity of animal-mediated seed dispersal as a determinant of plant diversity.

The lemur frugivore communities across the forest edge to the interior gradient in this system showed the highest species richness and variability in body sizes at intermediate distances. These patterns may be a result of the variable preferences and tolerance levels of animal species to the environmental conditions across the gradient (Murcia, 1995; Haddad et al., 2015). While our data is inconclusive, intermediate distances may harbor the highest diversity in species and body sizes because it may be frequented by species preferring either edge or interior habitats as well as generalist species.

While we did not find an overall association of larger frugivore body size with distance to the edge habitat as we expected, our seed dispersal data suggests that the most effective dispersers of large seeds in this system may be reduced or missing in edge habitat. Indeed, the forest edge in our study site was not frequented by the most frugivorous large-bodied lemur species in this system, *Varecia variegata,* which is one of the most important seed dispersers for large-seeded tree species in many of Madagascar's ecosystems (review in Razafindratsima et al., in press), nor by the two fruit-eating pigeons known to swallow large seeds (**Supplementary Table 2**). In contrast, the edge habitat is frequented by small-sized omnivorous and folivorous lemurs (such as *Microcebus* and *Avahi*) and by the large-sized folivorous *Indri indri* (**Supplementary Table 2**). Species within the genera *Microcebus* and *Avahi* have been found to have a flexible diet and high tolerance to different types of forest, such as disturbed habitats (Rendigs et al., 2003; Murphy et al., 2016; Knoop et al., 2018; Ramananjato et al., 2020; Ramananjato and Razafindratsima, 2021), and they are frequently observed to forage in forest edge habitats. Primate species that have a high proportion of leaves in their diet, such as *Indri indri*, are also often less vulnerable to habitat disturbance than species with a diet dominated by fruits because of the relatively high density and quality of foliage that is often available in disturbed habitats (Glessner and Britt, 2005; Irwin et al., 2010; Seaman et al., 2018).

Many of the bird species in this study site also seem to avoid the forest edge, as only 30% of all the encountered bird species ($n$ = 60) were observed in the forest edge, and none of them were edge-specialists (**Supplementary Table 2**). Of the frugivorous birds, only 11 out of 21 species were observed in the edge habitat. As with lemurs, a majority of the large-sized frugivorous bird species, such as fruit-eating pigeons (*T. australis* and *A. madagascariensis*) and a frugivorous coua (*C. serriana*), may be interior specialists as they were absent from forest edges. The absence of these species in forest edges in Madagascar may have consequences for the many plants that specialize in bird dispersal in Madagascar's diverse rainforests (Rakotomanana et al., 2003; Razafindratsima, 2014). These results also suggest forest fragmentation may be an important threat to some frugivore bird populations in Madagascar where increasing and extensive habitat disturbance and fragmentation of forested habitat (Harper et al., 2007; Vieilledent et al., 2018) may limit their populations. These results were surprising, in part because none of the frugivorous birds are currently listed as threatened by the IUCN Red List (IUCN, 2021); however, further research and assessment may be necessary.

These differences in frugivores visiting edge vs. interior forests were associated with different rates of animal-mediated seed dispersal, estimated from the seed rain, between the two habitat types. The forest edge received a lower rate of animal-mediated seed dispersal than the interior habitats. The reduced number of large and highly frugivorous animals in edge habitats has been suggested to drive the lower seed dispersal rates in edge habitats in other systems (Magrach et al., 2014).

The median size of seeds reaching the forest edge habitat through dispersal by animals was smaller than those dispersed into the forest interior. Edge habitats had a higher proportion of small seeds than interior habitats, which had a higher proportion of medium-sized seeds. This result corroborates findings in other systems, showing a higher percentage of medium, large, and very large seeds in the seed rain received by forest interior than forest edge habitats (Oliveira et al., 2004; de Melo et al., 2006). This pattern of endozoochorous seed dispersal we observed was unlikely to be a result of the distribution of seasonally fruiting trees on the edge vs. interior forest because we found no significant difference in the number of these trees between habitat types. However, future work should also consider fruit crop size, which may vary between habitat types because of differences in abiotic conditions that may influence fruit production (Restrepo et al., 1999; Dunham et al., 2018; Gonçalves da Silva et al., 2018). We also found that seeds of several plant species were only actively dispersed by animals in one or the other habitat, which may reflect the differences in frugivore species composition between the edge and interior habitat.

Despite the bias in size distributions of seeds falling in edge and interior forests, the rate of seedling recruitment of animal dispersed plants did not differ overall, and there was no bias in recruitment of small-sized plant species on the edge habitat. It is also possible that historical seed bank composition (Klanderud et al., 2010) and other ecological processes, such as competition or alteration of the seed bank through secondary dispersal and/or seed predation (Dausmann et al., 2008; Razafindratsima, 2017), may have important influences on patterns of recruitment (Eriksson, 1995; Eriksson and Eriksson, 1997; Wenny, 2000). For example, research in the Amazonian rainforest has shown that tree seedling recruitment in cleared habitat is less likely to originate from seed rain than from the seed bank (Young et al., 1987; Lawton and Putz, 1988), most likely due to the high rates of seed predation of newly fallen seeds by animals (Uhl, 1987). Regardless of the mechanism, these findings support the general concept that although seed species supply through dispersal is important, it is not sufficient on its own to structure plant communities; it interacts with local environmental conditions (Myers and Harms, 2009). Further, we also suspect that the short duration of this study may have limited our ability to detect significant impacts or even to differentiate the existence of competitive exclusion from environmental filtering.

While we found no differences in recruitment patterns in our study, further work should examine how biases in dispersal patterns may translate into differences in seed bank compositions and successional impacts in forest edges over longer time scales. Size-biased patterns of seed rain may influence the successional dynamics of plant communities in forest edges over time because these patterns may create opportunities for some species with particular traits to become more prevalent in the edge community (Brodie and Aslan, 2012; Kurten et al., 2015). Seed rain also may influence the composition of the seed bank for future recruitment (Wandrag et al., 2015). The forest edge habitat could become dominated by small-seeded pioneer plants, lose rare tree species, and become homogeneous in terms of floristic composition over time (Oliveira et al., 2004; Melo et al., 2010; Lôbo et al., 2011). Small seed size is also associated with lower aboveground carbon storage in trees in this region (Razafindratsima et al., 2018b); thus, future succession of edges toward fast-growing species with smaller seed sizes could result in a large-scale reduction of carbon storage from fragmented forests. It is also possible that the differences in the biotic processes and abiotic environment in edge habitat may outweigh any impacts that more subtle differences in seed rain may have (Balcomb and Chapman, 2003; Orrock et al., 2006). Further studies examining the role of seed dispersal limitation on the dynamics and biodiversity of tree communities in edge habitats may help resolve this.

## Conservation and Management Implications

If seed supply is limiting for forest edge communities, management of large-seeded species may need to be considered in some areas. For example, encouraging key seed dispersers to frequent the edge habitats could be useful for conserving rare, large-seeded species and increasing plant diversity in these areas (Couvreur et al., 2004; Cosyns et al., 2005; Chapman and Dunham, 2018). Generalist seed dispersers could increase the odds of many species reaching and establishing in these areas, thereby increasing local species richness (Myers and Harms, 2009; McConkey et al., 2012; Carlo and Morales, 2016). This is especially important to consider in current human-modified landscapes, as land-use transformations pose critical concerns for a large number of plant populations worldwide (Murcia, 1995; Tylianakis et al., 2008; Haddad et al., 2015), and particularly in the tropical forests of Madagascar (Harper et al., 2007; Razafindratsima et al., 2018a; Morelli et al., 2020). Plus, in the long-term, if not properly managed, the forest edge could recede into the core of the forest, affecting forest regeneration and succession (Gascon et al., 2000). Encouraging seed dispersal into these habitats could be possible by increasing the attraction of animal frugivores to visit them – for example, by planting specific food trees and/or installing human-made perching structures (Wunderle, 1997; Martinez and Razafindratsima, 2014; Mantia et al., 2019).

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## AUTHOR CONTRIBUTIONS

OR conceived the idea, designed the project, analyzed the data, and drafted the first version of the manuscript, with inputs from AD. OR, NR, RH, RN, and TR collected the data. All authors made revisions in the writing.

## FUNDING

This fieldwork was supported by funding from the American Philosophical Society (Franklin Research Grant), the National Geographic Society (#9881-16), Harvard University Herbaria, Hrdy Fellowship, and the College of Charleston (Research and Development departmental funding) to OR.

## ACKNOWLEDGMENTS

We thank the "Ministère de l'Environnement et du Developpement Durable" in Madagascar for research permission (permit N° 291/16/MEEF/SG/DSAP/SCB and 190/17/MEEF/SG/DSAP/SCB.Re). We also thank Rainer Dolch, Alain Alimarisy, Sylvie Andriambololonera, Lantoarisoa Faranirina, Zafimahery Rakotomalala, Jacques Rakotoarisoa, and Tahiana Rakotondrainibe for logistical supports, and the elders of the surrounding villages and the community-based management association VOI FITAMA for permitting us to work in their forest. We are grateful to Jean Rafalimandimby, Jerome Rolland, Joseph Randrianantenaina, Tovo Randrianantenaina, Evangeliste Randrianantenaina, Gerdaya Ratonganirina, Justin Randriamandimby, and Abel Manampisoa for sharing with us their indigenous knowledge of the plants and animals in the region and for their valuable help with data collection. We are also grateful to Mad Randrianasolo and Anjaratiana Tafitamahandry at Association Mitsinjo, Benja Rakotonirina at the Institut Malgache de Recherches Appliquées (IMRA), and the botanists at the Malagasy National Herbarium at the Parc Botanique et Zoologique de Tsimbazaza (PBZT) and MBG Madagascar for their significant help with plant species identification. Special thanks to Angelo Andrianiaina, Veronarindra Ramananjato, Mahandry Andrianarisoa, Solofo Andrianarimalala, Herilanto Ramaroson, Mihajatiana Raoelison, and Hajatiana Rabarison for helping with data collection, Tomás Carlo and Colin Chapman for their insights in the conception of this project, Finaritra Randimbiarison for the illustrations, and Haldre Rogers and two reviewers for their feedback that improved an earlier version of this manuscript.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.655441/full#supplementary-material

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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in Ecology and Evolution

HYPOTHESIS AND THEORY
published: 06 December 2021
doi: 10.3389/fevo.2021.752110

# A Mechanistic Framework for Understanding the Effects of Climate Change on the Link Between Flowering and Fruiting Phenology

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**Edited by:**
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Consejo Superior de Investigaciones Científicas (CSIC), Spain

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Estación Biológica de Doñana (EBD), Spain
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**Specialty section:**
This article was submitted to Population, Community, and Ecosystem Dynamics, a section of the journal Frontiers in Ecology and Evolution

**Received:** 02 August 2021
**Accepted:** 08 November 2021
**Published:** 06 December 2021

**Citation:**
Sandor ME, Aslan CE, Pejchar L and Bronstein JL (2021) A Mechanistic Framework for Understanding the Effects of Climate Change on the Link Between Flowering and Fruiting Phenology. Front. Ecol. Evol. 9:752110. doi: 10.3389/fevo.2021.752110

Phenological shifts are a widely studied consequence of climate change. Little is known, however, about certain critical phenological events, nor about mechanistic links between shifts in different life-history stages of the same organism. Among angiosperms, flowering times have been observed to advance with climate change, but, whether fruiting times shift as a direct consequence of shifting flowering times, or respond differently or not at all to climate change, is poorly understood. Yet, shifts in fruiting could alter species interactions, including by disrupting seed dispersal mutualisms. In the absence of long-term data on fruiting phenology, but given extensive data on flowering, we argue that an understanding of whether flowering and fruiting are tightly linked or respond independently to environmental change can significantly advance our understanding of how fruiting phenologies will respond to warming climates. Through a case study of biotically and abiotically dispersed plants, we present evidence for a potential functional link between the timing of flowering and fruiting. We then propose general mechanisms for how flowering and fruiting life history stages could be functionally linked or independently driven by external factors, and we use our case study species and phenological responses to distinguish among proposed mechanisms in a real-world framework. Finally, we identify research directions that could elucidate which of these mechanisms drive the timing between subsequent life stages. Understanding how fruiting phenology is altered by climate change is essential for all plant species but is particularly critical to sustaining the large numbers of plant species that rely on animal-mediated dispersal, as well as the animals that rely on fruit for sustenance.

**Keywords: global change, flowering, fruiting, life history stages, phenological shifts, seed dispersal**

## INTRODUCTION

Phenological shifts are among the most visible ecological effects of global climate change. Research on individual species (e.g., CaraDonna et al., 2014), meta-analyses (Root et al., 2003; Munguía-Rosas et al., 2011), and community-wide taxonomic surveys (Ovaskainen et al., 2013) demonstrate that phenological, or the timing of life history, events in most species and stages are advancing in response to warmer temperatures. Well-studied life history stages include migration (Mayor et al., 2017) and breeding (Burger et al., 2012) in birds, hibernation in mammals (Sheriff et al., 2011), adult emergence in insects (Bartomeus et al., 2011; Renner and Zohner, 2018), and green-up and flowering in plants (Calinger et al., 2013; Rafferty and Nabity, 2017; Ettinger et al., 2018). In contrast, other life history stages, such as fruiting, have received relatively little attention (Chuine and Régnière, 2017; Mendoza et al., 2017; Ettinger et al., 2018). An important gap in our understanding concerns whether the latter of successive events in the life histories of an organism (e.g., fruiting which follows flowering) are shifting independently of, or are functionally constrained by, earlier stages.

It is reasonable to predict that climate-induced shifts in the timing of early life history stages result in parallel shifts in subsequent life-history stages of the same organism. The timing of life history stages is driven by a combination of external (climate) and internal (physiological or endogenous) factors. It is largely unknown, however, to what extent each factor drives the timing of one life history stage relative to earlier ones (McDermott and DeGroote, 2017; Ettinger et al., 2018; Gougherty and Gougherty, 2018; Augspurger and Zaya, 2020; Buonaiuto et al., 2021). With the exception of a few pioneering studies (e.g., Menzel et al., 2001; Post et al., 2008a,b; Haggerty and Galloway, 2011; Jiang et al., 2016; Segrestin et al., 2018), little research has yet explored whether successive life history stages predictably shift in concert with each other in response to climate change, nor, when they do, what drivers underlie this relationship (Chuine and Régnière, 2017; Ettinger et al., 2018). Parallel shifts might occur either if the interval between successive life-history events is constant or nearly so (likely a product of internal factors such as physiology or development), or if the external, proximate climatic cues for the two events are the same or tightly associated. Parallel shifts in successive life history stages could be related to reproductive strategy and traits (Forrest and Miller-Rushing, 2010; Ettinger et al., 2018; reviewed in Gougherty and Gougherty, 2018; Buonaiuto et al., 2021), genetics and selective processes (Crozier et al., 2008; Wilczek et al., 2010), and temporal boundaries on the growing or breeding season (Morales et al., 2005). Alternatively, successive life history stages could respond to different climatic conditions (Kingsolver et al., 2011), resulting in a changing interval between them as the climate changes (Lany et al., 2016). Determining which internal or external mechanisms are acting on the timing of life history events should offer critical predictive insights into whether and how climate change will affect the persistence not only of individual taxa, but also of the interactions among them.

Perhaps the most thoroughly documented phenological shift in response to climate change involves flowering. Advances in flowering time (conventionally noted by opening of flowers on an earlier date) have been found both across communities and within them, and contrasting responses across regions and taxa have been explored in some depth (e.g., Fitter and Fitter, 2002; Root et al., 2003; Miller-Rushing and Primack, 2008; CaraDonna et al., 2014; Rafferty and Nabity, 2017). Here, we use this wealth of knowledge on flowering phenology to explore whether subsequent life stages are linked, and if so, how they are linked. Although several investigators have speculated about whether advances in flowering time are accompanied by parallel shifts in fruiting time (Primack, 1987; Eriksson and Ehrlén, 1991; Forrest and Miller-Rushing, 2010), there are as yet few tests of these ideas (Chmielewski et al., 2004; Sherry et al., 2007; Segrestin et al., 2018). As a consequence, the response of fruiting phenologies to a changing climate remains poorly understood (Chuine and Régnière, 2017).

Experimental warming studies have shown that with higher temperatures, most species fruit earlier in the season (Sherry et al., 2007; Post et al., 2008a,b). However, observational studies of fruiting phenology, particularly those using datasets that span decades, vastly lag behind the number published for flowering. One possible reason for the focus on flowering phenology over fruiting is that assigning a date to fruiting is less straightforward because fruit morphology is more diverse across taxa than flower morphology, and stages of fruit development are less easily identified through observation. We follow convention of previous studies and, unless otherwise noted, use "fruiting" or "fruiting time" to denote the first date on which the presence of mature fruits or seeds is observed (e.g., Gordo and Sanz, 2009; Haggerty and Galloway, 2011; Ettinger et al., 2018), and, in our discussions of published studies and within the case study we present, we compare across taxa with different fruiting structures (e.g., fleshy, dry, indehiscent, dehiscent, animal-dispersed, and wind-dispersed; Menzel et al., 2006, 2020; Ge et al., 2015).

Here, we explore promising pathways for advancing understanding of which and how fruiting times are linked to flowering times. In animal-dispersed plants, shifts in fruiting phenology have the potential to affect or even disrupt seed-dispersal interactions (Forrest, 2014; Rafferty et al., 2015). Thus, an understanding of shifting fruiting phenologies is important to our ability to predict the effects of global change on plant-animal communities (Rogers et al., 2021). We incorporate plants that are animal-dispersed as well as those that employ abiotic seed dispersal, but we focus on the interaction and community repercussions for animal-dispersed plants. First, we synthesize current understanding of the linkages between flowering and fruiting stages across angiosperms. Second, to ground our discussion of life history stage linkages in a real-world framework, we report on a case study designed to determine how the flowering and fruiting times of individuals tracked over multiple decades at the same location are linked. Third, we leverage our extensive knowledge of climate-mediated shifts in flowering phenology to develop a conceptual model for how successive life history stages are linked and how climate change could affect these linkages. We then return to our case study results to demonstrate how to distinguish and eliminate proposed mechanisms for these linkages. Finally, we suggest directions for future research to test these mechanisms, and discuss the implications of climate change-driven shifts in fruiting phenology for ecological communities.

## CLIMATE CHANGE AND POTENTIAL LINKAGES BETWEEN FRUITING AND FLOWERING

Few studies have determined the extent to which flowering and fruiting phenology are linked or physiologically constrained. We argue here that examining the flowering-to-fruiting interval can provide insight. We define the flowering-to-fruiting interval (hereafter, FTFI) as the period from flower opening to the first date on which the presence of mature fruits or seeds is observed. The FTFI is "constrained" if it remains fixed in length, including within warming experiments or in a changing climate. The FTFI is a trait defined at the level of the reproductive structure; i.e., it is the length of time from flowering to fruiting measured on a single flower developing into a fruit on an individual plant. In order to make use of historical datasets in which observations were not collected at this level, most previous studies have largely used population- or species-level measurements, with a few examples of individual-level measurements (**Table 1**). If flowering and fruiting are linked, internal drivers will dictate that fruiting times are related to flowering times as well as to the FTFI. If external factors are instead driving phenology, the FTFI could lengthen or shorten as flowering and fruiting respond to these external factors independently. If flowering and fruiting are responding independently to the same external factors, or if by chance these two life stages are responding in similar ways to different independent factors, the FTFI will stay the same.

**TABLE 1 |** Evidence from previous observational and experimental studies on the length of the flowering to fruiting interval (FTFI), the time between the flowering and ripe fruit phenophases, and for external and internal drivers of FTFI duration.

| Authors | Year | Time | Level | Driver |
|---|---|---|---|---|
| Lechowicz (1995) | 1995 | 30 years | Not reported | External |
| Post et al. (2008b) | 2008b | 1 year | Individuals | External |
| Jiang et al. (2016) | 2016 | 3 years | Individuals | External |
| Sethi et al. (2020) | 2020 | 5 years | Plots | External |
| Ettinger et al. (2018) | 2018 | 1 year | Individuals | Internal |
| Segrestin et al. (2018) | 2018 | 12 years | Population | Internal |
| Post et al. (2008a) | 2008a | 2 years | Plots | Both |
| Menzel et al. (2006) | 2006 | >15 years | Country | Both |
| Menzel et al. (2020) | 2020 | 67 years | Country | Both |
| Haggerty and Galloway (2011) | 2011 | 1 year | Individuals | Both |

*The citation for each study is listed in the first two columns. The third column lists the span of the dataset in number of years. The fourth column lists at what level phenology was tracked: individuals, plots, or population. "Individuals" denotes that phenological stages were tracked on marked individuals, and the mean across many individuals of the same species was analyzed. "Plots" denotes that phenological stages were recorded at the level of experimental or observational plots, across all individuals of the same species within a plot, and means were analyzed across multiple plots. "Population" denotes that phenological stages were recorded at the level of a site, which generally included multiple individuals of the same species, and means were analyzed across multiple sites. "Country" denotes that phenological stages were tracked by country, and means were analyzed across multiple countries. When it was unclear within the study whether individuals, plots, population, or country was tracked, we listed "not reported" within the column. The last column summarizes whether the study provides evidence for internal, external, or both internal and external drivers of FTFI duration.*

The few previous studies of how flowering and fruiting are linked provide evidence for internal drivers, but also evidence for independent responses of flowering and fruiting to external factors (Post et al., 2008a,b; Ettinger et al., 2018; Segrestin et al., 2018; **Table 1**). Studies that present evidence for external factors independently driving fruiting and flowering times often show some evidence for internal drivers as well (Lechowicz, 1995; Menzel et al., 2006, 2020; Haggerty and Galloway, 2011; Jiang et al., 2016; Sethi et al., 2020; **Table 1**). **Table 1** synthesizes the results of these previous studies and the mixed evidence for internal and external drivers of flowering and fruiting. An early study of the FTFI based on a nearly 30-year dataset found no evidence for a linear relationship between flowering and fruiting dates across many temperate species, even when grouped by time of season in which fruiting occurs (Lechowicz, 1995). In contrast, a more recent single-year study did find evidence for this linear relationship in 25 U.S. species (Ettinger et al., 2018), as did a 12-year study of 100 European species (Segrestin et al., 2018). Even concurrent studies on the same species have produced mixed evidence for internal and external drivers of the FTFI. A 1-year warming experiment revealed that higher temperatures shortened the entire reproductive cycle of *Betula nana* by 27 days on average, from flower bud set to fruit set (Post et al., 2008b), thereby also shortening the FTFI. However, a concurrent 2-year warming experiment showed that higher temperatures did not decrease the FTFI in *B. nana* (Post et al., 2008a), even though flowering and fruiting were advanced.

Other experimental studies have yielded evidence for both internal and external drivers of the FTFI. Alpine plants transplanted to warmer and cooler altitudes for 3 years flowered earlier and later, respectively, but fruiting times remained the same (Jiang et al., 2016). These results support external drivers of the FTFI, but a different elevation transplant study (Haggerty and Galloway, 2011) showed evidence for both external and internal drivers: in a single-year common garden experiment, *Campanulastrum americanum* populations planted at lower elevations had a shortened FTFI relative to those planted at higher elevations. However, populations from low elevations planted at either elevation had a longer reproductive cycle overall than those from high elevations (Haggerty and Galloway, 2011).

Observational studies have similarly yielded mixed evidence. A recent observational study on 28 species of U.S. alpine plants showed mostly shortened FTFI with climate change, comparing observations taken in 2015 to those taken in 2011–2014 (Sethi et al., 2020). Shorter FTFI were shorter by 3–15 days, but two species showed an FTFI change of a day or less, and the FTFI of three species increased by 3–5 days (Sethi et al., 2020). Menzel et al. (2006) used phenological records to determine (1) how timing of life history stages was changing in 14 European countries over 30 years and (2) how these phenological changes correlated with temperature across 9 European countries over 15 years. While flowering time was largely negatively correlated with temperature, fruiting time was more variable: the correlation with temperature was negative for most species, but positive for a few others. A recent update of this study incorporating data on the same European species from 1951 to 2018 showed the same pattern (Menzel et al., 2020). As temperature increases and flowering times advance, Menzel et al.'s (2006, 2020) findings imply that the FTFI will shorten for some species, remain the same for some, and lengthen for others, depending on how internal and external factors interact to determine fruiting times.

## CASE STUDY: FRUITING PHENOLOGY SHIFTS AND LIFE STAGE LINKAGE IN EUROPEAN SPECIES

Nearly all of the few studies that directly analyze the FTFI do so on the scale of a year or a few years, and in the case of multi-year analyses, only for a single species (**Table 1**). Publicly available fruiting data at time scales longer than 20 years are particularly sparse (Chuine and Régnière, 2017; see **Supplementary Appendix 1** for descriptions of publicly available phenology databases). Yet, time series > 30 years are needed to estimate robust trends (Dose and Menzel, 2004; Menzel et al., 2020). Here, we use a three-and-a-half-decade dataset (from the PEP725 database; Templ et al., 2018) to assess how the FTFI is changing in individual plants. Our case study both illustrates the challenges of analyzing the phenology of certain life history stages on a decadal scale using publicly available data. At the same time, it provides compelling evidence that such data, when available, can be used to analyze changes to the length of time between phenophases with climate change. We do not present here a comprehensive treatment of FTFI across species. Although more long-term data are needed to assess changes in the FTFI across diverse taxa and regions (Mendoza et al., 2017; **Supplementary Appendix 1**), we use this case study to illustrate the potential power of analyzing relationships between the timing of different life history stages.

### What Are the Relative Changes Between Flowering and Fruiting Life Stages?

We examined whether a close temporal link exists between the flowering and fruiting life stages, and assessed the strength of this linkage. To determine how the FTFI has changed over time, we used phenology data from the PEP725 database (Templ et al., 2018) over 35 years (1980–2015). The FTFI is defined at the level of a single reproductive structure on an individual plant. Our data did not allow for this level of precision so instead we used the next most precise level, that of the individual. We used all six tree and shrub species with > 100 individuals per year for which both first flowering and first ripe fruits were recorded in the database: *Aesculus hippocastanum*, *Sorbus aucuparia*, *Vaccinium myrtillus*, *Sambucus nigra*, *Ribes grossularia*, and *Ribes rubrum*. These species represent both animal-dispersed (*Vaccinium myrtillus*, *Sambucus nigra*, *Ribes grossularia*, and *Ribes rubrum*) and wind-dispersed (*Aesculus hippocastanum* and *Sorbus aucuparia*) species; given that we had access to data on only a few species, we were unable to compare FTFI responses in biotically vs. abiotically dispersed species. We calculated the FTFI by subtracting the first flowering day from the first day at which ripe fruits were noted, for each individual within each species for each year. Number of days between flowering and fruiting became our response variable for a single multi-species analysis with a Bayesian regression framework (for analysis details see **Supplementary Appendix 2**: Methods 2).

The FTFI remained constant over time in three of the six species: *Sorbus aucuparia*, *Vaccinium myrtillus*, and *Sambucus nigra*. The other three species experienced either a significant increase (*Aesculus hippocastanum*) or significant decrease (*Ribes rubrum* and *Ribes grossularia*) in FTFI over time (**Figure 1**). If a close linkage exists, we would expect no change in FTFI. If, however, a close linkage does not exist because flowering and fruiting are responding to different external cues and a changing climate independently, the result could be a shortening or lengthening or lack of change in the interval between flowering and fruiting. The divergent results between species suggests that a range of mechanisms might be determining the role of climate change on the FTFI.

### How Is Fruiting Phenology Shifting?

The magnitude of a shift in fruiting phenology for a species, and in which direction this shift occurs, can provide additional information about the mechanism driving the FTFI. To determine whether or not fruiting alone was advancing over the same time period as our FTFI analysis, we performed an additional analysis on fruiting for the same 6 plus an additional 8 European species. We analyzed fruiting at the level of the population, to be consistent with previous analyses of fruiting, which allowed us to include species that did not have enough individual observations spanning multiple years to be in the FTFI analysis. The incorporation of these additional 8 species allowed us to more broadly compare the results of our fruiting analysis to previous studies. We selected native, broad-leaved woody plant species that had records that occurred consistently from 1980 to 2015 in one location (for additional methods see **Supplementary Appendix 2**: Methods 1).

We found that fruiting had advanced by an average of 4.2 days per decade (days/decade; 95% CI: 2.4–6.2 days/decade, **Figure 1**), or 14.7 days from 1980 to 2015 (for additional results see **Supplementary Appendix 2**: Results 1). The 4.2 days/decade advancement in fruiting in our species is consistent with Ge et al.'s (2015) meta-analysis of 104 Chinese tree, shrub, and herb species, in which spring/summer phenophases, including fruiting, advanced by an average of approximately 2 days for trees and shrubs and 5.5 days/decade for herbs from 1960 to 2011. It is also consistent with Gordo and Sanz's (2009) findings of 3.2 days/decade for 29 perennial Spanish species from 1943 to 2003. It is likewise consistent with Menzel et al.'s (2006) findings of 2.4 days/decade for 542 European species from 1971 to 2000 and 1–2.5 days/decade, depending on the season of fruiting,

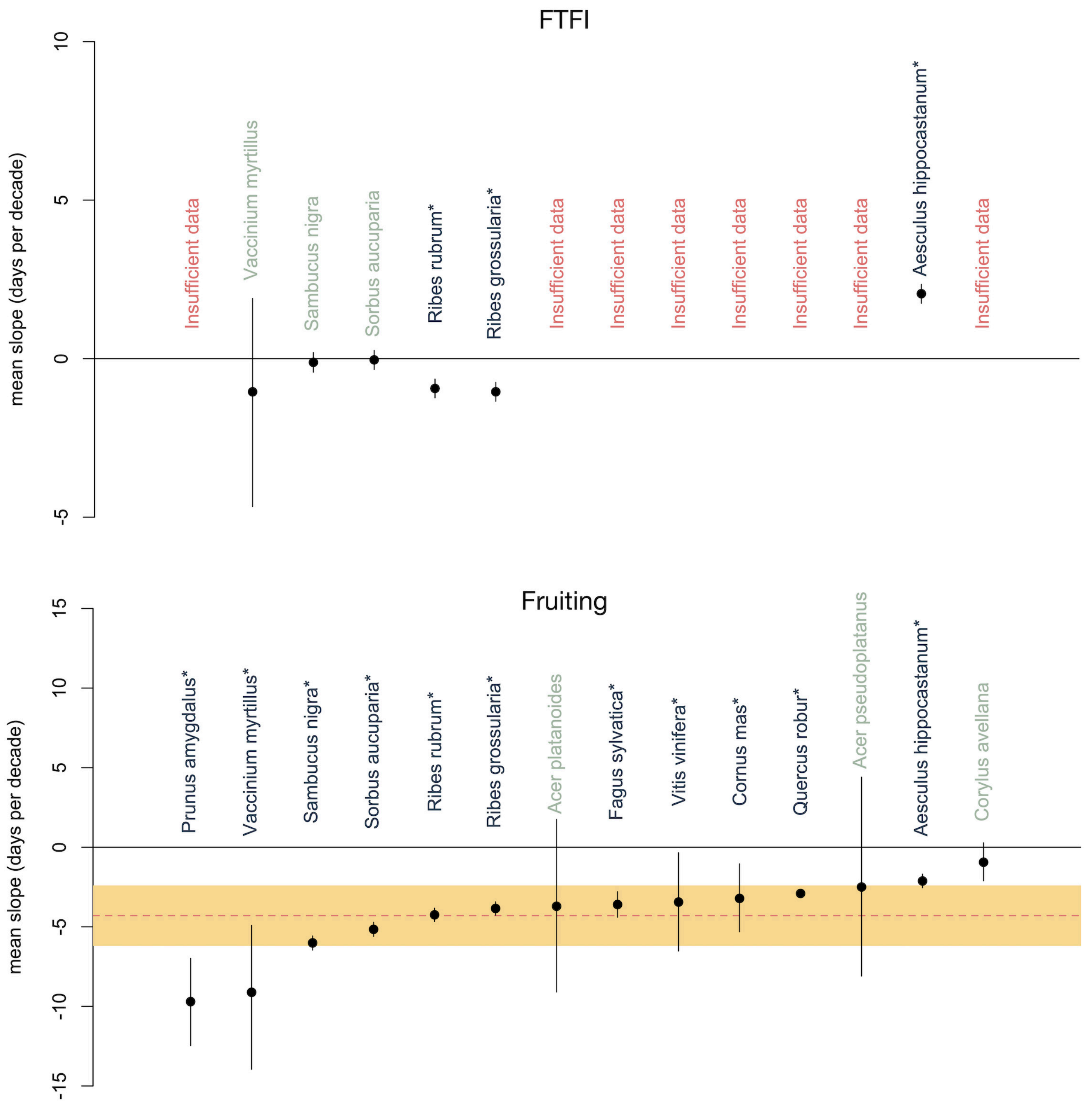

**FIGURE 1 |** Mean phenological changes in the flowering to fruiting interval (FTFI; top) and fruiting time (bottom) of 6 and 14 European trees and shrubs, respectively, from 1980 to 2005. Mean changes are represented with dots and 95% credible intervals are represented with bars. Species names for which the FTFI or fruiting time changed significantly are starred and in dark gray. Species names for which the FTFI or fruiting time did not significantly change are in green. Across-species mean of the shift in fruiting time (–4.2 days/decade) is denoted by the red dotted line (bottom) with the 95% credible interval in orange.

for the same species from 1951 to 2018 (Menzel et al., 2020). Collectively, these findings highlight the need to conduct similar, long-term, studies of how fruiting phenology is responding to climate change.

Fruiting advanced for all of the six species in our FTFI analysis (**Figure 1**). The species in which fruiting advanced the most were also the species for which the FTFI remained constant. *Vaccinium myrtillus*, *Sambucus nigra,* and *Sorbus aucuparia* advanced fruiting by an average of 9.0, 6.0, and 5.2 days/decade, respectively, while their FTFI did not significantly change. For *Ribes rubrum* and *R. grossularia*, fruiting advanced by an average of 4.3 and 3.8 days/decade while the FTFI shortened by a mean of 1.0 and 0.9 days/decade, respectively. Fruiting in *Aesculus hippocastanum* advanced by 2.1 days/decade, while the FTFI lengthened by a mean of 2.0 days/decade. The similar responses of fruiting in these six species but differing responses of the FTFI suggest that separate mechanisms may be operating for each species.

## WHAT MECHANISMS COULD EXPLAIN HOW SUCCESSIVE LIFE HISTORY STAGES ARE LINKED?

If the timing between flowering and fruiting is physiologically constrained, a shift in timing in flowering should have a direct effect on the timing of fruiting. This could result in flowering and fruiting times advancing in parallel, as in three of the six species in our case study. However, we cannot rule out the possibility that separate, possibly correlated external factors are acting on each of the life history stages. Furthermore, external factors could act on flowering and fruiting separately or jointly, such that the FTFI lengthens or shortens, as seen in the other three species in our case study. Below we propose four general mechanisms for how flowering and fruiting times may jointly or independently shift with climate change. While the specifics of these mechanisms can relate more strongly to one seed dispersal type than another, as noted below, all four mechanisms apply to plants with either biotic or abiotic seed dispersal. We additionally recognize that multiple mechanisms could be operating at once.

### Mechanism 1: Physiological and Developmental Processes Constrain FTFI, Such That Fruiting Time Will Shift Only in Parallel With Flowering Time

The FTFI will always be somewhat variable across individuals and reproductive structures for any given species, but internal constraints such as developmental processes or seed and fruit size should impose limits on the variability possible for this interval and result in parallel shifts in flowering and fruiting, as shown in **Table 2** and **Figure 2**. Certain fruit or seed traits such as size could be correlated with, and could possibly dictate, the length of the fruit development and maturation periods (Primack, 1987; Segrestin et al., 2018). For example, animal-dispersed seeds, which are on average larger than wind-dispersed seeds, have a physiologically constrained lower limit on seed development time that is greater than that of smaller seeds (Primack, 1987), and a longer FTFI on average than wind-dispersed seeds (Segrestin et al., 2018). The minimum developmental time for fruits theoretically constrains the FTFI, regardless of external environmental effects like drought (Gordo and Sanz, 2010; Segrestin et al., 2018).

Similarly, seed mass and the amount of time that fruits need from flowering to ripening are phylogenetically constrained and positively correlated (Heydel and Tackenberg, 2016), suggesting that certain evolutionary histories dictate fruit development and maturation periods. Plants with life history stages such as leaf-out and flowering that occur earlier in the spring display traits that are a product of rapid growth, which may translate to a shorter FTFI, resulting in smaller seeds or fruits due to the shorter development time (Wolkovich and Cleland, 2014). If phenology is phylogenetically constrained, we would expect the FTFI to be phylogenetically constrained. Studies have found phylogenetic signals in leaf-out, flowering, and fruiting phenology, but their strengths are highly variable, with some clades exhibiting a stronger pattern than others (Marco and Páez, 2002; Wolkovich and Ettinger, 2014; Gougherty and Gougherty, 2018). While this signal seems to indicate phylogenetic constraints, it may instead be that phenology is correlated with other plant traits that are themselves evolutionarily conserved (Marco and Páez, 2002; Davis et al., 2010; Wolkovich and Ettinger, 2014; Gougherty and Gougherty, 2018).

### Mechanism 2: Stabilizing Selection on Fruiting Time Results in a Longer or Shorter FTFI

Stabilizing selection could be indirectly acting on fruiting times by promoting genotypes linked to a particular disperser-fruiting time relationship (e.g., Palacio et al., 2021) or climate-fruiting time (e.g., Inouye et al., 2019). If physiological and developmental constraints were keeping the FTFI the same length and stabilizing selection via an external driver were maintaining fruiting times, we would expect to see both unchanging fruiting times and unchanging flowering times in the face of climate change. However, we know that flowering time is largely advancing; therefore, we would expect stable fruiting times and thus lengthened FTFIs if fruiting time were driven by stabilizing selection (**Table 2** and **Figure 2**). When a fruit reaches maturity could be tightly associated with the historical activity of seed dispersers (e.g., bird-dispersed fruits, Noma and Yumoto, 1997) and could remain constant, even though the timing of disperser activity is itself shifting with climate change (e.g., in birds, Thomas and Lennon, 1999; Cotton, 2003; Marra et al., 2005; Tingley et al., 2009).

The second way in which stabilizing selection could be acting is if the fruiting time-climate relationship evolved in response to an abiotic driver that is unlikely to be affected in a direction predictable by climate change, such as solar irradiance and photoperiod (Hamann, 2004; Chapman et al., 2005; Zimmerman et al., 2007; Mendoza et al., 2017; Ettinger et al., 2021) or the onset of monsoon rains (Singh and Kushwaha, 2006). If fruit ripening times are synchronized by a biotic or abiotic event that does not significantly shift over time with climate change, whereas flowering phenology is responsive to shifting environmental cues, an increase or decrease in the length of the interval could result (**Table 2** and **Figure 2**).

### Mechanism 3: Genotypic Variation Results in a Changing of the FTFI, Including Changes to Fruiting Time

The genetic underpinnings of flowering phenology, and less so fruiting phenology, in response to temperature have been

**TABLE 2 |** Proposed mechanisms for how the flowering to fruiting interval (FTFI), the time between the flowering and ripe fruit phenophases, will change or remain stable with climate change.

| Mechanism | Internal vs. External | Change to FTFI | Pathway(s) and evidence for mechanism |
|---|---|---|---|
| **1—Physiological and developmental constraints** | **Internal** | **Shortened** | None |
| | | **No change** | **Physiological and developmental constraints on the FTFI** (Primack, 1987; Gordo and Sanz; 2010; Segrestin et al., 2018) |
| | | **Lengthened** | None |
| **2—Stabilizing selection** | **External** | **Shortened** | **Fruit timing does not change from current with respect to other climactic or phenological event (but flowering is delayed)** (Hamann, 2004; Chapman et al., 2005; Singh and Kushwaha, 2006; Zimmerman et al., 2007) |
| | | **No change** | None |
| | | **Lengthened** | **Fruit timing does not change from current or with respect to other climatic or phenological event** (Noma and Yumoto, 1997; Hamann, 2004; Chapman et al., 2005; Singh and Kushwaha, 2006; Zimmerman et al., 2007) |
| **3—Directional selection** | **External** | **Shortened** | **Higher temperatures advance fruiting** (Chuine et al., 1999; Sherry et al., 2007; Misson et al., 2010; Haggerty and Galloway, 2011; Darbyshire et al., 2014; Gallinat et al., 2015) |
| | | **No change** | **Changes in precipitation do not alter fruiting times** (Sherry et al., 2007) |
| | | **Lengthened** | **Higher temperatures delay fruiting** (Sherry et al., 2007; Gordo and Sanz, 2009)<br>**Changes in precipitation delay fruiting** (Peñuelas et al., 2004; Mazer et al., 2015; Dunham et al., 2018) |
| **4—Environmental controls** | **External** | **Shortened** | **Phenotypic plasticity (response to temperature and precipitation)**<br>**Phenotypic plasticity (flower longevity shortened)** (Nagahama et al., 2018)<br>**Resource acquisition takes less time, advancing fruiting** (Chuine and Beaubien, 2001; Klapwijk et al., 2013; Tripathi et al., 2016; Guillaume et al., 2018) |
| | | **No change** | **Phenotypic plasticity (response to temperature and precipitation)** |
| | | **Lengthened** | **Phenotypic plasticity (response to temperature and precipitation)**<br>**Phenotypic plasticity (flower longevity lengthened)** (Arroyo et al., 1981; Moore and Lauenroth, 2017)<br>**Resource acquisition takes more time, delaying fruiting** (Klapwijk et al., 2013; Guillaume et al., 2018) |

*Numbers beside each of the mechanisms correspond to the numbers of the proposed mechanisms within the text. For each mechanism, the evidence for the expected change(s) to the FTFI are summarized in bold in the rows corresponding to the expected change(s): shortened, no change, and lengthened. Supporting evidence and references are listed under the bold summaries.*

well-studied in certain species, most of which are commercially grown (e.g., Usenik and Štampar, 2011; Satake et al., 2013; Marrano et al., 2019; Bernard et al., 2020; Chen et al., 2020). These studies show diverse responses of life history stages to climate change between closely related species and even individuals of the same species, and suggest that the underlying genetic differences influencing these life history stages dictate whether or not the FTFI is altered by climate change. In contrast, there is poor understanding of the genetics of fruiting phenology in wild species, and experiments on intraspecific genetic variation interacting with climate change-induced environmental conditions are rare (e.g., Faticov et al., 2020). Most of our understanding is based on observational and experimental studies of plant responses to temperature and precipitation without knowledge of the underlying genetic architecture, which suggest either selection favoring genotypes that are able to fruit earlier in the growing season, or plastic phenological responses to temperature and precipitation. In conjunction with flowering responses to climate change, we can make inferences about how selection on or plasticity of fruiting times will affect the flowering to fruiting interval (**Table 2** and **Figure 2**). In multiple studies, higher temperatures generally advanced flowering and advanced fruiting by a greater magnitude than flowering, which shortened the FTFI (Sherry et al., 2007; Misson et al., 2010; Haggerty and Galloway, 2011; Gallinat et al., 2015; Carbognani et al., 2018), but in some U.S. and European species, fruiting advanced less than flowering, resulting in a longer FTFI (Sherry et al., 2007; Gordo and Sanz, 2009). A temperature-based modeling framework for flowering phenology (e.g., Chuine et al., 1999) extended to the FTFI largely predicts a shorter FTFI (Darbyshire et al., 2014).

Both increased and decreased precipitation have led to variable effects on FTFI in observational and experimental studies (Peñuelas et al., 2004; Sherry et al., 2007; Mazer et al., 2015; Dunham et al., 2018; **Table 2** and **Figure 2**). Doubling precipitation had no effect on fruiting times and FTFI in a controlled experiment (Sherry et al., 2007). In three studies that did not track the FTFI, increased precipitation resulted in delayed fruiting (Peñuelas et al., 2004; Mazer et al., 2015) as did decreased precipitation during the dry season (Dunham et al., 2018). If we

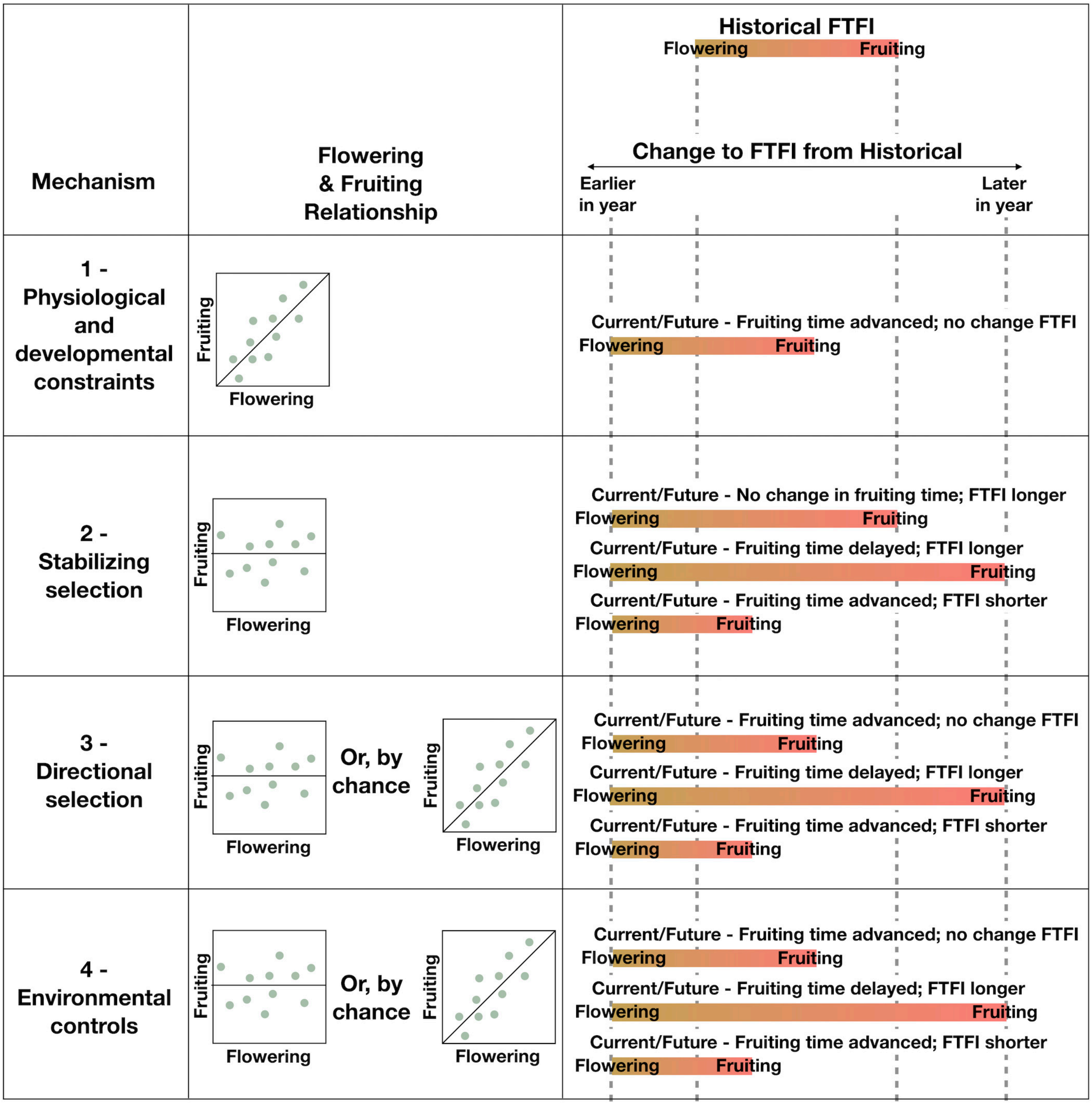

**FIGURE 2 |** Each proposed general mechanism (column 1) is expected to result in changes to the FTFI (column 3), based on the relationship between flowering and fruiting time (column 2) of single reproductive structures on single individual plants (dots). An internal mechanism results in a tight correlation between flowering and fruiting (Mechanism 1), whereas an external mechanism results in no clear relationship (Mechanisms 2–4). Although Mechanisms 3 and 4 are driven by external processes, these processes could by chance result in a relationship between flowering and fruiting that appears tightly correlated like an internal mechanism. An internal mechanism results in a shift of the FTFI from historical (top of column 3), but the length of the FTFI is preserved. External mechanisms result in several possible changes to the FTFI from historical wherein timing of flowering, fruiting, or both are altered. Bars represent the beginning of the flowering stage (yellow) to the fruit maturity stage (red) for a single reproductive structure on a single individual. Advanced flowering is represented by the dashed line labeled as earlier in year, and delayed fruiting is represented by the dashed line labeled as later in year. Historical flowering and fruiting times are represented by the two central dashed lines. All possible options for changes to fruiting, given advanced flowering currently and in the future, are displayed for each mechanism.

assume that most species in these studies experienced advanced flowering, we can then assume that FTFI has lengthened as a consequence. However, more studies on the effects of precipitation generally are needed, especially because studies on the effects of decreased precipitation on fruiting specifically are lacking (but see Sethi et al., 2020).

## Mechanism 4: Environmental Controls Result in a Changing of the FTFI, Including Changes to Fruiting Time

As previously stated, many of the responses of the FTFI to climate listed under Mechanism 3 could have been due to phenotypic plasticity in response to environmental conditions, since genetic responses were not tracked. Segrestin et al. (2018) found that across plant species, the onset of fruiting was more variable than the onset of flowering, perhaps indicating that the timing of fruiting is more plastic. Warmer air temperatures and earlier snow melt were found to be correlated with reductions in the FTFI of alpine plants (Sethi et al., 2020), indicating a plastic response to these environmental changes. Phenotypic plasticity could allow the FTFI to lengthen, shorten, or remain the same in response to biotic interactions or a variety of environmental conditions, including flowering and fruiting phenologies responding independently to these drivers (**Table 2** and **Figure 2**).

Plasticity in flower longevity, i.e., how long an individual flower is open, is another way in which plants could respond to environmental conditions and might result in a change to the FTFI. Some species can show variable flower longevity in response to pollen receipt, wherein the flower closes anywhere from a few hours to a few days after successful pollination (Primack, 1985; Proctor and Harder, 1995; van Doorn, 1997; Niu et al., 2011; Trunschke and Stöcklin, 2017). The time from pollination to fruiting could thus be fixed by physiological and developmental constraints on fruit development, as discussed above, but the FTFI may instead reflect the time from flower opening to pollination (Segrestin et al., 2018). If a species' flowering time becomes partially mismatched from pollinator availability, time to pollination could lengthen, resulting in a longer FTFI (**Table 2** and **Figure 2**).

Flower longevity, and thus the FTFI, could also be influenced by environmental factors such as warmer or cooler temperatures (Arroyo et al., 1981; Molau, 1997; Steinacher and Wagner, 2010; Nagahama et al., 2018) or increased precipitation (Moore and Lauenroth, 2017), resulting in a shortened or lengthened FTFI (**Table 2** and **Figure 2**). For example, Nagahama et al. (2018) found that climate warming causes flowers to senesce earlier, which could result in a shorter FTFI. Flower longevity increases due to lower temperatures (Arroyo et al., 1981), meaning that advanced flowering could result in flowers periodically experiencing lower than normal temperatures, which could result in longer flower longevity and thus a lengthened FTFI. Increased precipitation resulted in increased flower longevity for late-season species (Moore and Lauenroth, 2017), indicating that changes to precipitation in either direction could also alter flower longevity, and in turn, the FTFI.

A third way in which environmental conditions could influence the FTFI is through resource acquisition pathways. When a fruit matures may depend on how quickly a plant is able to acquire resources from photosynthesis, resulting in a shorter or longer FTFI. Resource acquisition via photosynthesis, in turn, can be impacted by an array of factors (**Table 2** and **Figure 2**). Warmer temperatures could result in fewer leaves damaged by frost, allowing plants to acquire resources more quickly, thus advancing fruiting times and shortening the FTFI (Chuine and Beaubien, 2001; Guillaume et al., 2018). Alternately, warmer temperatures could result in earlier leaf-out times, which could lead to greater frost damage of leaves, leading to a slower acquisition of resources, thus delaying fruiting times and lengthening the FTFI (Guillaume et al., 2018). Changing climate and the timing of insect emergence or population booms could either result in decreased or increased herbivore damage, leading to faster or slower acquisition of resources, respectively (Klapwijk et al., 2013). Lastly, with warmer temperatures, plants could produce more leaf mass or area, or thicker leaves, increasing photosynthetic capacity and leading to a faster acquisition of resources and advanced fruiting times (Tripathi et al., 2016).

## USING THE CASE STUDY TO DISTINGUISH POTENTIAL MECHANISMS FOR CLIMATE-DRIVEN SHIFTS IN FRUITING AND FLOWERING

Several of the mechanisms proposed above for how fruiting and flowering phenology respond jointly or independently to climate change could produce identical results in the FTFI over time (**Table 2** and **Figure 2**). Here we return to our case study to demonstrate how we could use the FTFI in combination with additional research to tease apart the mechanisms behind changes, or lack thereof, to the FTFI (**Figure 3**). For example, an absence of change in the FTFI, such as we found in three of our six species (**Figure 1**), indicates that the mechanism driving the FTFI for these species could be internal. Stabilizing selection is expected to produce a change in the FTFI, so that mechanism can be discarded. Constancy in the FTFI is consistent with Mechanisms 3, directional selection, and 4, environmental controls. The mechanism of environmental controls is difficult to rule out because, for example, an incremental increase in temperature across many years could have an equally strong correlation with fruiting as flowering has with fruiting. If, over many years and across yearly temperature and/or precipitation fluctuations, fruiting time is more strongly correlated with flowering time than with environmental cues, we could rule out environmental controls. The other processes affecting environmental controls are either difficult to track or require vastly more data. If length of flowering time, specifically from flower opening to pollination, does not affect fruiting time and thus FTFI, environmental controls via flower longevity could be ruled out. If any of the processes related to the acquisition of resources, like frost or herbivore damage to leaves, are not correlated with FTFI, we can rule out environmental controls. However, collecting data to discriminate multiple processes related to environmental controls is a massive undertaking. This will make discerning the importance of environmental controls via resource acquisition difficult. If we find no genetic evidence for directional selection on fruiting times (e.g., see Giménez-Benavides et al., 2011;

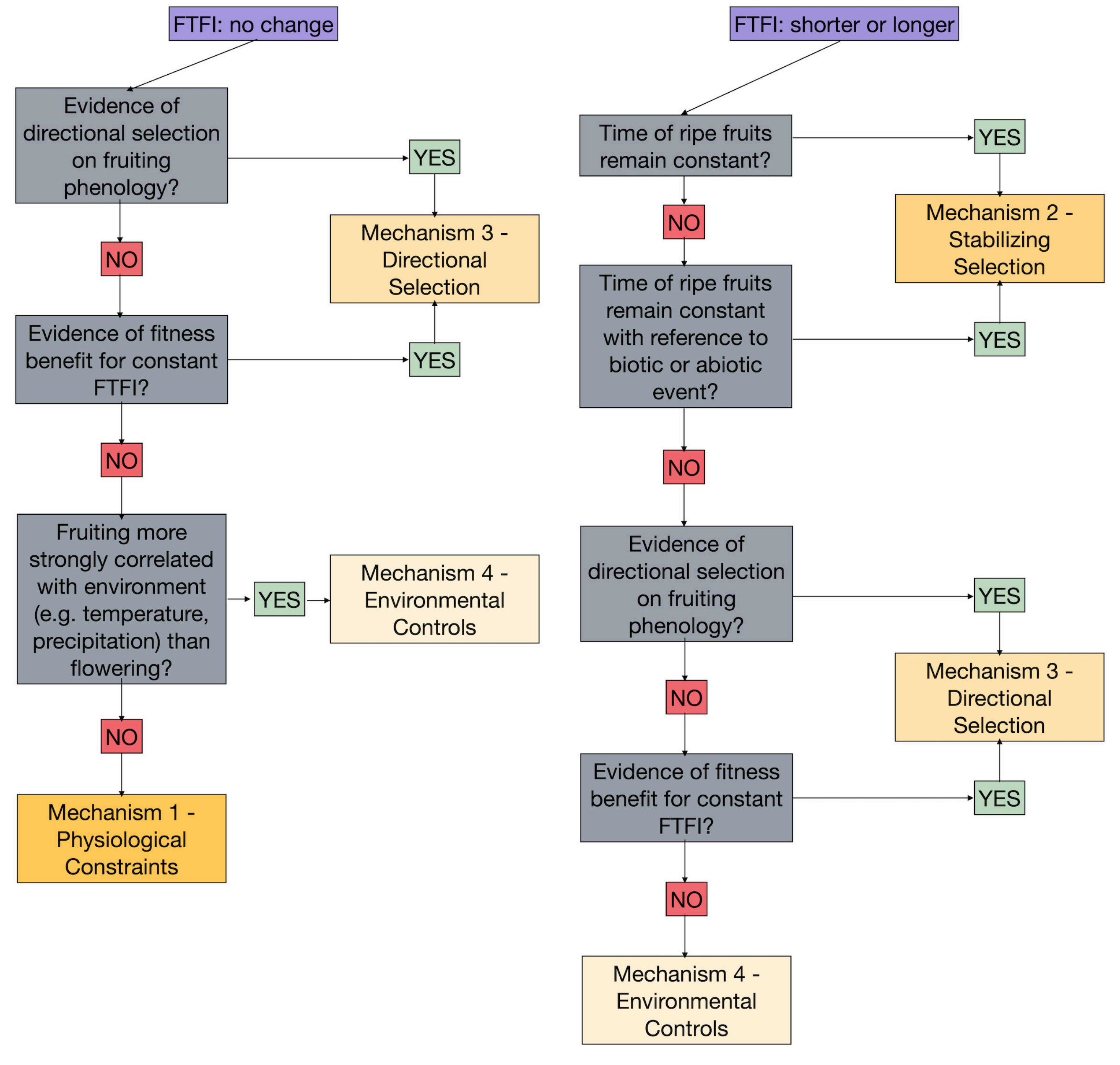

**FIGURE 3 |** Framework for using the observed changes, or lack thereof, in the flower to fruiting interval (FTFI) over time along with additional data and analyses to distinguish the underlying mechanism driving the FTFI. This flowchart only illustrates how the four general mechanisms can be distinguished. Ways to tease apart and eliminate the pathways through which each general mechanism could operate is described within the main text. The order shown here in which mechanisms can be progressively eliminated is suggested; there are multiple approaches to eliminating the possible mechanisms driving the FTFI. For example, directional selection could be eliminated after environmental controls or before stabilizing selection for the absence of a change or a lengthening or shortening of the FTFI, respectively.

Munguía-Rosas et al., 2011; Anderson et al., 2012; Yeoh et al., 2017, for studies on flowering), or if we find no fitness benefits for those individuals for which the FTFI remains constant, we can rule out directional selection.

Ruling out stabilizing selection, directional selection, and environmental controls leaves only physiological and developmental constraints as mechanisms producing a constant FTFI over time and in the face of climate change. Comparing the FTFI among species within the same genus and to species of genera with similar fruit or seed sizes could point to whether phylogenetic or developmental constraints are operating. Accounting for phylogenetic signal in the analysis of changes in FTFI across multiple years, species, and continents (i.e., Davis et al., 2010 for flowering) could point to the relative influence of evolutionary history on phenological responses to changing climate. However, none of these approaches permits elimination of either pathway through which the internal mechanism could be operating.

One of the six species in our case study, *Aesculus hippocastanum*, experienced an increase in FTFI over time (**Figure 1**). We expect physiological and developmental constraints to result in a constant FTFI over time, suggesting that the other three mechanisms we have proposed—stabilizing selection, directional selection, or environmental controls—could be responsible. Stabilizing selection could be acting if the time of first ripe fruits remains constant. However, for *A. hippocastanum* the timing of fruiting shifted earlier (**Figure 1**). Another indication of stabilizing selection might be fruit ripening that remains constant in reference to an abiotic or biotic event. If we found no correlation between fruiting times and abiotic or biotic events, or no genetic evidence for stabilizing selection on fruiting times, we could rule out this mechanism. Furthermore, if we found no genetic evidence of directional selection on fruiting times, or if we found no fitness benefits for those individuals for which the FTFI increases, we could rule out directional selection. If flower longevity was driving the lengthening of the FTFI by an increased amount of time between flower opening (the beginning of the FTFI, as defined above) and flower closing, we would expect to observe a lengthening of the first flowering to flower senescence interval over multiple years. We would also potentially expect to see a strong correlation between date of pollination and first ripe fruit dates. If neither this correlation nor a correlation between pollination and first ripe fruits were found, environmental controls via flower longevity could be rejected. If both flowering and fruiting were correlated with the same or independent environmental conditions, with no evidence for selection on either, environmental controls might be driving the increased FTFI. However, correlations with environmental conditions may be direct (e.g., temperature and/or precipitation acting directly on flowering and fruiting) or indirect, via resource acquisition. A number of pathways that affect photosynthesis could be involved in this indirect relationship, and teasing them apart would require extensive data collection in, for example, leaf damage and cloudless days.

Two of the six species represented in this case study experienced a decreased FTFI (**Figure 1**), again suggesting that stabilizing selection, directional selection, or environmental controls could be responsible. As with *A. hippocastanum*, in *Ribes rubrum,* and *R. grossularia* fruiting occurred earlier (**Figure 1**), indicating that stabilizing selection could only be acting if fruit ripening time remained constant in reference to an unknown abiotic or biotic event. The same process of eliminating mechanisms just described for *A. hippocastanum* could be applied to these *Ribes* species.

## RECOMMENDATIONS FOR FURTHER RESEARCH ON THE PROPOSED MECHANISMS DRIVING THE FTFI

Throughout this paper, we have stressed that additional research is needed to further distinguish which mechanisms are driving the FTFI, and through what pathways. Here we discuss research directions for internal drivers of the FTFI, how genetics and selection influence the FTFI and will determine future fruiting responses to climate change, and the interactions among different environmental controls and how these affect fruiting both now and with climate change. Although different species may be driven by different mechanisms, and all of the mechanisms discussed here may be operational in nature, simultaneous, standardized studies of multiple species could provide evidence for or eliminate possible mechanisms on a broad scale. We additionally emphasize that long-term studies of phenology are essential because short-term interannual variation could reflect phenological plasticity rather than evolutionary change.

Further research could elucidate how physiological mechanisms determine the FTFI, and to what extent different mechanisms co-occur and can interact. Taking a trait-based approach to determine differences in seed and fruit development time across the range of seed and fruit traits could advance our understanding of the extent to which the time of fruit development is constrained, or varies, along trait axes (e.g., Singh and Kushwaha, 2006). Determining which families or clades have long fruit or seed development times closely linked to the number of growing degree days within a season could shed light on the extent to which phylogenetic constraints work in concert with environmental controls to determine the length of the fruit development period. Similarly, studies that compare the relative influence of evolutionary history and environmental controls could tease apart the extent to which internal vs. external process are driving phenology (e.g., Staggemeier et al., 2015), particularly with the incorporation of phenological shifts due to climate change (Davis et al., 2010). We suggest incorporating standardized FTFI measures into existing phenological data collection protocols so as to more accurately compare across datasets, as Buonaiuto et al. (2021) have suggested for the interval between flowering and leaf-out.

If fruit or seed dispersal mode does not predict the strength of selection on fruit ripening times (e.g., Schluter, 1988; Kingsolver et al., 2001; Palacio et al., 2021), it is less likely that stabilizing selection is acting on fruit ripening times across many species. Additionally, if peak activity of seed dispersers and fruit ripening times are shifting and becoming out of phase with climate change, instead of shifting in parallel, biotic interactions driving stabilizing selection in animal-dispersed fruits can be discarded as a general pattern. To determine how peak activity of dispersers and fruit ripening times are responding to each other and climate change, more long-term studies that track disperser phenology along with fruiting phenology, particularly in the tropics where biotic interactions are assumed to play a greater role than climatic factors, are sorely needed (Mendoza et al., 2017).

Mechanistic research that addresses the molecular and regulatory basis of fruit ripening in wild species would further disentangle selection for particular genes associated with ripening from changing environmental cues and altered physiological processes (Chen et al., 2020). Additionally, determining how selection is acting on the reaction norm for fruit ripening times (Inouye et al., 2019) as climate changes, and how genetic variation in wild species of genes associated with fruiting responds to changes in temperature, precipitation, and other climatic factors, would help further our understanding of how a changing environment is interacting with selective pressures. Agricultural scientists are exploring the genetic underpinnings of plant phenology (e.g., Satake et al., 2013; Marrano et al., 2019; Chen et al., 2020) and phenological responses to climate change of cultivated species (e.g., Chmielewski et al., 2004; Darbyshire et al., 2014); future research that draws on this body of literature could inform our understanding of wild plants in natural systems. Additionally, studies that correlate climatic cues with gene expression in genes associated with fruiting in wild plants (e.g., see Kudoh, 2016; Yeoh et al., 2017 for this type of research with genes associated with flowering) would increase our ability to tease apart the pathways and extent to which different climatic variables impact the genetic component of fruiting. Lastly, in animal-dispersed species, disperser activity could directionally select for fruiting phenology, and further studies are needed to explore to what extent and in which species dispersers drive phenotypic selection, particularly with reference to the FTFI and population-level traits like crop size and fruiting duration (Palacio et al., 2021).

We have emphasized that different types of environmental controls can interact with each other. Further studies are needed to understand and differentiate among these effects. For example, in an analysis of the midpoint of listed fruiting dates of 11,605 Chinese species within a compiled flora, Du et al. (2020) found that mean annual precipitation, precipitation seasonality, temperature seasonality, and temperature of the coldest quarter were included in the best supported models of the variation in fruiting times across species. Reduced soil moisture was a climate-related variable that was correlated with shortened FTFI in some alpine plant species, as was increased temperature in most species, although the interaction between them was not specifically tested (Sethi et al., 2020). Further, interactions between temperature and precipitation (e.g., Misson et al., 2010; Butt et al., 2015; Mazer et al., 2015) with climate change could be additive and cause fruiting times to advance more than changes to temperature or precipitation alone. Further research is also needed on how precipitation affects fruiting phenology, including the effect of higher or lower precipitation on the FTFI and how timing of precipitation affects fruiting (e.g., Molau, 1997; Fitter and Fitter, 2002; Parmesan and Yohe, 2003; Du et al., 2020). Additionally, seasonality of precipitation and how it relates to fruiting phenology in tropical wet forests needs to be further investigated, as current studies show contradictory patterns (Mendoza et al., 2017). Finally, environmental controls could interact with other factors influencing a species' phenology and our other proposed mechanisms. For example, higher temperatures due to climate change could interact with biogeography (e.g., Loarie et al., 2009; Butt et al., 2015; Ge et al., 2015, reviewed in Du et al., 2020), phylogeny (Davis et al., 2010), and life form (Du et al., 2020; Ganjurjav et al., 2021) to produce patterns in the FTFI across many species.

Lastly, we recommend that future research precisely define the beginning and end of the FTFI and that these definitions are incorporated into study design. Because FTFI is defined at the level of a single reproductive structure on an individual plant, the start and end of the FTFI can be defined in terms of flower opening, closing, or pollen deposition, and the end of the FTFI can be defined as when the individual fruit is ripe, with the recognition that "ripeness" will need to be precisely delineated for each species. While our case study was limited by available phenological data, future studies can tease apart flower longevity by defining the beginning of the FTFI at the time of pollen deposition or flower closing and can investigate intraspecific, and even intraindividual, variation in FTFI. Long-term studies using these definitions on the boundaries of the FTFI, which are currently lacking, can then be used to determine interannual variation.

## IMPLICATIONS FOR ANIMAL-MEDIATED SEED DISPERSAL AND DISPERSAL COMMUNITIES

The mechanisms we propose that could drive the FTFI in the context of a changing climate are relevant to all plants with biotic and abiotic seed dispersal. However, FTFI and fruiting phenology changes in animal-dispersed plants are particularly important to study since they have larger repercussions for the communities of which these plants are a part. For example, changes in fruiting phenology and animal abundance or activity could result in a temporal mismatch between partners, especially if plants and animals are responding to different environmental cues or responding in different ways (Forrest, 2014; Rafferty et al., 2015; Palacio et al., 2021). If the timing of peak disperser activity is shifting in response to environmental changes in a similar direction to fruiting times, with fruiting times responding via either genotypic variation or environmental controls in plants, no phenological mismatch will result. However, if the FTFI is internally driven, fruiting times will shift earlier in the season in conjunction with earlier flower times. If the FTFI is externally driven, changing environmental conditions could result in fruiting times that are out of step with peak disperser activity, resulting in a similar phenological mismatch outcome as if FTFI were internally driven. The same is true if the FTFI is a result of stabilizing selection, unless other selective pressures act to re-align fruiting times with peak disperser activity. Phenological mismatch could result in dispersal failure via fruiting occurring before dispersers are active (Warren and Bradford, 2013), lower plant fitness via reduced disperser activity (McConkey and Drake, 2006; Traveset et al., 2012; Rogers et al., 2017), animal population declines via reduced fecundity or increased mortality (van Schaik et al., 1993; Wright et al., 1999; Saino et al., 2011), or changes in community composition (Moegenburg and Levey, 2003; Peralta et al., 2020).

Phenological mismatch is not the only way in which plant and animal populations, communities, and ecosystems could be affected by climate change impacts on the FTFI. Plant and seed-disperser populations could be affected by reduced fruit production resulting from higher than average temperatures, increases or decreases in precipitation, or a combination

(Young et al., 2004; Augspurger, 2009; CaraDonna and Bain, 2016; Babweteera et al., 2018; Benlloch-González et al., 2018; Chapman et al., 2018; Lee et al., 2018; Mendoza et al., 2018; Pardee et al., 2018; Nussbaumer et al., 2020; Rogers et al., 2021) if internal or external drivers of the FTFI pull fruiting times into less than ideal climatic conditions. Additionally, fruiting phenology could shift earlier at different magnitudes across elevation or space (e.g., Rafferty et al., 2020), leading to shifting spatial activity of seed dispersers in response to changed resource levels across the landscape (Curran and Leighton, 2000). Lastly, major shifts in frugivorous animal activity could lead to a cascade of effects that impact ecosystem function (Rogers et al., 2021), even if seed dispersal is not directly affected. For example, red elderberry fruiting early in Alaska caused bears to leave salmon runs to gorge on fruits, disrupting a strong predator-prey interaction (Deacy et al., 2017) and likely causing a reduction in nitrogen influx to the forest (Helfield and Naiman, 2006).

## CONCLUSION

We have leveraged here the extensive data on flowering phenology to explore how flowering and fruiting phenologies are linked. We found strong evidence in some species but not in others for a link between flowering and fruiting times. These results suggest that we should rapidly expand our understanding of the FTFI and shifts in fruiting phenology to enable better predictions for future climate change-influenced conditions. For those plant species demonstrating climate-driven phenological shifts in either direction, we need to explore *when* and *to what extent* those shifts will affect the ecological functioning and conservation concerns of plant and/or animal populations and/or mutualisms (e.g., Saino et al., 2011; Rafferty et al., 2015; Renner and Zohner, 2018). A better understanding of the magnitude of the effects on plant and animal populations and communities, as well as the factors that produce those effects, could enable parameterization of fitness models, and increase our ability to predict population trajectories and community composition in a changing climate.

## DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. Data were provided by the members of the PEP725 project: http://www.pep725.eu (PEP725).

## AUTHOR CONTRIBUTIONS

MES performed case study analyses. MES, CEA, LP, and JLB jointly wrote the first draft of the manuscript. All authors contributed substantially to revisions.

## FUNDING

The ideas for this paper emerged from an NSF-funded workshop (Grant DEB-1548194) at the National Socio-Environmental Synthesis Center. This work was supported by the NSF (Grant DEB-1701858).

## ACKNOWLEDGMENTS

We would like to extend our gratitude to all participants of the NSF-funded workshop at the National Socio-Environmental Synthesis Center, and especially Noelle Beckman, Haldre Rogers, Janneke Hille Ris Lambers, Florian Hartig, Sebastian Schreiber, and Rebecca S. Snell. We are grateful to two reviewers for helpful comments on an earlier version of the manuscript.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.752110/full#supplementary-material

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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ORIGINAL RESEARCH
published: 16 December 2021
doi: 10.3389/fevo.2021.719566

# Multiple Anthropogenic Pressures Lead to Seed Dispersal Collapse of the Southernmost Palm *Jubaea chilensis*

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***Specialty section:***
*This article was submitted to*
*Population, Community,*
*and Ecosystem Dynamics,*
*a section of the journal*
*Frontiers in Ecology and Evolution*

***Received:*** *02 June 2021*
***Accepted:*** *24 November 2021*
***Published:*** *16 December 2021*

***Citation:***
*Cordero S, Gálvez F and Fontúrbel FE (2021) Multiple Anthropogenic Pressures Lead to Seed Dispersal Collapse of the Southernmost Palm Jubaea chilensis. Front. Ecol. Evol. 9:719566. doi: 10.3389/fevo.2021.719566*

Seed dispersal is a critical process for plant reproduction and regeneration. Successful recruitment depends on pre- and post-dispersal processes that complete a seed's journey until becoming a new plant. However, anthropogenic stressors may disrupt the seed dispersal process at some stages, collapsing plant regeneration and hampering its long-term persistence. The Chilean palm tree *Jubaea chilensis* is the southernmost and the only non-tropical palm species, which currently relies on the scatter-hoarding rodent *Octodon degus* for seed dispersal. We assessed seed fate by measuring predation and dispersal rates through experimental fieldwork in the Palmar de Ocoa site (located within La Campana National Park) and the Palmar El Salto. We also used previous reports on seed harvest and seedling herbivory to depict the whole *J. chilensis* seed dispersal process and assess the relative importance of different anthropogenic pressures. We asked the following questions: (1) What is the effect of human harvesting on *J. chilensis* recruitment? (2) Do native and exotic rodents predate *J. chilensis* seeds in the same way? and (3) Does post-dispersal herbivory matter? We found that *J. chilensis* fruits are harvested for human consumption, reducing pre-dispersal available seeds by removing about 23 tons per season. Then, post-dispersal seeds at the Ocoa palm grove are heavily predated by exotic (*Rattus rattus*) and native (*Octodon* spp.) rodents; only 8.7% of the seeds are effectively dispersed by *Octodon degus*. At Palmar El Salto, 100% of the seeds were predated by *Rattus rattus*, precluding further analysis. Finally, 70% of the seedlings were consumed by exotic herbivores (mainly rabbits), resulting in a success rate of 1.81%. Only 7.9% of the surviving seedlings become infantile plants (4 year-old). Our assessment suggests that *J. chilensis* has aging populations with very few young individuals in disturbed sites to replace the old ones. For those reasons, we suggest increasing its conservation category to critically endangered as land-use change is rapidly fragmenting and shrinking the extant *J. chilensis* populations. We urge to take urgent actions to protect this relict palm, which otherwise may go extinct in the next decades.

**Keywords: central Chile, exotic species, *Jubaea chilensis*, overharvesting, seed predation, extinction**

# INTRODUCTION

Seed dispersal is an important process for plant reproductive success, playing a key role in the functioning and dynamics of communities (Traveset and Richardson, 2006; Corlett, 2017) as well as in maintaining biodiversity (Bascompte and Jordano, 2007; Valiente-Banuet et al., 2015). This mutualistic interaction benefits both parties, by providing food resources for animals, and promoting the colonization of new ranges and escaping from competing siblings for plants (Farwig and Berens, 2012). Plants are highly dependent on animals for seed dispersal in different biomes, with 90% of trees being dispersed by animals in tropical regions (Howe and Smallwood, 1982) and up to 60% in temperate regions (Willson et al., 1990). Seed dispersers facilitate natural plant regeneration. Therefore, the disruption of dispersal interactions can decrease gene flow among populations and limit seedling recruitment and establishment, leading to the collapse of regeneration and subsequent local extinction (Rodríguez-Cabal et al., 2007; Gomes et al., 2008; Aslan et al., 2013; Fontúrbel et al., 2019; Meadley-Dunphy et al., 2020).

Habitat alteration caused by anthropogenic activities usually induces changes in the composition of frugivorous animals that perform seed dispersal services (Figueroa-Esquivel et al., 2009; Vizentin-Bugoni et al., 2019). Disturbed habitats are more susceptible to being invaded by exotic animal species, interfering with ecological interactions such as seed dispersal and predation (Shiels et al., 2014). Sometimes exotic animals invade new habitats and establish novel interactions with native and exotic plants, altering seed dispersal, and recruitment patterns (Foster and Robinson, 2007; Devenish et al., 2019; Meadley-Dunphy et al., 2020). However, in many cases, exotic species behave like seed predators, limiting seed banks of native species (Towns et al., 2006; Stachurska-Swakon et al., 2018). In this regard, exotic rats (*Rattus* spp.) are important seed predators (Yabe et al., 2010) and are considered as one of the most harmful invasive species worldwide (Drake and Hunt, 2009). *Rattus* spp. are common in disturbed forests (Meyer and Shiels, 2009; Fontúrbel, 2012) and usually exert negative ecological impacts by acting as major seed predators, consequently affecting forest dynamics and composition (Campbell and Atkinson, 2002). These rodents may have been responsible for many past plant extinctions, as in native palm forests in Hawai'i (Athens et al., 2002) and Easter Island (Hunt, 2007).

Some relict plant species depend on rodents for seed dispersal, as is the case of *Jubaea chilensis*, an endangered palm species from central Chile. Although it is believed that the fruits of *J. chilensis* were dispersed by extinct megafaunal species in the past (González et al., 2017), its seeds are currently dispersed by the native scatter-hoarding rodent *Octodon degus*. Scatter-hoarding behavior implies that uneaten seeds can germinate under beneficial conditions, but many seeds are destroyed when the embryo is damaged (Vander Wall, 1990; Lichti et al., 2017). This scatter-hoarder rodent uses palm seeds as a key resource during juvenile and pre-reproductive stages (Zunino et al., 1992). However, this interaction is disrupted by the black rat (*Rattus rattus*), a major palm seed predator (Perez et al., 2008; Maron and Pearson, 2011).

*Jubaea chilensis* is the southernmost palm tree, endemic to continental Chile, and the only species of the monotypic genus *Jubaea* (Arecaceae), whose populations from the Easter Island became extinct in the sixteenth century presumably due to seed predation by Pacific rats (*Rattus exulans*). Although this palm species was highly abundant in the past, its presence is currently restricted to three main populations within protected areas and some private lands in central Chile (Flores-Toro and Aguirre-Saavedra, 2008). Thus, it is considered as Vulnerable according to the IUCN threat categories. During the last decades, *J. chilensis* populations have suffered a substantial decline as a consequence of multiple anthropogenic pressures, which include fruit and seed overexploitation for human consumption, reduction of vegetation cover, wildfires, and exotic species (González et al., 2009; Guzmán et al., 2017). However, the impacts of exotic species may be largely underestimated as they are usually associated with herbivory by lagomorphs and domestic cattle. Therefore, the ecological consequences of seed predation by exotic rodents remain largely unknown. Here we assessed the whole seed dispersal process (from fruits to seedlings) of the largest *J. chilensis* population, related to different anthropogenic stressors that may lead to a reproductive collapse in this species, we used camera-trap monitoring, experimental field tests, and demographic information gathered from previous reports to answer the following questions: (1) What is the effect of human harvesting on *J. chilensis* recruitment? (2) Do native and exotic rodents predate *J. chilensis* seeds in the same way?, and (3) Does post-dispersal herbivory matter? We hypothesized that *J. chilensis* recruitment would be negatively affected by multiple anthropogenic stressors, being seed predation by rodents the most critical factor.

# MATERIALS AND METHODS

## Study Area

The study was conducted at the Palmar de Ocoa site, located within La Campana National Park (32°57.26′ S 70°35.18′ W; **Figure 1**). This public protected area is located in the mountain range of La Campana-El Roble, in the Valparaíso region (central Chile). The study area is encompassed in the Central Chile biodiversity hotspot (Myers et al., 2000), characterized by a Mediterranean climate type, with rainy winters reaching around 480 mm annually and a prolonged dry period with only 120 mm of rain (Quintanilla et al., 2012). The average annual temperature is ∼ 18°C (Pliscoff, 2009). This site contains the largest *J. chilensis* population with ∼ 70,000 individuals (including all age classes) (Gallardo et al., 2006; González et al., 2009, 2017). The dominant vegetation is composed of sclerophyllous forests and scrubland formations, where *J. chilensis* is mainly associated with *Cryptocarya alba*, *Quillaja saponaria*, *Lithrea caustica*, *Peumus boldus*, and *Retanilla trinervia*. Further, the Santuario de la Naturaleza Palmar El Salto (Palmar El Salto hereafter; 33°04.00′ S 71°31.00′ W) is a protected site that contains the third-largest *J. chilensis* population with ∼ 7,000 individuals (Flores-Toro and Aguirre-Saavedra, 2008), which we

**FIGURE 1 |** A panoramic view of the Ocoa palm grove (left panel) and a *Jubaea chilensis* adult individual (right panel).

also studied for comparison purposes. This site is fragmented into four areas: Rodelillo, Altos del Tranque, Quebrada El Quiteño, and Quebrada Las Siete Hermanas; the latter was selected for the study as it is the one with the highest number of *J. chilensis* individuals (Möder et al., 1997). The vegetation at Palmar El Salto is represented mainly by scrubland formations, dominated by *Nassella chilensis*, *Chusquea cumingii*, and *Retanilla trinervia* (Flores-Toro and Aguirre-Saavedra, 2008). This study area is characterized by a Mediterranean climate with coastal influence and annual precipitation of up to 400 mm (Flores-Toro and Aguirre-Saavedra, 2008). Despite the fact there is a protected site, it was not designated for preservation and conservation purposes; thus, multiple anthropogenic pressures as domestic cattle, frequent intentional fires, and fruit extraction are some of the greatest threats to this *J. chilensis* metapopulation.

## Demography

Based on the latest available censuses reported by González et al. (2017) and Bravo et al. (2018), the current *J. chilensis* population at the Palmar de Ocoa, within La Campana National Park, comprises 70,308 individuals. From those, 31,143 individuals belong to reproductive adults (75–250 years; 44.3%), 7,078 to juveniles (35–75 years; 10.1%), 2,359 to infantile (4–35 years; 3.4%), and 29,728 to seedlings (<4 years; 42.3%) (accordingly to the 2017 census of Bravo et al., 2018). On the other hand, the *J. chilensis* population at the Palmar El Salto comprises 6.947 individuals, with 6,441 individuals belonging to reproductive adults (92.7%), 504 to juveniles (7.8%), 2 to infantile (0.03%), and 0 to seedlings (0%) (accordingly to the census of González et al., 2017). Age classes were defined following the criteria of González et al. (2017). Fruiting on *J. chilensis* occurs on palm trees of at least 75 years old (González et al., 2017) producing ∼10,000 fruits per palm (information obtained from Guzmán et al., 2017). On average, the seed germination rate is ∼70%, but it may take up to 4 years as these seeds have a long dormancy period (Guzmán et al., 2017).

## Seed Dispersal and Predation Assessment

During the austral fall of 2018 (March–May, corresponding to the *J. chilensis* fructification period), we installed feeding stations (25 in the Palmar de Ocoa and 5 in the Palmar El Salto) under breeding palm individuals (palm seeds naturally fall and can be found in the ground, but now they are particularly scarce due to human harvesting practices), separated by at least 100 m from each other to ensure independence. We used seeds because (i) fruits are extremely scarce in palm groves due to anthropogenic harvesting pressures, and (ii) rabbits usually consume the remaining pulp, modifying seed arrangement and making it impossible to recognize seed identity afterward. The *J. chilensis* fruits are yellowish-green ovoid monospermous drupes of 4-cm in diameter, which fall from the palm when ripe and release the seeds when dehydrated. When the pulp is not completely detached from the seed, rabbits sometimes eat it, leaving bare seeds intact in the site; contrarily, rodents remove pulp and discard it to obtain the seeds.

Each feeding station contained 24 palm seeds, provided by the Hacienda Las Palmas de Cocalán, due to the fruit scarcity and difficulty obtaining intact seeds within the study area. Seeds were marked by attaching a 65-cm nylon thread using epoxy glue, with a 15-cm adhesive tape attached to the distal end, containing a unique identification code in indelible ink. We placed those 24 seeds in a correlative numerical order at each station along a circle with the labels facing outward. The first seed was pointed as a reference to identify the complete set of seeds. Tags were covered with litter from the surrounding area to make them less visible. Then, to assess seed removal by rodents, we used infrared camera traps (Browning Strike Force HD Pro) placed 10–20 cm above the ground and 1.5 m away from the feeding station and separated at least 100 m from each other to minimize data independence problems. Camera traps were operated in video mode with 20-s captures and a 10-s delay between captures. After the depletion of the stations, we conducted exhaustive searches for labels in a 40-m radius on days 2, 4, 6, 8, 16, and 32, verifying and recording seed destination. Post-removal seed destination was classified as:

(i) cached, if the seed was buried intact, (ii) eaten, if threads were still attached to seed fragments or if fragments were found near the tags, and (iii) unknown fate, if not found the label within the study area (those seeds were excluded from the analysis).

## Data Analysis

We used a sequential analysis from fruit production to seedling survival. We used the demographic information described in section "Demography" (information obtained from previous reports) and the seed dispersal and predation information obtained through the camera-trap assessment (described in section "Seed Dispersal and Predation Assessment"). We compared seed fate (either disperser, eaten, or missed) data for each rodent species detected by our camera traps using one-way ANOVAs and Tukey *post hoc* tests. We were able to make such comparisons only for the Palmar de Ocoa site, as at Palmar El Salto, all seeds were eaten by a single species, precluding any comparison. Further, we estimated the impact of human harvesting using information from the Chilean Ministry of Environment (Gallardo et al., 2014), which reports that 200 people were authorized to extract *J. chilensis* fruits during the masting period, such extraction represents ∼23 tons of fruits per year [we estimated the number of fruits using the average mass of the seeds (7.66 g) used for the camera-trap assessment]. Then, we used the average germination rate reported by Guzmán et al. (2017) to estimate the number of seedlings produced. Seedlings are known to be largely affected by exotic herbivores in the study area (Guzmán et al., 2017). Therefore, to estimate the final number of surviving seedlings, we used sapling herbivory estimations from Cabello (2006), which were around 70% and mainly caused by rabbits and cows (Marcelo, 2007). Finally, we estimated the overall success rate as the ratio between the number of surviving seedlings and the number of seeds produced.

# RESULTS

We obtained valuable information on *Jubaea chilensis* demography and seed dispersal from different data sources (**Table 1**). We used the data of the Palmar de Ocoa site, which has 70,308 *Jubaea chilensis* individuals according to the last census (González et al., 2017). Of those individuals, 44.3% correspond to breeding adults, representing 31,143 individuals. Considering that each adult palm can produce 10,000 fruits on average, the overall number of seeds produced (considering that these are single-seeded fruits) is 311,430,000.

**TABLE 1 |** Data sources of the seed dispersal variables included in this study.

| Stage | Variable | Sources |
|---|---|---|
| Pre-dispersal | Fruit production | Guzmán et al., 2017 |
| | Seed harvest | Gallardo et al., 2014 |
| | Population census | Bravo et al., 2018 |
| | Age structure | González et al., 2017 |
| Dispersal | Seed predators | Our camera trap data |
| | Seed dispersers | Our camera trap data |
| | Seed fate | Our camera trap data |
| Post-dispersal | Germination rate | Guzmán et al., 2017 |
| | Herbivory rate | Cabello, 2006; Marcelo, 2007 |

## Human Harvesting Impact

From the 311,430,000 fruits produced per year, human harvesting (the main pre-dispersal threat) is responsible for extracting 3,002,611 fruits –based on official reports from the Ocoa palm grove– stating that 23 tons of *J. chilensis* fruits are extracted each year (average seed mass 7.66 g). Thus, human harvesting equals depleting ∼300 *J. chilensis* individuals, leaving 308,427,389 seeds (99%) to be dispersed (**Figure 2A**). There are no official records of fruit extraction for the Palmar El Salto site.

## Seed Predation and Dispersal Patterns

We found three rodent species feeding on *J. chilensis* seeds in our camera trap records at the Palmar de Ocoa (**Figure 3**). From the total 596 photographic records –obtained from 25 independent camera traps– 236 corresponded to the exotic black rat (*Rattus rattus*; 39.6%), 232 corresponded to the native nocturnal rodent *Octodon lunatus* (38.9%), and 128 corresponded to the native diurnal rodent *Octodon degus* (21.5%). Some camera traps recorded visitation events of a single rodent species, while others recorded two or three species within the same station. All seeds placed in the experimental stations were depleted within 1 week. Both *R. rattus* and *O. lunatus* acted only as seed predators, with 213 seeds (91.8%) eaten (i.e., predated) and 23 (8.2%) with unknown fate in the case of *R. rattus,* and 200 seeds (86.2%) eaten and 32 (13.8%) with unknown fate in the case of *O. lunatus* (**Figure 4**). However, *O. degus* had a dual role as seed disperser and predator. From the 128 seeds collected by *O. degus*, 71 (55.5%) were eaten, 46 (35.9%) were effectively dispersed (no larder-hoarding was observed at our study site), and we were unable to determine the fate of 11 (8.6%) seeds. Seeds were dispersed at distances of 6.2 m ± 0.4 (mean ± standard error; range: 1.0–20.3). Therefore, 91.32% of the seeds with known fate were predated, while only 8.7% of the seeds (15,545,653) represent 8.5% of the seeds produced and 8.68% of the seeds available post-harvesting) were effectively dispersed by *O. degus* (**Figure 2B**). By extrapolating these values to the population, 15,545,653 seeds may be effectively dispersed, representing 8.5% of the seeds produced and 8.68% of the post-harvesting seeds. In the case of the Palmar El Salto, the only rodent species present was *R. rattus*, which depleted feeding stations within 48 h. From the 120 seeds placed there, 118 (98%) were predated by *R. rattus*, and two were missed (2%) after rodents removed them.

## Germination and Seedling Herbivory

Assuming an average germination rate of 70% (values obtained from previous reports), the 26,771,497 seeds would yield 18,740,048 seedlings. From those, 70% is lost by exotic herbivores (the main post-dispersal threat according to the published literature), leaving only 5,622,014 seedlings that may be capable of surviving and recruiting to the population. Therefore, these figures represent a success rate of 1.81% (**Figure 2C**). Furthermore, following the population age structure from the last

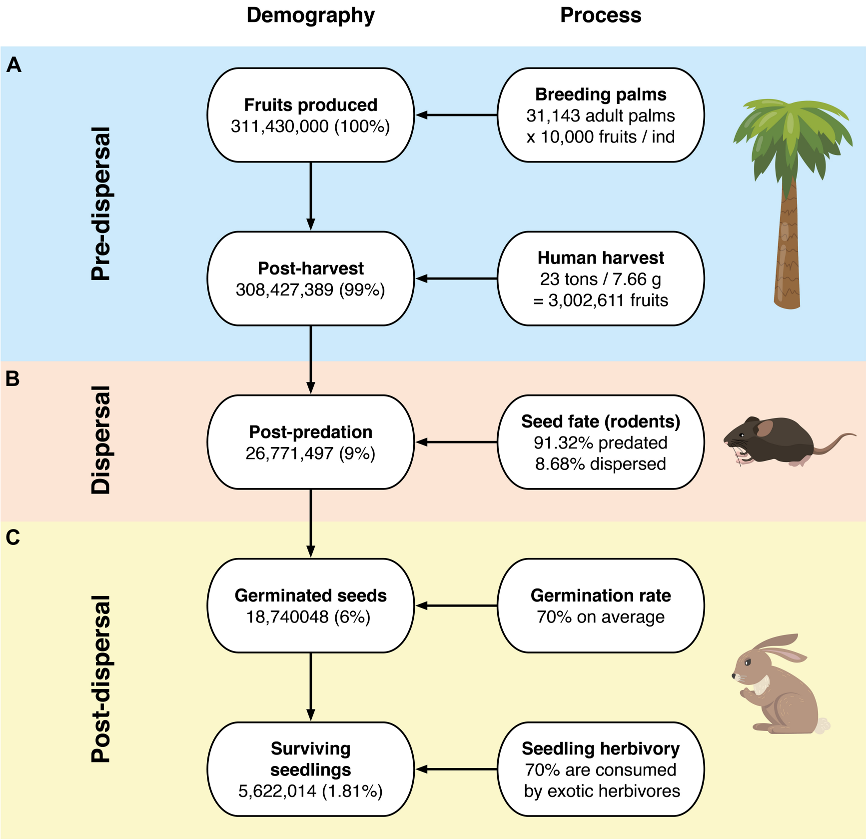

**FIGURE 2 |** *Jubaea chilensis* recruitment process at: **(A)** pre-dispersal, **(B)** dispersal, and **(C)** post-dispersal stages.

census (Bravo et al., 2018), only 7.9% of the seedlings will survive until the fourth year to become infantile individuals, resulting in 444,139 potential recruits (equivalent to 0.14% of the total fruits produced). We could not perform a similar analysis for the Palmar El Salto site, as there is no regeneration at this site.

## DISCUSSION

We found that the *Jubaea chilensis* seed dispersal process is affected by anthropogenic stressors at all stages of recruitment. This chain of events begins with human harvesting, which acts at the pre-dispersal stage. Although local people remove 23 tons of fruits each year, it only represents ∼1% of the fruits produced by the adult palms in our study area. However, those numbers may be underestimated for two reasons. First, our estimations are based on official extraction reports (based on management plans with extraction quotas defined *a priori*). However, some illegal extraction cannot be quantified and it is expected to vary over the years. Second, besides fruit extraction, people also extract palm sap to produce a sweet syrup commonly called "palm honey." González et al. (2009) indicate that up to 400 L of sap can be extracted from each palm tree, but the actual sap extraction values are unknown as no management plan is associated with this kind of use. While sap extraction does not kill the palm, it is likely to impact fruit production, but those effects have not been quantified yet. There are official reports of fruit extraction at our site because it is a public, protected area. However, quantitative data is largely absent from other localities where we found remnant palm populations.

Examining the next phase of the seed dispersal process, we found that seed predation is by far the most relevant threat for *J. chilensis* regeneration. Currently, *J. chilensis* seed dispersal relies on the native rodent *Octodon degus*, responsible for dispersing about half of the seeds caught by scatter-hoarding (i.e., burying the seeds) and eating the other half. However, its close relative, *O. lunatus*, predate all seeds taken as this species does not bury seeds for consuming them later (Zunino et al., 1992; Kelt et al., 2004). Besides those two native rodents, the exotic *Rattus rattus* is a common invasive species in this area responsible for predating ∼34% of the seeds (236 out of 696 seeds). The proliferation of *Rattus* spp. is positively associated with habitat disturbance (Cusack et al., 2015), becoming major seed predators across different ecosystems (Campbell and Atkinson, 2002; Hunt, 2007; Yabe et al., 2010; Shiels and Drake, 2011). In this regard, native vegetation of central Chile has been largely impacted by habitat fragmentation and degradation process, derived from a rapid land-use change (Echeverría et al., 2006, 2007), which may explain the increasing abundances of exotic rats to the detriment

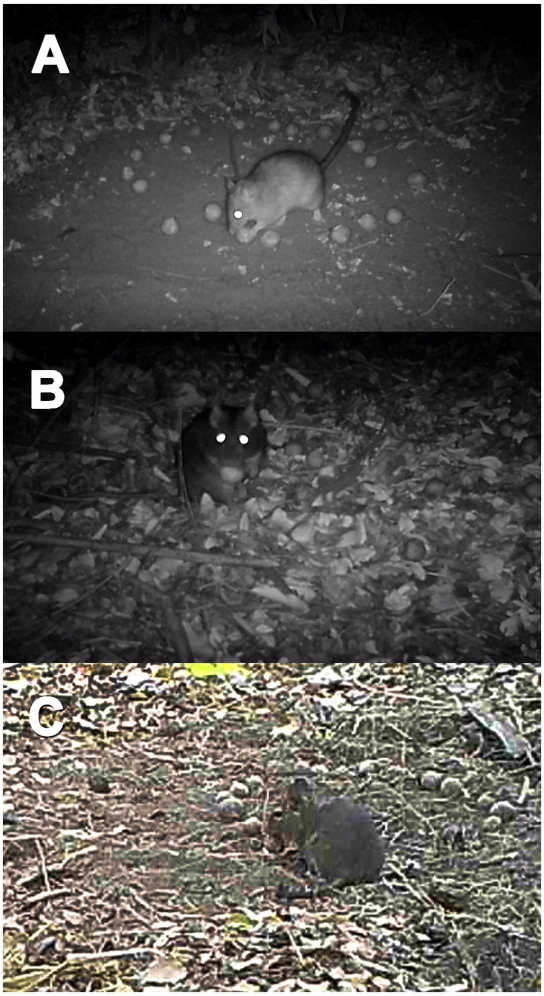

**FIGURE 3 |** The three rodent species consuming *Jubaea chilensis* seeds found during the camera-trap survey: **(A)** *Rattus rattus*, **(B)** *Octodon lunatus*, and **(C)** *Octodon degus*.

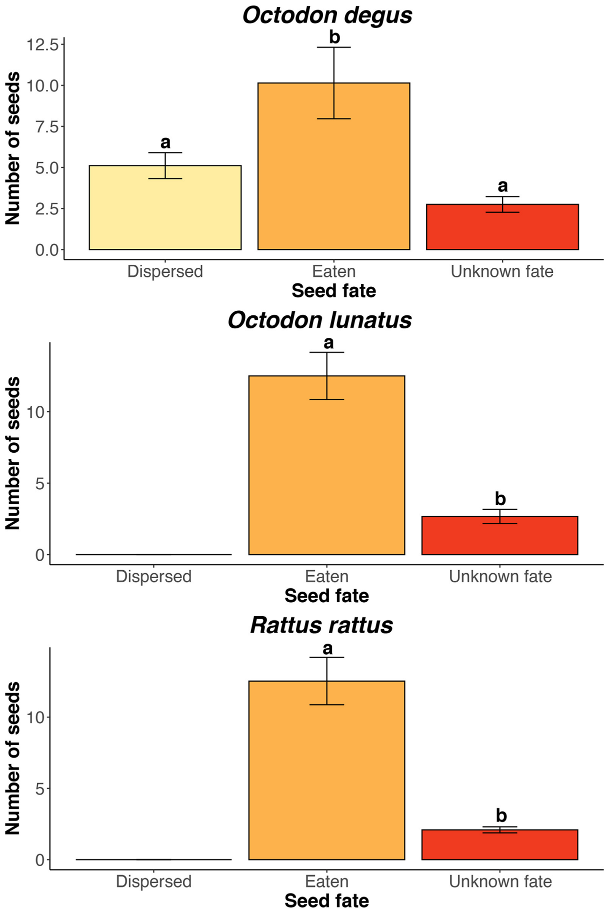

**FIGURE 4 |** *Jubaea chilensis* seed fate by rodent species at the Ocoa site. Bars represent mean ± SE number of seeds per feeding station ($N = 24$). Letters above bars represent significant differences ($P < 0.05$) after Tukey *post hoc* tests.

of native small mammals (Fontúrbel, 2012). Large *Rattus rattus* densities have been reported in the extant *J. chilensis* populations nearby urban centers of Valparaíso and Viña del Mar (i.e., Palmar El Salto site), largely subsidized by palm fruits and favored by the neighboring urban habitats. To the best of our knowledge, no other native seed disperser *J. chilensis* seeds besides *Octodon degus*, which could compensate for seed predation if exotic rodents were eventually removed, and no pathogens have been reported to affect seed viability. Thus, there is reasonable to assume that exotic rodents are responsible for limiting seed dispersal.

Although low seed dispersal rates may allow plant regeneration, limited dispersal can lead to alterations and collapse gene flow, affecting population connectivity, as well as the regional-scale distribution of genetic variation (Pérez-Méndez et al., 2018). This is a major threat for *J. chilensis* since current populations have been affected by genetic erosion and high levels of inbreeding, with populations as different genetic pools and loss of genetic variability (González et al., 2017). Therefore, the reduced genetic diversity of *J. chilensis* populations caused by limited dispersal and habitat fragmentation may reduce the ability of the species to persist under land use change and climate change scenarios.

Then, in the post-dispersal process, those few seeds effectively dispersed by *O. degus* must face two additional filters. The first filter is germination, as *J. chilensis* has slow germination. The seeds suffer a physiological dormancy due to the immaturity of the embryo, and germination takes up to 4 years (Guzmán et al., 2017). Within this period, seeds can be predated, reducing actual germination rates. Also, seeds can be re-cached, reaching sites with more suitable or unsuitable conditions for germination and can be pilfered by other rodents as well (Zhang et al., 2014). In the latter scenario, pilfered buried seeds may have a fate different than germination, as both *O. lunatus* and *R. rattus* act as seed predators. While the average germination rate is ~70% (Cabello, 2006), microclimatic conditions largely influence it (Fleury et al., 2015). Seeds covered by a thick litter layer have larger germination rates

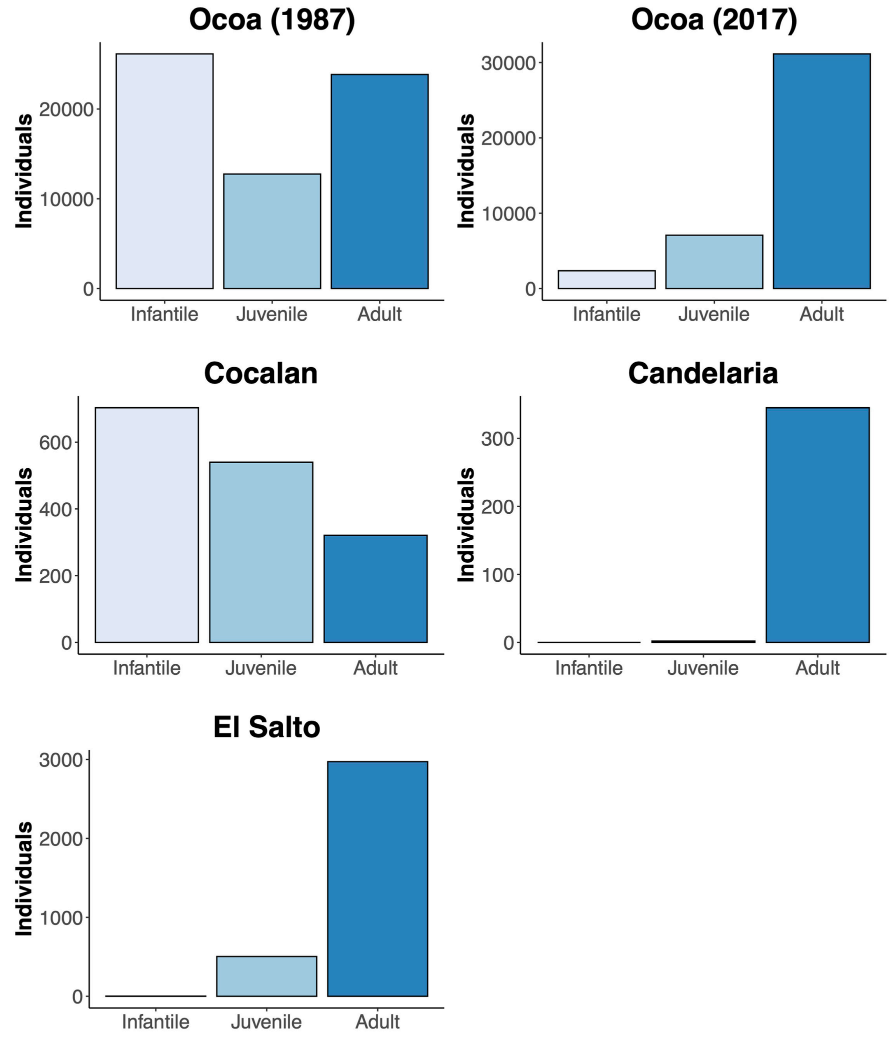

**FIGURE 5 |** Age structure (infantile: 4–35 year-old, juvenile: 35–75 year-old, adult > 75 year-old) of the four largest *Jubaea chilensis* populations in central Chile. Demographic information based on González et al. (2017) and Bravo et al. (2018).

and seedling survival rates (Miranda et al., 2016). Also, denser native vegetation protects seedlings from abiotic stress and animal consumption, increasing their survival probabilities (Fleury et al., 2015). Thus, the fragmentation and degradation of the native sclerophyllous scrublands that characterize the extant *J. chilensis* remnants facilitate the invasion by exotic rats and reduce germination and seedling survival probabilities. Along with rats, other exotic species have intentionally and unintentionally been introduced to the study area and the proliferation of human settlements. Hence, rabbits and cows are very common herbivores in palm grove fields and, at the Palmar de Ocoa, are responsible for ∼70% of seedling mortality. An experiment conducted by Fleury et al. (2015) showed that excluding exotic herbivores increased seedling survival by 39%. Also, herbivores consume other plant species that act as nurse plants for *J. chilensis* seedlings (Holmgren et al., 2000; Saiz and Alados, 2012), worsening the current scenario. Even if seedlings escape herbivory, infantile individuals are still subject to other anthropogenic pressures such as fire regimes, as they cannot cope with these events, unlike adults (González et al., 2017). Considering the pre-dispersal, dispersal, and post-dispersal processes affecting *J. chilensis* recruitment, we estimated a success rate of 1.81%, which is consistent with a recent regeneration estimation made by the Chilean Forestry Corporation (CONAF, public entity responsible for the La Campana National Park); based on 113 plots, palm regeneration was estimated to be 1.1 ± 0.3% (Bravo et al., 2018). Those estimations were based on monitoring individuals and determining age classes in several plots, but the factors explaining that result were not quantified yet.

## Future Perspectives

The extant 120,980 *J. chilensis* individuals reported by González et al. (2009) represent ∼2.5% of the original population (estimated that there were 5 million individuals by 1550; Hechenleitner et al., 2005). Once, the Valparaíso region was

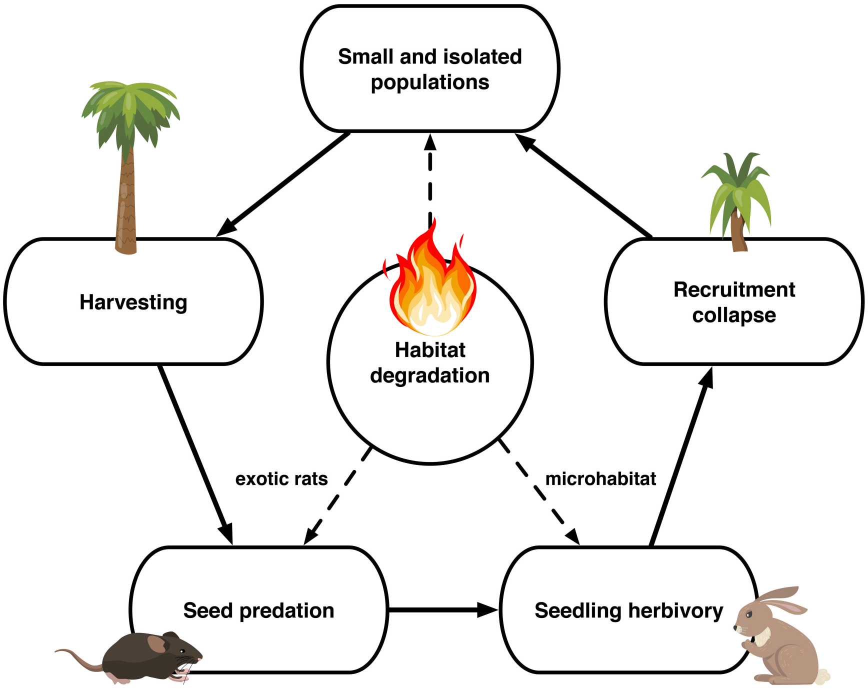

**FIGURE 6 |** *Jubaea chilensis* extinction process due to anthropogenic stressors affecting the seed dispersal process.

covered by dense palm groves, from which a few scattered populations remain. Of those, 87.5% are preserved within La Campana National Park (70,308 individuals) and Las Palmas de Cocalán National Park (35,500 individuals). The remaining 15,172 palm individuals are scattered among ten isolated populations, ranging between 2 and 7,200 individuals [mean abundance 1,664 ± 729 individuals (mean ± 1 SE)], and immersed in a heavily degraded anthropogenic matrix. Comparing the age classes of the four largest *J. chilensis* populations (**Figure 5**), we observe that the number of infantile and juvenile individuals in Ocoa has decreased between 1987 and 2017, while they are quite scarce at Candelaria and El Salto populations. The only exception is the Cocalán population, where infantile and juvenile individuals outnumber adult palms, which is because this site has a management plan for palm honey extraction that requires planting 10 new individuals for each adult palm harvested (Serra et al., 1986). Based on the evidence compiled and our data, we propose that currently, *J. chilensis* is undergoing through an extinction process (**Figure 6**) that can lead to a collapse in the next decades if no actions are taken.

Imposing harvesting quotas would have little impact on improving palm regeneration. What is urgent now is controlling exotic species that consume palm seeds and seedlings, collapsing its regeneration. Seed predation by rats and seedling herbivory by cows and rabbits occur even within protected area boundaries, putting the two largest *J. chilensis* populations at great risk (Fleury et al., 2015; Miranda et al., 2016). These extant palm groves can be considered as living dead because we have aging populations with little regeneration, facing increasing levels of anthropogenic disturbance. Thus, we may be witnessing an extinction debt here (Tilman et al., 1994; Carroll et al., 2004), as with the current recruitment levels, *J. chilensis* may become extinct within a century. Currently, this relict palm is categorized as Vulnerable by the International Union for Conservation of Nature (IUCN) red list. However, considering the facts presented here, we strongly suggest reconsidering such a category and changing it to Critically Endangered instead.

## CONCLUSION

We conclude that the Chilean palm, *Jubaea chilensis*, is a critically endangered species due to the collapse of the seed dispersal process, hampering its recruitment. This is the southernmost palm species in the world, and the extant populations may represent an extinction debt. The anthropogenic pressures that this species faces may be leading to an inevitable collapse. We urge local authorities to take urgent actions to increase its protection and control those exotic species responsible for seed predation and seedling herbivory, which are the main recruitment decline drivers. The presence of exotic rats is positively correlated with habitat disturbance (Fontúrbel, 2012). Therefore, avoiding the degradation of the extant palm groves would be the best option to limit their populations. Furthermore, using exclusion meshes could significantly reduce rabbit herbivory on seedlings. However, the most relevant action would be to increase reforestation efforts by planting 4-year-old saplings since these are less susceptible to the potential damage caused by exotic herbivores. Together, these actions will contribute to recovering the extant populations and avoid a dark fate for this emblematic species.

## DATA AVAILABILITY STATEMENT

Original data associated to this submission is available at the figshare digital repository: https://doi.org/10.6084/m9.figshare.14717676.

## ETHICS STATEMENT

Ethical review and approval was not required for the animal study because we only used camera-traps.

## AUTHOR CONTRIBUTIONS

SC and FG conceived the study and collected field data. SC, FG, and FF designed the methodology. FF performed data analysis and wrote the first draft of the manuscript with contributions of SC and FG. All authors read and approved the submitted version.

## FUNDING

SC was supported by a Ph.D. scholarship granted by the Chilean Agency of Research and Development (ANID, 21211752).

## ACKNOWLEDGMENTS

We are grateful to the Chilean Forestry Corporation (CONAF) for granting us access to the study area. We are indebted to Piero Vitta, Gerardo Jara, Franco Verdessi, Javiera Rodríguez, Constanza Andía, Manuel López, and Joaquín Gómez, who assisted in the field and to Hacienda Las Palmas de Cocalán for providing us the seed material for the study. Comments of three reviewers largely improved the manuscript.

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
in Ecology and Evolution

ORIGINAL RESEARCH
published: 16 December 2021
doi: 10.3389/fevo.2021.782462

# Persistence of Seed Dispersal in Agroecosystems: Effects of Landscape Modification and Intensive Soil Management Practices in Avian Frugivores, Frugivory and Seed Deposition in Olive Croplands

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OPEN ACCESS

**Edited by:**
*Anna Traveset,*
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**Specialty section:**
*This article was submitted to*
*Population, Community, and*
*Ecosystem Dynamics,*
*a section of the journal*
*Frontiers in Ecology and Evolution*

***Received:*** *24 September 2021*
***Accepted:*** *10 November 2021*
***Published:*** *16 December 2021*

**Citation:**
*Rey PJ, Camacho FM, Tarifa R,*
*Martínez-Núñez C, Salido T, Pérez AJ*
*and García D (2021) Persistence of*
*Seed Dispersal in Agroecosystems:*
*Effects of Landscape Modification and*
*Intensive Soil Management Practices*
*in Avian Frugivores, Frugivory and*
*Seed Deposition in Olive Croplands.*
*Front. Ecol. Evol. 9:782462.*
*doi: 10.3389/fevo.2021.782462*

Farming impacts animal-mediated seed dispersal through mechanisms operating on at least two spatial scales. First, at the landscape scale, through habitat loss and land conversion to agriculture/livestock grazing, and second, at the farm scale, via a local intensification of agricultural practices. These two scales of farming impact seed dispersal function but have rarely been integrated. In particular, studies evaluating the effect of agriculture on the seed dispersal function of frugivorous birds in Mediterranean ecosystems are lacking. This study evaluates the role of landscape transformation, from fruit-rich woodland habitats to olive grove landscapes, together with local intensive practices of soil management on the persistence of the seed dispersal function for Mediterranean fleshy-fruited plants in olive landscapes of south Spain. We used bird censuses, mist-nets, and seed traps to characterize avian frugivore assemblages, frugivory, and seed deposition in the seminatural woodland habitat (SNWH) patches and olive fields of 40 olives farms spanning 20 localities distributed across the whole range of olive cultivation in Andalusia (southern Spain). We found that despite the remarkable dispersal function of olive grove landscapes, avian frugivore abundance and diversity, frugivory, and seed arrival decreased in olive fields compared to SNWH patches. Likewise, SNWH cover loss and/or olive growing expansion decreased avian frugivory and seed arrival. Interestingly, the habitat effects in the olive farms often depended on the landscape context. In particular, less diverse fruit-eating bird assemblages pooled in SNWH patches as olive grove cover increased or SNWH decreased in the landscape, while remaining relatively invariant in the olive fields. Finally, compared to conventional intensive agriculture, low-intensity management increased frugivory and seed deposition. We conclude that olive fields are less permeable to frugivores than expected due to the agroforest-like nature of these landscapes and that the presence of SNWH patches is crucial for the maintenance of frugivory and seed dispersal in agricultural landscapes. These results evidence that woodland habitat loss by olive expansion and intensive practices seriously threaten the dispersal service in olive-dominated landscapes. Maintenance, restoration, and promotion of woodland patches should be prioritized for the conservation of seed dispersal service and for enhancing the functional connectivity in human-shaped olive landscapes.

**Keywords: seed dispersal, frugivorous bird, olive grove, agriculture intensification, landscape transformation, habitat loss, agroforest ecosystem, frugivory**

## INTRODUCTION

Seed dispersal by frugivores is an important ecosystem service that promotes spontaneous natural regeneration of vegetation and contributes to shaping natural plant communities and their dynamics in many regions (Herrera, 1985; Lázaro et al., 2005; Tomback, 2016). In particular, the seed dispersal of woody plants by frugivorous vertebrates is a major driver of the dynamics of tropical forests and Mediterranean woodlands (e.g., Herrera, 1995; Muller-Landau and Hardesty, 2005) where frugivores act as mobile links for connecting plant populations across landscape patches (Henry et al., 2007; Pérez-Méndez et al., 2017; Parejo-Farnés et al., 2020). In current real-world (human-shaped) landscapes, ensuring the persistence of seed dispersal by frugivorous vertebrates is crucial for maintaining connected plant metapopulations and metacommunities across remnant landscape patches (Mueller et al., 2014; González-Varo et al., 2017), and for promoting plant population recovery through rescue effects (García et al., 2010).

Farming is a major driver of global change and biodiversity crisis (Tilman et al., 2001; Foley et al., 2005, 2011), giving rise to the predominance of human-shaped, biologically homogenized landscapes in many regions (Green et al., 2005; Tscharntke et al., 2005, 2012; Arroyo-Rodríguez et al., 2020). Farming has also affected the ecosystem functions and the delivery of biodiversity-based ecosystem services (Letourneau et al., 2011; Gurr et al., 2017; Landis, 2017), among which seed dispersal by frugivores is not an exception. Conceptually, farming impact on animal seed dispersal is expected to operate through mechanisms, acting at least at two spatial scales (Martin et al., 2019). First, at the landscape scale, land conversion to agriculture and/or livestock grazing provokes the direct loss of natural habitats, decreasing the population sizes of frugivores and fleshy-fruited plants, and increasing isolation among remnant habitat patches, This causes plant metapopulations to collapse as it restricts animal mobility and alters seed deposition patterns. Second, at the local (farm) scale, intensification of farming practices makes lands dedicated to intensive agriculture or livestock production qualitatively inhospitable or scarcely permeable (because of pesticide application and/or drastic modification of habitat conditions and food availability) for many frugivores compared to low-intensively managed farmlands, impeding seed arrival. Reduced levels of frugivory at these two scales are also expected as fruit-bearing plants become scarce in the landscape and in the areas dedicated to crop yield. Although some of these effects have been described in both temperate and tropical systems (e.g., Lozada et al., 2007; Pejchar et al., 2008; Martínez and García, 2017), they have not been considered together (in an integrative way), which would provide a comprehensive view of the impact of farming on the persistence of animal-mediated seed dispersal. Importantly, considering the operating mechanisms at these two scales together enables us to directly associate patterns of seed dispersal decay with specific human actions (expansion of croplands, destruction or substantial modification of habitat features, intensification of agricultural practices), which allows researchers to formulate appropriate corrective measures.

The impact of farming on seed dispersal by frugivores may be expected to differ with the type of farmland, depending on how suitable the agroecosystem is for provisioning appropriate habitat structure and food resources for frugivores (Lozada et al., 2007). Namely, more structural protection is expected in woody permanent croplands than in arable lands, and more food is expected in fruit-provisioning woody croplands compared to other woody croplands. This means that fruit-provisioning woody croplands are potentially permeable to different frugivorous animals and could ease the persistence of seed dispersal function. Some of these fruit-provisioning woody croplands can be managed according to biodiversity-friendly agroforestry/agroecology principles to preserve biodiversity (including frugivorous and insectivorous vertebrates) and ecosystem services without incurring necessarily in a loss in fruit yield. Examples exist, mainly in tropical agroforest systems such as shade coffee and cocoa plantations (Lozada et al., 2007; Clough et al., 2011; De Beenhouwer et al., 2013; Maas et al., 2013; Sánchez-Clavijo et al., 2020; Araújo-Santos et al., 2021), but there are also some studies supporting the application of similar principles in temperate apple orchards (García et al., 2018), semiarid nopal agroecosystems (Mellink et al., 2016), or Mediterranean olive groves (Rey, 2011).

The olive agroecosystem is currently the most important woody crop in Europe in socio-economic and cultural terms, as well as by the extension of its cultivated area (Loumou and Giourga, 2003; European Commission, 2012). Vast areas of the Mediterranean lowlands have been progressively occupied through centuries by olive groves, rendering highly variegated natural landscapes into an olive monoculture (Weissteiner et al., 2011). Olive cropland has expanded at the expense of Mediterranean native forest, woodlands, and scrublands (seminatural woodland habitats, SNWH hereafter), particularly rich in fleshy-fruited plant species (Herrera, 1984a; Jordano, 1984) and where avian seed dispersal by birds represents a major driver of woody vegetation recovery and the long-term dynamics of ecological communities (Rey and Alcántara, 2000, 2014; Perea et al., 2021). However, unlike some notable information on avian frugivore abundance and diversity (Rey, 1993, 1995, 2011; Morgado et al., 2021), data on frugivory and seed dispersal in olive grove landscapes is anecdotal, restricted to fruit consumption during winter by the most abundant frugivores, or taken from a single or a small number of localities (Jordano and Herrera, 1981; Rey, 1992; Blanco et al., 1994; Rey et al., 1996; Rey and Valera, 1999; Delibes et al., 2012; Perea and Gutiérrez-Galán, 2016).

Besides the progressive landscape transformation, olive cultivation practices have experienced a continuous process of local (farm) intensification that has happened in two different non-mutually exclusive ways (Infante-Amate et al., 2016). A long-lasting and widespread form of olive farming intensification is based on the elimination of ground herb covers. This is typically done by using pre and post-emergence herbicides or tillage, usually accompanied by the use of other pesticides (insecticides) and fertilizers, regardless of whether the olive field is irrigated or rainfed. Ground herb cover (composed of so-called weeds) is considered to compete with the olive tree for soil nutrients and water in this form of intensive farming. However, the persistent removal of the herb cover has raised many concerns in terms of sustainability and the environment as it leaves soils persistently bare and increases soil erosion (Gómez et al., 2014), and impacts ecosystem function and services. A second, more recent, form of intensification is based on increasing the density of the planted trees. This form of intensification has raised environmental concerns, especially from the recent advent of the superintensive hedge-like plantations, because it involves important structural changes and increased input demand that affects biodiversity and sustainability. Moreover, the accelerated expansion of hedge-type plantations demands new agricultural lands and the transformation of other (typically annual) croplands and/or traditional olive groves of old trees into this novel form of plantation (Moreira et al., 2019; Morgado et al., 2020, 2021). Olive tree density based intensification is unevenly distributed in the Mediterranean region, with the traditional density frames still representing the vast majority in major olive producer countries, such as Spain, Tunisia, Italy, Greece, and Morocco (Vilar and Pereira, 2018).

Despite the relevance of olive crops, in agronomic and ecological terms, and differently to tropical agroforest systems (Lozada et al., 2007; Pejchar et al., 2008; Araújo-Santos et al., 2021), we lack information on the role of olive groves on the persistence of seed dispersal services in Mediterranean agricultural landscapes. Namely, the impact of SNWH loss derived from olive expansion and intensification is virtually unknown. Because of its savanna-like tree structure and fruit provisioning cropland nature, it could be presumed that olive groves would be relatively permeable for avian frugivore activity and seed dispersal. Olive groves are known to play a crucial role in the lifecycle of many avian frugivores that winter in the circum-Mediterranean area but that have found increasingly shortened natural wintering quarters by habitat loss during in recent centuries (Rey, 1993, 1995). This is favored by the fact that cultivated olive derives from the human selection of one of the more frequent and rewarding fruits of the Mediterranean scrublands, the wild olive (Rey, 2011). Nonetheless, evidence also exists, indicating that the avian frugivore assemblage and diet in olive groves are notably simplified compared to wild olive forests and other Mediterranean scrublands (Rey, 1993; Rey et al., 1996; Rey and Valera, 1999).

This study evaluates these effects at the regional level, focussing on Andalusia (in the south of Spain), to what extent olive expansion and the loss of SNWH—that is, landscape modification towards olive-dominated landscapes- and the intensive agriculture—that systematically removes herbaceous ground cover with herbicides and/or tillage, leaving the soils uncovered–compromise the seed dispersal function supplied by frugivorous birds. This study does not evaluate olive farming intensification based on increased tree density (which considered traditional, intensive, and superintensive tree density frames) since in Andalusia (the largest region/area devoted to olive groves in the world) traditional tree density frames still represent 75% while superintensive hedge-like plantations only reach 2.5% of the land devoted to this crop (Vilar and Pereira, 2018). The removal of ground cover has raised strong environmental, sustainability and biodiversity concerns in Andalusia (Gómez and Giráldez, 2009; Moreno et al., 2009; Paredes et al., 2013; Sánchez-Moreno et al., 2015; Parras-Alcántara et al., 2016; Martínez-Núñez et al., 2020a,b; Gómez-Rosado et al., 2021; Tarifa et al., 2021; for some examples). Awareness of the environmental impact caused by bare soils in the olive cultivation sector is reflected also in European Common Agriculture Policy (CAP) (Díaz et al., 2021). More specifically, we evaluate how landscape and the farm scale, in terms of anthropogenic impact, have affected avian frugivore abundance and diversity, frugivory and seed deposition into the productive (olive field) and non-productive (seminatural woodland) habitats of these human-shaped landscapes. We intend to provide insights for plant regeneration and enhancement of connectivity in these landscapes that could be applied by farmers and land managers. To do this, this study used information from bird censuses and mist-netting (which provide data on avian abundance and seed occurrence in bird droppings) and seed fall traps (for seed deposition data) from 20 olive grove localities widely distributed in Andalusia (southern Spain). The study considered: (i) three levels of the gradient of SNWH cover at the landscape scale (low, intermediate, and high SNWH cover); (ii) two types of habitat patch within each olive farm (seminatural woodland and olive field); and (iii) two types of agricultural practices in paired olive farms per locality (intensive practices of persistent removal of ground herb cover vs. low-intensity management, which maintains the herbaceous cover most of the year). We expected there to be a decrease of frugivore abundance and diversity with SNWH cover loss by olive grove expansion (Rey, 1993) and higher abundance and diversity in seminatural woodland remnants, especially in olive-dominated landscapes (Rey and Valera, 1999). We further expect a higher degree of frugivory in seminatural woodland remnants than in the olive fields and in landscapes with higher SNWH cover than in olive-dominated landscapes with low SNWH cover (Rey and Valera, 1999). Concomitant with these predictions, we also predicted stronger seed deposition in the woodland remnants compared to isolated perching trees or the olive fields, especially in landscapes of high SNWH cover. Finally, because of the mobility of birds at local and landscape scales, we do not expect strong effects of agricultural practices on bird abundance, frugivory and seed deposition.

## MATERIALS AND METHODS

### Study System

Olive groves are a woody crop that occupies a large part of the Mediterranean region. With more than 1.5 million ha, Andalusia is the region with the highest area dedicated to this crop in the world. Olive growing has continued to expand in Andalusia in the last several decades, to the detriment of other crops and areas of natural vegetation. This has caused the simplification of the landscape. Over vast areas of the Guadalquivir Valley countryside, olive groves occupy the entire cultivable area and have become a monoculture, which implies a completely homogeneous human-shaped landscape. In areas where the relief is more undulating and parent rock emerges, the olive tree plantation is not viable and small patches of natural vegetation have survived clearing. This has leds to a more heterogeneous landscape in which the olive grove matrix predominates but is dotted by patches of semi-natural vegetation. Another source of heterogeneity in these landscapes are areas where the olive grove is interspersed with other crops, forming an agricultural mosaic. Finally, in the foothills and piedmont of the mountains of the region, large forest, woodland, and scrubland masses can occur along with other woody crops, and olive groves do not comprise the majority of land use. Therefore, a noticeable gradient of olive grove cover and semi-natural habitat cover can be envisaged across the whole olive growing area of the region.

Regarding crop management practices, the conventional and most widespread practice is the intensive and persistent removal of herbaceous cover using chemical methods (herbicides) (hereafter, "intensive management"). These types of practices are very aggressive for the soil, causing erosion and loss of tons of fertile soil every year (Castro et al., 2008; Gómez and Giráldez, 2009; Gómez et al., 2009; Gómez-Rosado et al., 2021). Conversely, other more environmentally respectful practices are notably increasing in the region, since the application of the most recent update to European Common Agricultural Policy (CAP), which entails the maintenance of spontaneous herbaceous cover, that is left to grow for most of the year (hereafter, "low-intensity management"). In an increasing number of cases, this maintenance of herbaceous cover is combined with organic farming, but the area dedicated to organic olive growing is still a minority in the region. Modern hedge-like plantations, although rapidly proliferating are still marginal in the region, and convey a new and different facet of the intensification of olive production that is not considered in the present study (see Morgado et al., 2021, for the effects of superintensive olive farming in avian frugivore abundance and richness). Olive trees in all the localities were more than 30-years old and were grown with a plantation frame of 7 × 8 m or frequently higher.

### Experimental Design

To integrate the landscape and local (farm) scale effects of agricultural intensification, this study included 40 olive farms, which were paired in 20 localities (see details in **Supplementary Table 1**). Each pair of farms was composed by an intensive and a low-intensity farm that were embedded within a same landscape. The mean distance between localities was 105 ± 63.1 km (average ± 1SD). Martin et al. (2019) have shown that both crop and non-crop habitats influence functional biodiversity and agroecosystem services in Europe. We considered these two types of habitat cover for the selection of our study localities, the characterization of their landscapes, and the differentiation of habitats within each olive farm. In particular, the study localities were chosen to cover a gradient of loss of seminatural woodland habitat (SNWH) cover and land use conversion to olive groves, across the main olive growing areas of Andalusia (south Spain). We considered three categorical levels of landscape modification based on information of SNWH cover, landscapes with low ($< 5\%$), intermediate (from 5 to 20%) and high ($> 20\%$) SNWH cover (localities ranging between $<1\%$ and ca. 70% of SNWH cover). In total, 8 localities fell within the Low, 8 in the Intermediate, and 4 in the High SNWH cover categories. Similar categorical approaches based on natural habitat loss (and relatively similar cutting points) have been used for ecological/agroecological conceptualization of the landscapes (e.g., McIntyre and Hobbs, 1999) or for characterizing the complexity of the agricultural landscapes (Tscharntke et al., 2005; Concepción et al., 2008). We further used the olive grove cover of each landscape as an estimator of olive expansion. We used recent land use cartography of the region, based on SIOSE data (http://www.siose.es) and a Geographic Information System software, QGIS v.2.14 (QGIS Development Team, 2018), to calculate the proportion of area occupied by forests, woodlands, scrublands (all integrating the SNWH), and olive groves in an area within a circle of 2 km radius, centered between the paired farms of each locality. We further estimated olive and SNWH covers within a 1 km radius circle around the center of each farm. A 1 km-scale is commonly used for analyses of the effects of landscape heterogeneity on birds. Available studies on seed dispersal kernels by frugivorous birds in the Iberian peninsula, including human-altered landscapes, report that most seeds are dispersed at distances notably shorter than 1 km and that seed dispersal events beyond 2 km are extremely rare (Jordano et al., 2007; Rey and Alcántara, 2014; González-Varo et al., 2017, for Mediterranean landscapes, see also Morales et al., 2013, for Cantabrian range).

Intensive and low-intensity management were defined based on the ground herb cover management, whether persistently eliminated during the whole year with herbicides or maintained for most of the year and eventually removed in late spring by mechanical mowing or livestock grazing (Rey et al., 2019; Martínez-Núñez et al., 2020a,b, for further information of characterization of these two categories of agricultural management). Finally, evaluations of the seed dispersal function (see below) were undertaken by distinguishing two types of habitats within each olive farm: the olive field, corresponding to the productive part of the farm; and semi-natural woodlands

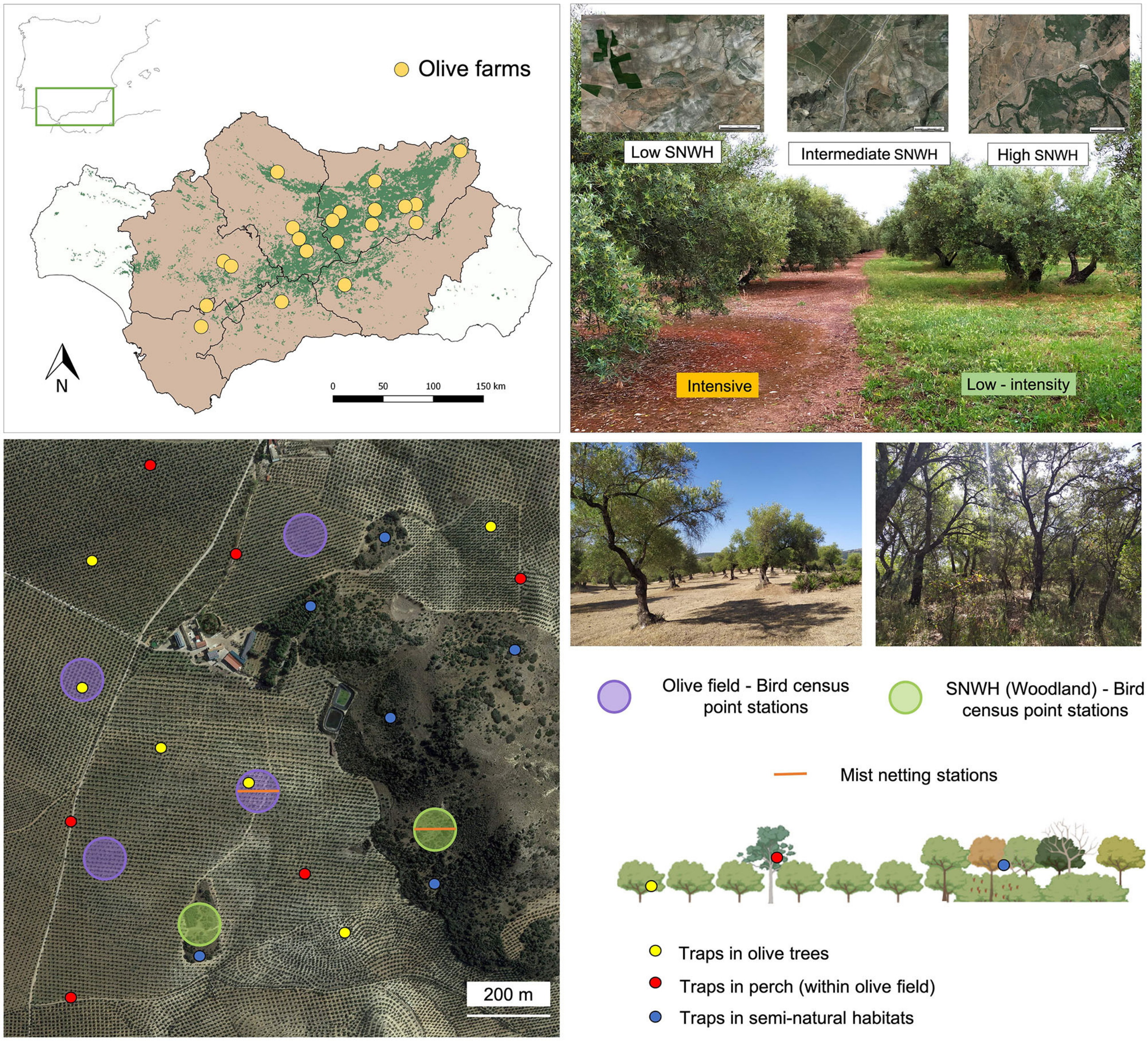

**FIGURE 1 |** Study sites and sampling schemes for avian frugivore assemblages, frugivory and seed deposition in olive grove landscapes of Andalusia (south of Spain). Upper left panel shows the study area in the south of Spain and the localities of study within Andalusia (indicated by yellow circles in the right panel, each locality with a pair of olive farms differing in agricultural management). Upper right panel illustrates the difference between intensive olive farms (note the bare ground by persistent removal of the herbaceous cover) and low-intensity management farms (with a developed ground herb cover for most of the year). Insets on the upper right panel show ortophotos of olive grove landscapes. From left to right these insets depict an increase in seminatural woodland habitat (SNWH) cover in the landscape (irregular green) and an increase in olive grove cover (although in ortophoto of the low SNWH cover some polygonal cereal croplands are appreciated). Lower panels identify sampling stations within an olive farm, distinguishing between stations of bird censuses (large open circles), seed fall traps stations (small solid circles), and bird mist-netting stations (lines). Colors of stations correspond to different habitats within the olive farm as indicated in the corresponding labeling.

patches, corresponding to the unproductive part of the farm (see **Figure 1** for the overall study design).

## Evaluation of the Dispersal Function

As described in the Introduction, agriculture may filter the dispersal function by: (1) filtering the bird species pool occurring in croplands; (2) modifying the foraging activity of the frugivorous birds (frugivory); and (3) modifying the spatial behavior (mobility) of these birds, and thus affecting where they deposit their droppings (seed deposition). We evaluated several parameters that characterize each of these components of the dispersal function.

### Avian Frugivore Abundance and Richness Estimate

From June 2019 to March 2020 (covering most periods of fruit availability in the Mediterranean region and the time in which birds majorly consume fruits), we conducted 5-min bird censuses at circular point census stations (with a 50 m radius), every month. We located 10 permanent census stations (6 in the olive fields and 4 in SNWH patches) in large olive farms (> 25 ha) and 6 stations (4 in olive fields and 2 in SNWH patches) in small farms (< 25 ha, typically less than 10 ha). All birds seen and heard within each station were recorded, although we only considered fruit-eating birds according to classifications outlined by Storchová and Horak (2018). Censuses were conducted within 4 h of sunrise.

We obtained for each census station an estimate of fruit-eating bird abundance and species richness and characterized the frugivore guild of the olive grove landscapes. Abundance and richness were estimated as the mean number of fruit-eating birds recorded across monthly surveys in each station.

## Frugivory

We intended to quantify the magnitude of frugivory of the pool of potential frugivores detected in censuses. From September 2019 to March 2020, we conducted bird mist-netting sessions in the study olive farms. Birds trapping was carried out for 3 h in each session (between mid-morning and noon). For each farm, two simultaneous capture zones, one in the olive field and the other in SNWH patches were considered, with a distance of 150 meters between zones. In each zone (habitat), we set two mist-nets of 12 × 2.5 meters and a mesh size of 16 mm (24 linear meters of the net in total per zone). In each mist-net zone, a sound call that emitted songs of the frugivorous species present was arranged at random to attract birds.

A 1-m wide strip of mosquito net was placed on the ground under the mist nets to collect the seeds excreted by the birds while they were trapped in the net (see details in **Supplementary Figure 1**). Once a bird was released from the net it was immediately introduced in a cloth bag with a paper cone located inside (where the excreted/regurgitated seeds were collected) until the moment of seed collection/identification. All birds were kept in the cloth bags for around 1 h and then ringed and released. The 1-h bird holding time intended to balance the time typically required for seed regurgitation/defecation of avian frugivores (temperate avian frugivores have seed retention times in their guts of less than 1 h, e.g., Herrera, 1984b; Sorensen, 1984; Fukui, 2003) while trying not to compromise the birds' daily energy requirements and food provisioning. For each individual captured, the total number of seeds of each species collected under the net and in the collector was recorded.

Bird trapping, handling, and banding were carried out by two expert ringers (FMC and RT) who had ringing licenses and authorization for the scientific banding of wild birds in Andalusia.

Logistic constraints hindered sampling in each olive farm of each locality every month. Therefore, we decided to conduct a monthly trapping session in one of the farms of each pair (all the localities thus having a bird trapping session per month). Consequently, the statistical analysis on frugivory will be based only on these 20 farms where birds were more actively trapped (see statistical analyses section). Among the farms sampled extensively for frugivory, there was a relatively even distribution between intensive and low-intensive farms and among SNWH categories. In the other member of each pair of farms, we set mist-nets just twice during the whole study period. Data from these last farms were used only for completing a general description of fruit species consumed by the frugivore assemblage in olive groves.

From this information, we reported three components/descriptors of the frugivory intensity: the proportion of the mist-netted frugivore species with seeds in their droppings, the abundance of seeds per dropping (including droppings without seeds) of fruit-eating birds, and the seed species richness per dropping. The first elucidates to what extent the potential frugivores can disperse seeds in olive grove landscapes, the second captures the magnitude of this activity, and the third provides information on the diversity of their fruit foraging activity.

Parallel to the sessions of bird mist-netting, we exhaustively surveyed the number of fruiting species around a 50 m radius circular plot. This allows us to gain an idea of the fruiting species richness in the neighborhood of the nets. Fruit species richness other than cultivated olive was frequently null around nets in the olive field while in the forest woodland patches decreased with the level of SNWH loss: average of 1.6 species (range: 0–7) in landscapes with low SNWH cover, 3.7 species (range 1–12) in intermediate landscapes, 6.8 species (range 4–8) in landscapes with high SNWH cover. Given the high avian mobility, 50 m-radius scales around nets may be too small to fully characterize fruit availability for avian frugivores in a locality. Consequently, we further assessed the fleshy fruit species detected in all 50 m radius census stations used for surveying frugivorous bird assemblages. We show the fruits available at each locality in **Supplementary Figure 2**. Again, the richness of the fruit species available at farm scale decreased with the level of SNWH loss in the landscape: average of 4.4 species (range: 0–16) in landscapes with low SNWH cover, 10.0 species (range 4–20) in intermediate landscapes, 12.0 species (range 9–16) in landscapes with high SNWH cover.

## Seed Deposition

To determine the probability of seed deposition into different habitats within the olive farm and to characterize seed rain, we used plastic plant pots of 40 cm diameter (0.1257 $m^2$ of collecting area) and 20 cm depth as seed fall traps. Traps were covered with a 1 × 1 cm wire mesh to avoid seed predation by micro mammals and perforated on their base to drain rainwater. To avoid seed drag by water drainage, we glued a mosquito mesh (1 cm above the trap button) that retained the seeds (e.g., *Ficus carica* seeds) to the trap wall. Eighteen seed fall traps were placed per farm in three different types of habitats (6 traps per habitat): beneath the olive tree canopy, beneath the canopy of isolated overtopping non-olive trees serving as perches to birds within the olive field, and within SNWH remnants. Traps were always set hanging from branches of trees and/or tall scrubs (**Supplementary Figure 1**).

The traps were active for 17 months, between October 2018 and March 2020 in 9 localities (3 in each of the landscape categories) out of the 20 study localities (18 olive farms). We collected the trapped seeds periodically (every 3 months, with monthly-bimonthly checks to make sure that the traps were active). All seeds collected were pooled at the trap level for these analyses.

From this data, we obtained for each trap three components of the seed deposition by birds: the event of seed arrival (1 vs. 0) as the trap collecting or not collecting seeds; the number of seeds collected, and the number of seed species collected.

## Statistical Analyses

For each response variable considered for frugivore assemblage (abundance and richness), frugivory, and seed deposition, we conducted Generalized Linear Mixed Models (GLMMs), considering habitat loss (i.e., level of SNWH cover: low, intermediate, and high SNWH), agricultural management (intensive vs. low-intensity), and habitat type (SNWH habitat vs. olive field) as fixed categorical effects. The olive grove cover at the 1-km circular radius was additionally considered as a continuous covariable since, although negatively correlated with SNWH ($r = -0.59$; $N = 40$ farms), such correlation was not strong enough to generate serious variance inflation factors in the models. Note that SNWH and olive cover, although related, describe different processes, habitat loss (not exclusively due to land conversion to olive grove), and olive expansion, respectively. We also considered all the possible interactions among these four fixed effects. The locality was considered a random blocking effect in all these analyses. In the case of bird abundance and richness per census station, we pooled data for all the study period (as a mean per census station). In the case of models of frugivory, the monthly trapping sessions conducted in the single station of each habitat within the farm were nested within a locality. We used Gaussian family distribution for a mean number of bird abundance (log-transformed) and richness per census station provided the non-negative continuous nature of the data averaged per point census station. Visual inspections of the residuals support that these response variables were distributed normally. In the case of frugivory, due to the frequency of zeros, we used negative binomial family models both for the number of seeds and number of seed species per dropping, and binomial models and logit link function for the proportion of frugivore species with seeds in their droppings. Finally, in the case of seed deposition, we used binomial family models and logit link function for probability of seed deposition, negative binomial for the total number of seeds collected, and Poisson distribution and log link function for the number of species collected per trap. All models were fitted using the R package *glmmTMB* (Brooks et al., 2017) and their $R^2_{GLMM(m)}$ values (the marginal $R^2$, that is, the variance explained by the fixed effects only) were obtained with the R package *MuMIn* (Barton, 2015). A model selection procedure based on AIC was used to choose the best model for each response variable among all models that were better than the null model (including only the random factor). When two or more models performed well but were indistinguishable (i.e., when differing in AIC $< 2$), we typically opted for the most parsimonious (attending to the behavior of the residuals of each model), or used the example that contained a biologically significant or marginally significant effect. The comparison of each competing model against the null model was conducted using the *dredge* function from *MuMIn* (Barton, 2015).

# RESULTS

## Variation in the Frugivore Abundance and Richness Across Olive Grove Landscapes and Agricultural Management Practices

Thirty five species of birds classified as frugivores occurred in the studied olive groves (**Table 1**) throughout the whole period of fruit availability considered (June 2019–March 2020). Among these species, some were resident, occurring in olive groves throughout all the year (Sardinian warbler, the European blackbird, or the European robin among the most common), while others occurred temporally, mainly as wintering (for example several thrushes, Blackcap or Black redstart), or during the post-reproductive period and migratory pass (several *Curruca* and *Sylvia* species, flycatchers, and the Common redstart), during summer and autumn. The wintering Blackcap and Song thrush and the resident Sardinian warbler, European blackbird, and European robin (the latter leaving the olive groves in the majority of localities during the reproductive season) were by far the most abundant and ubiquitous species. Overall, fruit-eating birds were more than two-fold more abundant in olive grove landscapes during the winter period compared to summer and autumn (**Table 1**), though the richness of frugivorous species was higher during summer-autumn (32 species in summer-autumn vs. 24 in winter).

*The abundance of fruit-eating birds* varied notably among olive groves (ranging from 6.6 individuals/10 ha to 24.1 individuals/10 ha). Our model selection procedure, considering effects of agricultural management (M), SNWH and olive grove cover (OGC) at the landscape level, and habitat within the olive farms (H), showed that only a single model was better than the null model (i.e., the model considering only the random factor). The selected model (**Table 2**) showed significant effects of H on avian frugivore abundance, being consistently higher in SNWH patches than in the olive fields (**Figure 2A**). Furthermore, we found an interdependent effect of H x OGC, meaning that frugivores increased their abundance in seminatural woodland patches but remained invariant in the olive field as olive grove cover increased in the landscape (**Figure 2A**).

In the case of *frugivore species richness*, two models were significantly better than the null model (**Supplementary Table 2A**). Both models showed that frugivore species richness was affected by H and by SNWH cover, but these effects were fundamentally interdependent. While frugivore richness was higher in seminatural woodland patches in all landscapes, it was invariantly low in the olive field (**Figure 2C**), that is, more SNWH cover did not increase frugivore richness in the olive field. Interestingly, this interaction effect also shows that frugivore richness in remnant SNWH patches was lower in landscapes with low SNWH cover compared to landscapes of intermediate or high SNWH. In addition to these effects, we also found a marginally significant effect of M (**Table 2A**). In particular, species richness was higher in low-intensive farms than in intensive ones (**Figure 2B**). In any case, this latter effect should be considered with caution since the alternative more parsimonious model, not including M, was not significantly worse (although it had higher AIC, $\Delta$AIC $< 2$).

**TABLE 1 |** List of frugivorous birds recorded in bird censuses in olive farms and their respective abundances during the period of fruit availability (June 2019 to March 2020).

| Species | Bird/10 ha | Bird/10 ha (low SNWH) | Bird/10 ha (intermediate) | Bird/10 ha (high SNWH) | Bird/10 ha (summer) | Bird/10 ha (winter) | Occurrence in olive farms |
|---|---|---|---|---|---|---|---|
| *Columba livia* | 0.35 | 0.21 | 0.65 | 0.00 | 0.12 | 0.48 | 3/40 |
| *Columba palumbus* | 1.01 | 0.72 | 1.62 | 0.39 | 1.00 | 1.02 | 22/40 |
| *Corvus corax* | 0.06 | 0.14 | 0.01 | 0.00 | 0.07 | 0.05 | 3/40 |
| *Corvus monedula* | 0.15 | 0.37 | 0.00 | 0.00 | 0.35 | 0.02 | 3/40 |
| *Curruca cantillans* | 0.05 | 0.02 | 0.07 | 0.06 | 0.12 | 0.00 | 8/40 |
| *Curruca communis* | 0.06 | 0.07 | 0.07 | 0.02 | 0.16 | 0.00 | 9/40 |
| *Curruca hortensis* | 0.02 | 0.04 | 0.02 | 0.00 | 0.06 | 0.00 | 3/40 |
| *Curruca melanocephala* | 12.79 | 9.91 | 15.72 | 12.70 | 14.20 | 11.95 | 40/40 |
| *Curruca undata* | 0.05 | 0.03 | 0.07 | 0.14 | 0.03 | 0.06 | 7/40 |
| *Cyanopica cyanus* | 0.44 | 0.53 | 0.57 | 0.00 | 0.45 | 0.43 | 6/40 |
| *Erithacus rubecula* | 5.61 | 3.72 | 6.00 | 8.60 | 2.39 | 7.54 | 40/40 |
| *Ficedula hypoleuca* | 0.09 | 0.18 | 0.03 | 0.02 | 0.23 | 0.00 | 9/40 |
| *Hippolais polyglotta* | 0.14 | 0.10 | 0.21 | 0.04 | 0.36 | 0.00 | 12/40 |
| *Iduna pallida* | 0.01 | 0.02 | 0.02 | 0.00 | 0.04 | 0.00 | 2/40 |
| *Luscinia megarhynchos* | 0.15 | 0.06 | 0.26 | 0.10 | 0.31 | 0.05 | 14/40 |
| *Muscipaca striata* | 0.18 | 0.10 | 0.23 | 0.23 | 0.47 | 0.00 | 14/40 |
| *Myiopsitta monachus* | 0.03 | 0.08 | 0.00 | 0.00 | 0.04 | 0.03 | 1/40 |
| *Oenanthe leucura* | 0.01 | 0.00 | 0.02 | 0.00 | 0.00 | 0.01 | 1/40 |
| *Oriolus oriolus* | 0.03 | 0.00 | 0.02 | 0.12 | 0.08 | 0.00 | 4/40 |
| *Phoenicurus ochruros* | 1.32 | 1.34 | 1.49 | 0.94 | 0.18 | 2.00 | 37/40 |
| *Phoenicurus phoenicurus* | 0.22 | 0.28 | 0.18 | 0.17 | 0.59 | 0.00 | 20/40 |
| *Pica pica* | 0.20 | 0.46 | 0.04 | 0.00 | 0.29 | 0.15 | 3/40 |
| *Psittacula krameri* | 0.03 | 0.07 | 0.00 | 0.00 | 0.04 | 0.02 | 1/40 |
| *Pyrrhocorax pyrrhocorax* | 0.02 | 0.00 | 0.00 | 0.10 | 0.05 | 0.00 | 1/40 |
| *Saxicola torquatus* | 0.22 | 0.19 | 0.20 | 0.31 | 0.24 | 0.21 | 15/40 |
| *Streptopelia decaocto* | 0.37 | 0.68 | 0.21 | 0.08 | 0.58 | 0.25 | 14/40 |
| *Streptopelia turtur* | 0.19 | 0.05 | 0.35 | 0.12 | 0.50 | 0.00 | 20/40 |
| *Sturnus unicolor* | 1.74 | 1.58 | 1.83 | 1.88 | 1.24 | 2.04 | 20/40 |
| *Sturnus vulgaris* | 0.06 | 0.00 | 0.15 | 0.00 | 0.00 | 0.10 | 1/40 |
| *Sylvia atricapilla* | 18.58 | 19.87 | 18.60 | 15.93 | 4.08 | 27.27 | 40/40 |
| *Sylvia borin* | 0.01 | 0.00 | 0.01 | 0.03 | 0.03 | 0.00 | 2/40 |
| *Turdus iliacus* | 0.03 | 0.05 | 0.01 | 0.05 | 0.00 | 0.06 | 5/40 |
| *Turdus merula* | 3.58 | 1.65 | 5.27 | 4.05 | 3.70 | 3.51 | 39/40 |
| *Turdus philomelos* | 12.34 | 10.90 | 12.54 | 14.79 | 2.13 | 18.46 | 40/40 |
| *Turdus viscivorus* | 0.26 | 0.03 | 0.26 | 0.70 | 0.22 | 0.28 | 11/40 |
| Total density | 60.38 | 53.45 | 66.73 | 61.57 | 34.36 | 75.99 | |

*Density of individuals per 10 ha is reported from 50 m radius bird census points. Data are averaged across point census stations and the 40 olive farms for the whole study period. Densities in each level of seminatural woodland habitat (SNWH) cover separately, and for summer-autumn (June to October) and winter periods (November to March) are also shown. The last column reports the number of farms in which each species occurs regarding the 40 studied olive farms.*

## Variation in Frugivory

Thirteen out of 17 mist-netted frugivorous bird species defecated/regurgitated seeds of fleshy fruit species in the whole set of olive groves studied (**Table 3**). They defecated/regurgitated 2,063 seeds of 27 different fleshy-fruited plant species (**Table 4**). Blackcap (*Sylvia atricapilla*) was by far the most important seed disperser, disseminating seeds of 26 out of the 27 species recorded (except *Juniperus oxycedrus*) in 31 olive groves and accounting for 90.4 % of total collected seeds in droppings. The other two major seed dispersers were the resident Sardinian warbler (*Curruca melanocephala*, dispersing seeds of 7 fleshy-fruited species in 18 olive groves), and the migrant Garden warbler (*Sylvia borin*, dispersing seeds of 4 species in 4 olive groves). Wild fruits accounted for a notable amount (62% in total) of seeds in bird droppings while cultivated olives represented 31% and other cultivated or naturalized species 7%. The plant species whose seeds were more frequent in bird droppings in olive groves were the cultivated olive (*Olea europaea* var.

**TABLE 2 |** Selected best model parameters after a selection procedure based in AIC for all the descriptors for avian frugivore assemblage abundance and richness (A), Degree of avian frugivory (B), and seed deposition (C) used as response variables in this study.

| | **(A) Frugivore assemblage** | | | | **(B) Frugivory** | | | | | | **(C) Seed deposition** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Frugivore abundance** | | **Frugivore richness** | | **% bird species with seeds in droppings** | | **Seed abundance in bird droppings** | | **Seed richness in bird droppings** | | **Probability of seed deposition** | | **Seed abundance** | | **Seed richness** | |
| **Model predictors** | **Estimate ± SE** | ***z*** | **Estimate ± SE** | ***z*** | **Estimate ± SE** | ***z*** | **Estimate ± SE** | ***z*** | **Estimate ± SE** | ***z*** | **Estimate ± SE** | ***z*** | **Estimate ± SE** | ***z*** | **Estimate ± SE** | ***z*** |
| (Intercept) | **0.713 ± 0.069** | **10.4***** | **3.32 ± 0.13** | **25.4***** | **–1.91 ± 0.61** | **–3.1**** | **–1.93 ± 0.69** | **–2.8**** | **–3.63 ± 0.57** | **–6.3***** | 0.64 ± 0.40 | 1.6 ns | **2.20 ± 0.33** | **6.6***** | –0.16 ± 0.2 | –0.66 ns |
| **Olive farm habitats (H)** | | | | | | | | | | | | | | | | |
| Olive field | **–0.128 ± 0.061** | **–2.1*** | **–1.7 ± 0.11** | **–15.8***** | | | **–0.83 ± 0.21** | **–4.1***** | **–0.65 ± 0.19** | **–3.4***** | | | | | | |
| **Seed traps habitat (H)** | | | | | | | | | | | | | | | | |
| Perch | | | | | | | | | | | –0.5 ± 0.3 | –1.6 ns | **–0.38 ± 0.18** | **–2.1*** | –0.20 ± 0.2 | –1.00 ns |
| Olive tree | | | | | | | | | | | **–1.86 ± 0.3** | **–5.8***** | **–1.50 ± 0.25** | **–6.1***** | **–1.7 ± 0.34** | **–4.96***** |
| **Ground cover management (M)** | | | | | | | | | | | | | | | | |
| Low-intensity | | | 0.1 ± 0.07 | 1.7· | **1.68 ± 0.49** | **3.4***** | **2.30 ± 0.54** | **4.3***** | **2.21 ± 0.60** | **3.7***** | 0.43 ± 0.25 | 1.7 · | | | **0.45 ± 0.20** | **2.59**** |
| **Landscape effects** | | | | | | | | | | | | | | | | |
| SNWH (Low) | –0.030 ± 0.035 | –0.9 ns | **–1.1 ± 0.18** | **–6.0***** | | | | | | | –0.4 ± 0.48 | –0.8 ns | | | –0.37 ± 0.3 | –1.21 ns |
| SNWH (High) | 0.013 ± 0.046 | 0.3 ns | –0.35 ± 0.2 | –1.6 ns | | | | | | | 0.69 ± 0.48 | 1.4 ns | | | **0.56 ± 0.28** | **2.00*** |
| OGC | **0.003 ± 0.001** | **3.1**** | | | **–0.01 ± 0.01** | **–1.9*** | –0.01 ± 0.01 | –1.7· | | | | | **–0.01 ± 0.01** | **–2.7**** | | |
| **Interaction effects** | | | | | | | | | | | | | | | | |
| H (Olive field) x OGC | **–0.002 ± 0.001** | **–3.0**** | | | | | | | | | | | | | | |
| H (Olive field) x SNWH (Low) | | | **1.15 ± 0.16** | **7.0***** | | | | | | | | | | | | |
| H (Olive field) x SNWH (High) | | | **0.36 ± 0.19** | **1.9*** | | | | | | | | | | | | |
| H (Perch) x M (Low-intensity) | | | | | | | | | | | | | | | –0.54 ± 0.3 | –1.95· |
| H (Olive tree) x M (Low-intensity) | | | | | | | | | | | | | | | 0.34 ± 0.42 | 0.82 ns |

*Adjusted p-values are shown (ns: non-significant; · $p < 0.1$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$). Effects of habitat within the olive farm (H), seminatural woodland cover, and olive grove cover at the landscape scale (SNWH and OGC), and ground herb cover management at the farm scale (M) are shown. All the effects were tested but only the coefficients for effects eventually incorporated in each selected best model during the model simplification procedure are shown. See* ***Supplementary Tables 2–4*** *for the other alternative competing models. Bold values identify significant effects at the $P < 0.05$ level of significance (or better).*

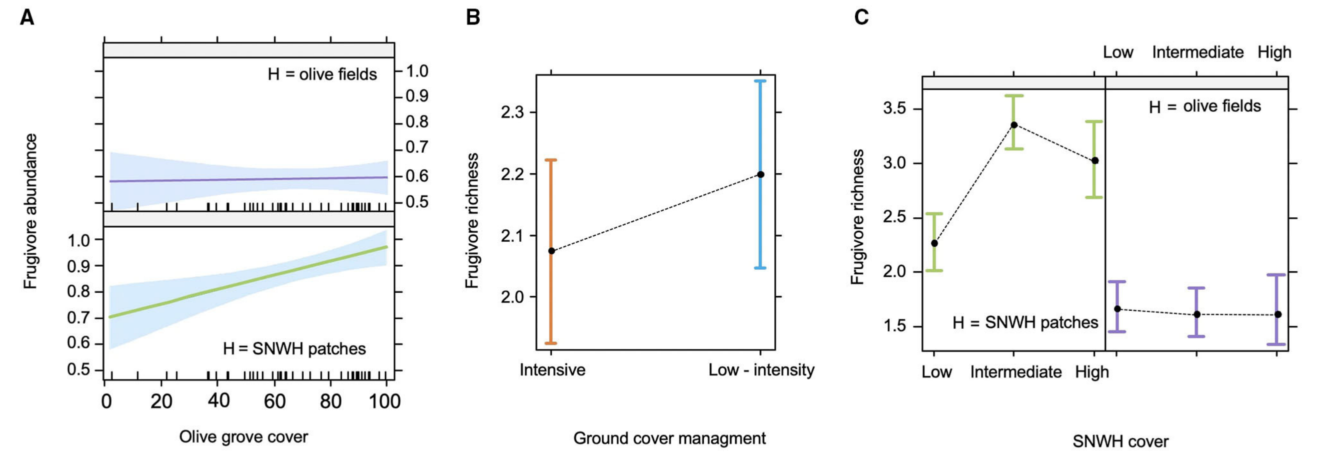

**FIGURE 2 |** Significant effects in the selected models for avian frugivore abundance **(A)** and species richness **(B,C)**. Note that in **(A)** the Y axis is in log10 scale, and that frugivore abundance ranges from $10^{(0.5+1)} = 30$ individuals, to $10^{(1+1)} = 100$ individuals/10 ha. Data represented are predicted least-squares means and 95% confidence intervals. Estimates of fixed effects are shown at the reference mean value for all the covariables. H refers to habitat (olive field and SNWH patches) where censuses and other samplings were conducted. SNWH refers to seminatural woodland habitat and SNWH cover refers to categories (low, intermediate, and high) of seminatural woodland cover in the landscape. OGC means olive grove cover at the landscape level and M is the local agricultural management (intensive and low-intensity ground herb cover management).

**TABLE 3 |** Frugivorous bird species dispersing seeds in olive groves and number of seeds occurring in their droppings/regurgitations.

| Species | N° of olive farms in which this species has dispersed seeds | Total number of dispersed seeds | Plant species dispersed | Three seed species more dispersed by each bird (number of dispersed seeds) |
|---|---|---|---|---|
| *Curruca cantillans* | 1 | 15 | 2 | *Rhamnus lycioides* (14)–*Rubus ulmifolius* (1) |
| *Curruca communis* | 3 | 14 | 4 | *Solanum* spp. (4)–*Olea europaea var. europaea* (4)–*Ficus carica* (4) |
| *Curruca melanocephala* | 18 | 85 | 7 | *Pistacia lentiscus* (40)–*Olea europaea* var. *europaea* (15)–*Rhamnus lycioides* (13) |
| *Curruca undata* | 1 | 1 | 1 | *Olea europaea* var. *europaea* (1) |
| *Cyanopica cyanus* | 1 | 4 | 1 | *Asparagus* spp. (4) |
| *Erithacus rubecula* | 1 | 1 | 1 | *Pistacia lentiscus* (1) |
| *Ficedula hypoleuca* | 1 | 2 | 1 | *Pistacia lentiscus* (2) |
| *Phoenicurus phoenicurus* | 1 | 1 | 1 | *Pistacia lentiscus* (1) |
| *Sylvia atricapilla* | 31 | 1,865 | 26 | *Olea europaea* var. *europaea* (615)–*Pistacia lentiscus* (359)–*Solanum* spp. (285) |
| *Sylvia borin* | 4 | 38 | 4 | *Ficus carica* (19)–*Rhamnus lycioides* (16)–*Olea europaea* var. *europaea* (2) |
| *Turdus merula* | 2 | 19 | 2 | *Ficus carica* (16)–*Rubus ulmifolius* (3) |
| *Turdus philomelos* | 3 | 11 | 2 | *Pistacia lentiscus* (9)–*Olea europaea* var. *europaea (2)* |
| *Turdus viscivorus* | 1 | 6 | 1 | *Juniperus oxycedrus* (6) |

*The number of olive groves where each frugivorous bird dispersed seed, the number of dispersed species by each frugivore, and the three seed species more frequently dispersed by each frugivore are also shown.*

*europaea*), *Pistacia lentiscus*, *Solanum* spp., *Rhamnus lycioides*, and the cultivated/naturalized *Ficus carica* (> 100 seeds in birds droppings for each of them) and *Phillyrea angustifolia*. The seed species occurring in bird droppings in more different localities (i.e., more ubiquitous in the orchards under study) were the cultivated olive (found in droppings in 29 olive groves), *Asparagus* spp. (15 olive groves), *F. carica* (15), *Pistacia lentiscus* (14), *Solanum* spp. (13) and *Rubus ulmifolius* (10). Sixteen fleshy fruit species appeared only in droppings of Blackcap, while one species (*Juniperus oxycedrus*) occurred only in droppings of Mistle Thrush (*Turdus viscivorus*). Apart from the cultivated olive (dispersed by 7 bird species) and *F. carica* (dispersed by 5 species), the fleshy-fruit species attracting a higher number of frugivorous birds were *P. lentiscus* and *R. lycioides* (occurring in droppings of 6 and 4 bird species, respectively).

Our models showed that frugivory varies fundamentally among habitats within olive farms and management regimes with some influence also of the olive grove cover, while the level of SNWH cover *per se* did not improve these models. Thus, in the case of the *proportion of frugivore species dispersing seeds* (i.e., with seed in droppings) no effect was detected (i.e., no model was better than the null model) when seeds of cultivated olives

**TABLE 4 |** Fleshy fruit species dispersed by birds in olive groves and number of dispersed seeds (i.e., collected in droppings) of each plant species.

| Fleshy-fruited plant species dispersed | N° of olive groves in which the species is dispersed | Total number of dispersed seeds | N° of birds species dispersing each plant species | The three bird species dispersing more seeds for each species (n° of dispersed seeds) |
|---|---|---|---|---|
| *Arbutus unedo* | 2 | 2 | 1 | *Sylvia atricapilla* (2) |
| *Asparagus* spp. | 15 | 45 | 3 | *Sylvia atricapilla* (36)–*Curruca melanocephala* (5)–*Cyanopica cyanus* (4) |
| *Crataegus monogyna* | 1 | 1 | 1 | *Sylvia atricapilla* (1) |
| *Cydonia oblonga* | 1 | 1 | 1 | *Sylvia atricapilla* (1) |
| *Daphne gnidium* | 3 | 6 | 1 | *Sylvia atricapilla* (6) |
| *Dioscorea communis* | 1 | 6 | 1 | *Sylvia atricapilla* (6) |
| *Ficus carica* | 15 | 119 | 5 | *Sylvia atricapilla* (77)–*Sylvia borin* (19)–*Turdus merula* (16) |
| *Jasminum fruticans* | 1 | 5 | 1 | *Sylvia atricapilla* (5) |
| *Juniperus oxycedrus* | 1 | 6 | 1 | *Turdus viscivorus* (6) |
| *Lantana camara* | 1 | 6 | 1 | *Sylvia atricapilla* (6) |
| *Myrtus communis* | 1 | 16 | 1 | *Sylvia atricapilla* (16) |
| *Olea europaea* var. *europaea* | 29 | 640 | 7 | *Sylvia atricapilla* (615)–*Curruca melanocephala* (15)–*Curruca communis* (4) |
| *Olea europaea* var. *sylvestris* | 9 | 26 | 1 | *Sylvia atricapilla* (26) |
| *Osyris alba* | 2 | 3 | 1 | *Sylvia atricapilla* (3) |
| *Phillyrea angustifolia* | 4 | 82 | 2 | *Sylvia atricapilla* (78)–*Curruca melanocephala* (4) |
| *Phillyrea latifolia* | 2 | 10 | 1 | *Sylvia atricapilla* (10) |
| *Pistacia lentiscus* | 14 | 412 | 6 | *Sylvia atricapilla* (359)–*Curruca melanocephala* (40)–*Turdus philomelos* (9) |
| *Pistacia terebinthus* | 1 | 2 | 1 | *Sylvia atricapilla* (2) |
| *Punica granatum* | 2 | 21 | 3 | *Sylvia atricapilla* (16)–*Curruca melanocephala* (3)–*Curruca communis* (2) |
| *Retama sphaerocarpa* | 1 | 1 | 1 | *Sylvia atricapilla* (1) |
| *Rhamnus alaternus* | 1 | 4 | 2 | *Sylvia atricapilla* (2)–*Curruca melanocephala* (2) |
| *Rhamnus lycioides* | 4 | 236 | 4 | *Sylvia atricapilla* (193)–*Sylvia borin* (16)–*Curruca cantillans* (14) |
| *Rubus ulmifolius* | 10 | 43 | 3 | *Sylvia atricapilla* (39)–*Turdus merula* (3)–*Curruca cantillans* (1) |
| *Schinus molle* | 1 | 5 | 1 | *Sylvia atricapilla* (5) |
| *Smilax aspera* | 2 | 40 | 1 | *Sylvia atricapilla* (40) |
| *Solanum* spp. | 13 | 320 | 3 | *Sylvia atricapilla* (315)–*Curruca communis* (4)–*Sylvia borin* (1) |
| *Vitis vinifera* | 1 | 5 | 1 | *Sylvia atricapilla* (5) |

*The number of olive groves where each plant species was dispersed, the number of frugivorous birds dispersing each seed species, and the three major avian dispersers for each plant are shown. Seeds occurring only in farms not considered in statistical analyses (see methods) are marked in blue.*

were considered (results not shown). Nonetheless, the exclusion of the seeds of cultivated olives from the analyses renders multiple models better than the null model. **Supplementary Table 3** shows the five models with the lowest AIC. The selected model (PFS3) incorporated significant effects of M (with low-intensity farms with ca. 4 times more proportion of frugivorous bird species dispersing non-cultivated olives, **Figure 3A**) and OGC in the landscape (**Table 2B**), with the frequency of species dispersing seeds decreasing with the olive cover (**Figure 3B**). This model was substantially similar to model PFS1 (with lower AIC, **Supplementary Table 3**) but was preferred because the latter incorporated the non-statistically significant effect of H.

In the case of the *abundance of seeds per frugivore dropping*, all five models that were better than the null model incorporated H and M as significant predictors, and four of them also included OGC. The best model (SAD2; see **Supplementary Table 3**) showed that the abundance of seeds in droppings was significantly higher in the seminatural patches than in the olive fields (a more than two-fold increase, **Figure 3C**), increased substantially in low-intensity management compared to intensive farms (**Figure 3D**) and decreased with OGC (**Figure 3E**), though this last trend was marginally significant (**Table 2B**). Likewise, all significant models for variation in *seed species richness in the bird droppings* incorporated H and M (some of them also OGC) (**Table 2B**), and the selected best model (SRD1) determined a significant increase in the diversity of seeds in droppings in seminatural patches compared olive fields (**Figure 3F**) and in farms under low-intensity management compared intensive farming (**Figure 3G**).

## Variation in Seed Deposition

In total, we collected 1,160 seeds of 34 fleshy fruit species in the 18 olive groves where traps were set up (see **Table 5**). The rate of seed deposition steadily increased over time: in 14 months (between 2018 and 2019) we collected 523 seeds in 186 (40.2%) out of 462 active seed fall traps (mean number of seeds collected

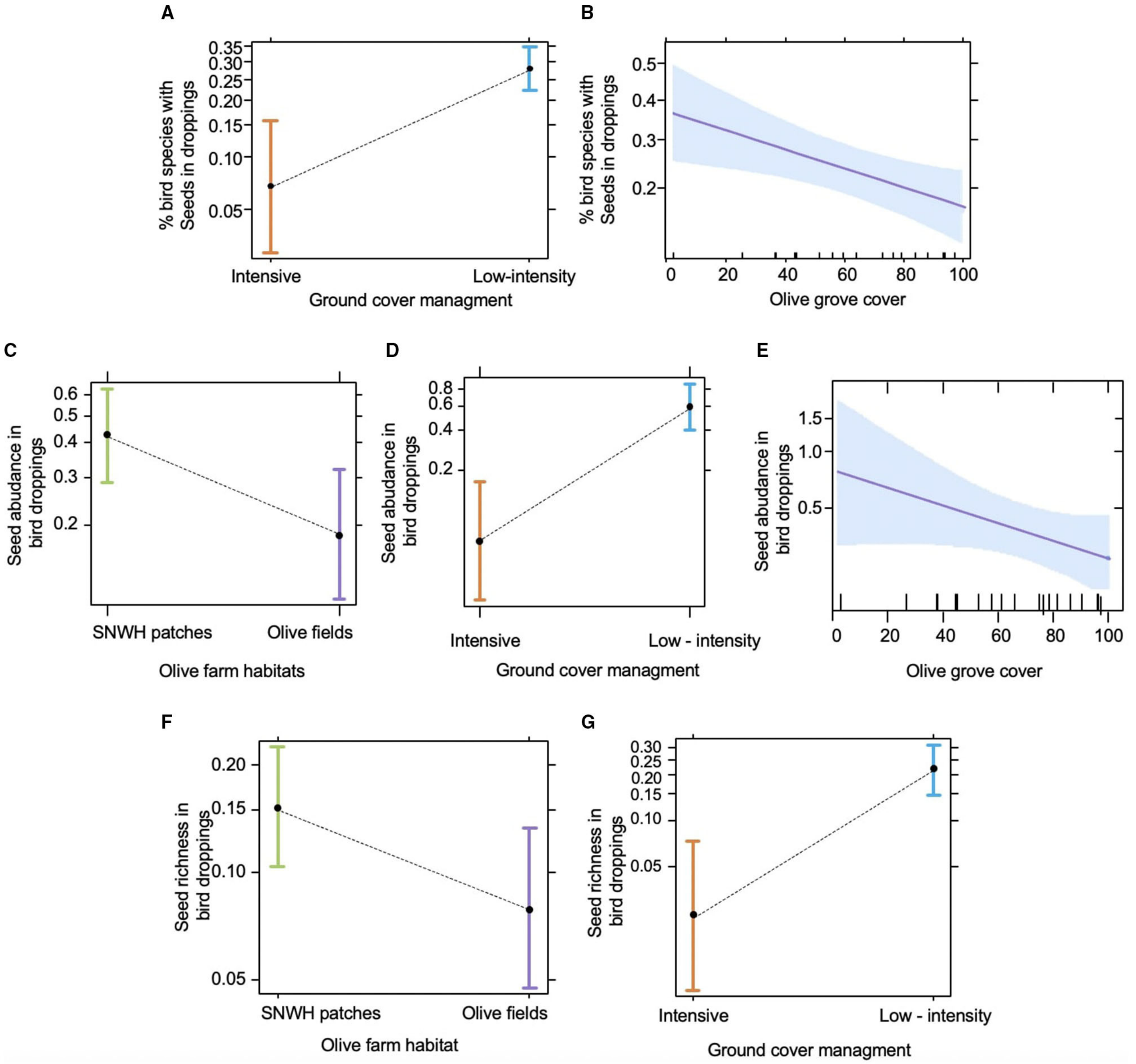

**FIGURE 3 |** Significant effects in the selected models for frugivory using as descriptors proportion of trapped frugivorous species with seeds in droppings (seed disperser probability in **A,B**), the abundance of seeds per dropping **(C–E)**, and seed species richness in droppings (species richness per dropping in **F,G**). Data represented are predicted least-squares means and 95% confidence intervals. Estimates of fixed effects are shown at the reference mean value for all the covariables. See **Figure 2** caption for code description.

per trap capturing seeds = 2.82 seeds), while in 3 months (winter) in 2020 we collected 637 seeds in 205 (47.4%) out of 432 active seed fall traps (mean number of seeds collected per trap capturing seeds = 3.11 seeds). Thirty traps were lost during the period of study. Referred to the total active traps in each period these figures represent 9.0 and 11.7 seeds/m$^2$ each period. We were unable to classify 9 out of the 34 species collected (**Table 5**). Unclassified seeds accounted for less than 1% of the collected seeds. Sixteen species were only collected in a single olive grove.

The most ubiquitous dispersed seeds were cultivated olive (collected in 17 out of 18 olive groves) and *F. carica* (13), and among the native species, *Asparagus* spp. and *Pistacia lentiscus* (both collected in 12 olive groves), *Rhamnus alaternus/lycioides* (11) and *Olea europaea* var. *sylvestris* (wild olives; 10). The cultivated and wild olives, *P. lentiscus*, *Asparagus* spp., *Rhamnus alaternus/lycioides* and *F. carica* seeds fell at least in 30 seed traps. In contrast, *R. ulmifolius*, although abundantly collected (85 seeds), was registered only in 2 olive groves and 5 traps. Notably, wild plant species accounted for 60% of the seed rain

**TABLE 5** | Collected seeds in the seed fall traps of 18 olive farms of 9 localities of study.

| Fleshy fruit species | Total number of seeds collected | Number of olive groves in which is collected | Number of traps collecting seeds |
|---|---|---|---|
| *Arbutus unedo* | 4 | 1 | 1 |
| *Arum italicum* | 2 | 1 | 1 |
| *Asparagus* spp. | 107 | 12 | 49 |
| *Capparis spinosa* | 26 | 1 | 2 |
| *Crataegus monogyna* | 18 | 5 | 12 |
| *Daphne gnidium* | 4 | 2 | 3 |
| *Dioscorea communis* | 2 | 2 | 2 |
| *Ficus carica* | 238 | 13 | 30 |
| *Jasminum fruticans* | 1 | 1 | 1 |
| *Juniperus oxycedrus* | 12 | 4 | 5 |
| *Myrtus communis* | 16 | 3 | 4 |
| *Ligustrum spp.* | 9 | 4 | 5 |
| *Olea europaea var. europaea* | 179 | 17 | 99 |
| *Olea europaea* var. *sylvestris* | 66 | 10 | 38 |
| *Opuntia* spp. | 43 | 3 | 6 |
| *Osyris alba* | 2 | 1 | 2 |
| *Phillyrea angustifolia* | 3 | 2 | 3 |
| *Pistacia lentiscus* | 86 | 12 | 54 |
| *Pistacia terebinthus* | 11 | 2 | 2 |
| *Rhamnus alaternus/lycioides* | 226 | 11 | 47 |
| *Rubus ulmifolius* | 85 | 2 | 5 |
| *Smilax aspera* | 1 | 1 | 1 |
| *Solanum* spp. | 6 | 2 | 2 |
| *Vitis vinifera* | 2 | 2 | 2 |
| *Viscum cruciatum* | 1 | 1 | 1 |
| *Unknown spp. 1* | 1 | 1 | 1 |
| *Unknown spp. 2* | 1 | 1 | 1 |
| *Unknown spp. 3* | 2 | 1 | 1 |
| *Unknown spp. 4* | 1 | 1 | 1 |
| *Unknown spp. 5* | 1 | 1 | 1 |
| *Unknown spp. 6* | 1 | 1 | 1 |
| *Unknown spp. 7* | 1 | 1 | 1 |
| *Unknown spp. 8* | 1 | 1 | 1 |
| *Unknown spp. 9* | 1 | 1 | 1 |

*Total number of seeds collected, number of olive groves, and seed fall traps where each seed species was collected are shown. Unknown classifications are also reported.*

while cultivated olives accounted only for 15% of the deposited seeds and other naturalized or cultivated species (*Ficus*, *Opuntia*, *Vitis*) represented the remaining 25%. These figures were, overall, congruent with data on bird droppings.

A set of models was significantly better than the null model for capturing the variation in each descriptor of seed deposition (**Supplementary Table 4**). Thus, in the case of the *probability of seed deposition* into seed fall traps, the models incorporated different combinations of H, M, and SNWH cover (**Supplementary Table 4**). The model with lower AIC (PSA1) showed significantly higher seed arrival to natural/seminatural patches and perches than the olive field (**Figure 4A**) and marginally significant differences between local management (**Table 2C**), with higher seed arrival in low-intensity farms (**Figure 4B**). This model also incorporated the effect of SNWH cover (**Table 2C**), with an increased gradient of seed deposition as SNWH cover increases in the landscape; although the corresponding estimated values were not statistically significant (**Figure 4C**). The effects of M and SNWH cover should be taken with caution since this model was not better than the more parsimonious PSA5, which only incorporated the effect of H but had higher AIC. Regarding the *abundance of seeds collected by each trap*, better models than the null model included combinations of H, M, SNWH cover, and OGC (see models SAT1 to SAT5 in **Supplementary Table 3**). The selected best model (SAT4) showed a significant increase in the number of seeds collected by traps in the SNWH patches and isolated perch trees compared to under the canopy of olive trees within the olive field (**Figure 4D**). The number of seeds per trap also significantly decreased as olive cover in the landscape increased (**Figure 4E**). Finally, for the *number of seed species collected per trap*, we achieved 3 models better than the null model.

The two models with less AIC (SRT1 and SRT2) were substantially equivalent. Thus, they showed both simple and interactive effects of H and M (**Table 2C**), with more diversity of seeds deposited into SNWH patches and isolated perch trees within the olive fields than under the canopy of olive trees, with low-intensive farms receiving more seed species than intensive farms only in the olive field (**Figure 4G**). The significant effect of SNWH cover (significant in the case of the best model based on AIC) and OGC (in the second model) in these two models were interchangeable, with this equivalence showing that SNWH loss and the associated expansion of olive groves reduced the diversity of seeds in the seed rain (**Figure 4F** for SNWH cover effect). The coefficient for OGC was negative in the alternative model (not shown).

## DISCUSSION

Avian-mediated seed dispersal is a fundamental function in the dynamics of Mediterranean woodland ecosystems (Herrera, 1995). In the Mediterranean lowlands, woodland ecosystems have been largely replaced by olive, vineyard, and cereal croplands throughout a millenary history of cultivation in the region. However, comprehensive studies of the effect of agriculture on seed dispersal by frugivorous birds in the Mediterranean are lacking. The present study shows the extent to which the landscape transformation from woodland habitats to olive grove landscapes (the large scale anthropogenic impact of agriculture) and the local (farm) scale of intensification of the

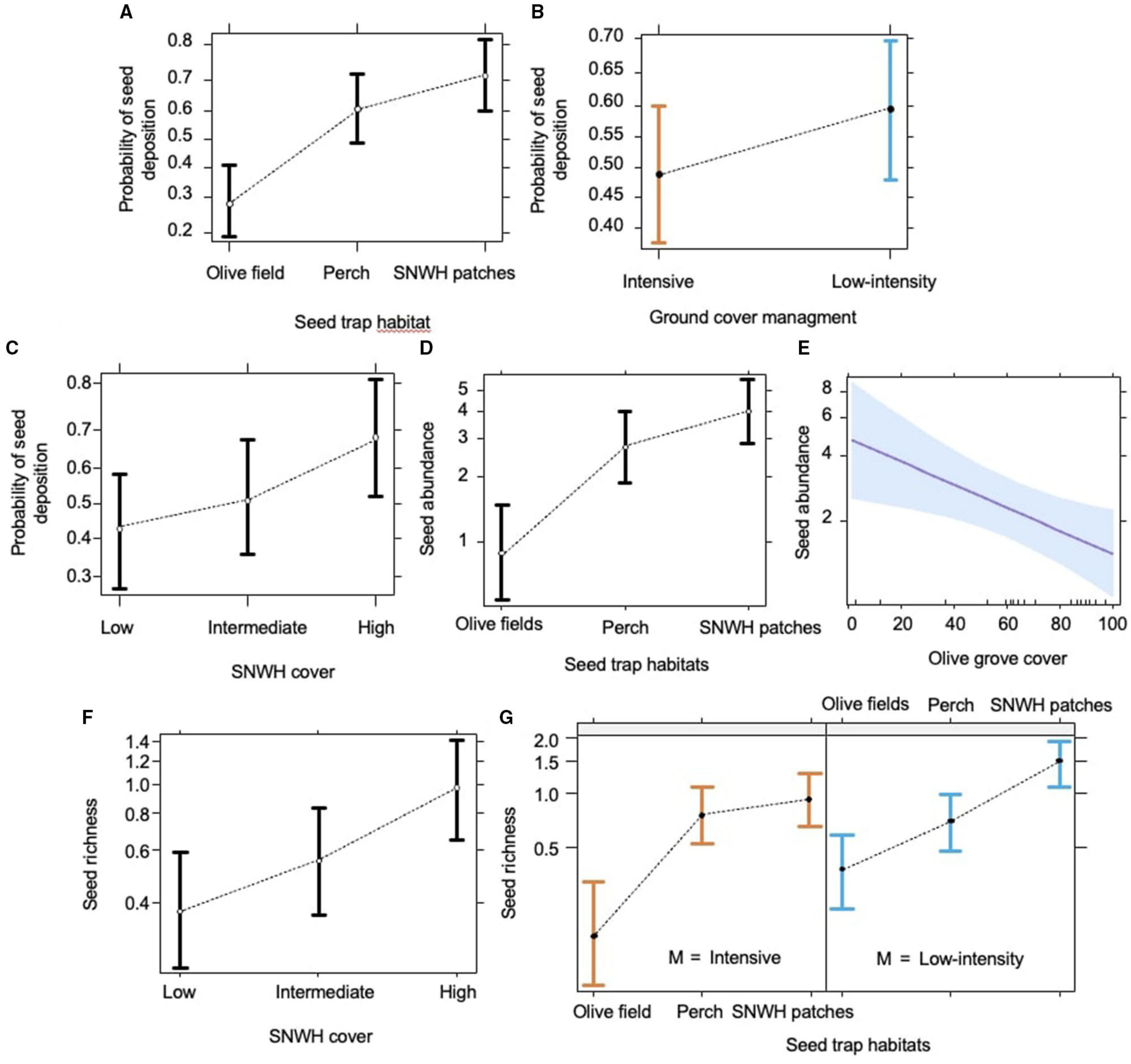

**FIGURE 4 |** Significant effects in the selected models of the seed deposition variability considering as descriptor seed arrival probability **(A–C)** seed abundance **(D,E)**, and species richness **(F,G)** in the seed fall traps. Data represented are predicted least-squares means and 95% confidence intervals. Estimates of fixed effects are shown at the reference mean value for all the covariables. See **Figure 2** caption for code description.

agriculture practices (i.e., ground herb cover removal that leaves soil uncovered), combine their effects to filter the assemblages of frugivorous birds and simplify their frugivory activity and seed deposition patterns. The data of the present study represents the most comprehensive and large-scale survey conducted on the effects of agriculture (specifically olive agriculture) on frugivory and seed dispersal in Mediterranean ecosystems.

The results show the remarkable persistence of the dispersal function of frugivorous birds for Mediterranean fruits in olive grove landscapes since more than 60% of seeds found in bird droppings and the seed rain were from wild native species, accounting for a relevant fraction of the regional flora with fleshy fruits. The results of this study also show that agriculture filters avian frugivore richness and abundance, causing a severe decay of the seed dispersal function. We confirmed our prediction on the important and pervasive role of seminatural woodland habitat remnants within farms for conserving the dispersal function through enhancing frugivore abundance and diversity, increasing frugivory, and attracting seed deposition. Agriculture impacted the seed dispersal function fundamentally by causing changes at the landscape scale, involving woodland habitat loss and conversion to olive fields (and other agricultural uses), and ultimately threatening this function in olive-dominated landscapes. Thus, in parallel to the habitat effect, we confirmed that an impoverished frugivore assemblage (in terms of diversity and abundance), decreased levels of avian frugivory, and decay in the abundance and species diversity of the seed rain are to be expected as seminatural woodland cover decreases or olive grove cover increases in these landscapes. Although we did not expect to find important effects of intensification of the local agricultural practices of ground cover removal, negative effects arose frequently, warning of the additional consequences of local agricultural management on frugivore activity and on the seed rains they generate in this agroecosystem. These results have important implications for the management and preservation of seed dispersal services at several scales.

### The Assemblage of Frugivorous Birds in Olive Grove Landscapes Is Functionally Heterogeneous but Locally Impoverished, Which Contributes to Bias Seed Dispersal, Limiting It to a Few Fruit Species

The role of olive groves as winter quarters for birds and for maintaining their frugivory activity in the Mediterranean Basin has been remarked on in several studies (Rey, 1993, 1995, 2011). These studies focused on the local scale of the olive fields rather than at the landscape scale and showed that olive groves host an impoverished assemblage of frugivorous birds that found this agroecosystem suboptimal. This is because olive groves are simplified compared to natural scrublands and forests, both structurally and in terms of fruit and other food resources, which affect the diet, the foraging behavior, and body condition of the frugivorous birds still able to settle in this agroforest-like agroecosystem (Rey and Gutiérrez, 1996, 1997; Rey et al., 1996, 1997; Rey and Valera, 1999). Our results confirmed previous suggestions. Thus, practically, the totality of avian seed dispersers in the lowlands of the region were represented in the olive grove landscapes considered as a whole (**Table 1**). They consumed and dispersed a substantial number of the fruiting species found in the region (**Tables 3–5**). However, frugivore assemblages became impoverished locally, as did seed dispersal function.

The frugivore assemblage of the olive grove landscapes seems to be characterized by its functional heterogeneity and the potential complementarity of seed dispersal services, which are fundamental for ensuring seed dispersal quality and resilience in real human-shaped landscapes (García and Martínez, 2012; García et al., 2013; Escribano-Ávila et al., 2014). Frugivores that contribute more significantly to seed dispersal in olive groves are either wintering birds (Blackcap) or resident species (Sardinian warbler and European blackbird), which consume fruit during all seasons and contribute to seed dispersal of the whole set of species available. Along with these species, some other bird species occur in olive groves dispersing fruits during short periods. They typically consume fruits and disperse seeds during a migratory pass, and include flycatchers and redstarts and especially, several *Sylvia* and *Curruca* species (Jordano, 1982, 1984, 1988; Herrera, 1984a).

The frugivore assemblage inhabiting olive grove landscapes varies considerably in body size with small, medium-sized, and large frugivores (Herrera, 1984b; Jordano, 1987), allowing complementary seed dispersal of all wild and cultivated fruits in the region (the latter pecked rather than swallowed in many cases, Rey and Gutiérrez, 1997). Finally, among common frugivores in olive groves, avian mobility and the ability to track fruit resources in human shaped landscapes is especially pronounced in Blackcap and Song thrush (Rey, 1995; Tellería et al., 2008). At the landscape scale, long distance flights between fragments of natural habits across olive groves are frequent in some large frugivorous birds of genus *Turdus*, *Columba*, and among Corvidae (authors pers. obs.; see also Perea and Gutiérrez-Galán, 2016).

These results indicate that the frugivore assemblage inhabiting olive grove landscapes has the potential to adequately disperse most fruit-bearing species in the region. However, the seeds in the droppings collected (**Tables 3**, **4**) and findings in the seed traps show that the fruits of many species are being consumed and dispersed extremely infrequently by most frugivores in olive grove landscapes, and that, in many cases, their dispersal is carried out by only one or two frugivorous bird species at most. Moreover, although our results illustrate remarkable frugivore activity, the low ratio of dispersed and available species (**Supplementary Figure 2**) indicates that a notable number of species remain undispersed at each locality (% of available but not dispersed species ranging from 13 to 100%). In addition, some species were not (or rarely) dispersed regionally, despite being present in these olive grove landscapes. Interestingly, these results also illustrate that some seeds were dispersed over long distances since they were found to be dispersed at the farm scale but seemed to be absent in the farms (this last phenomenon is identified as gray-colored empty cells in **Supplementary Figure 2**).

The frugivory activity was strongly dominated by two generalist frugivores, Blackcap and Sardinian warbler, which are also major seed dispersers in the native vegetation of the Mediterranean region (Jordano and Herrera, 1981; Herrera, 1984a). Frugivory activity and dispersal were biased toward some plant species, particularly the cultivated and the wild olives and *Pistacia lentiscus,* two lipid-rich winter fruits that account for more than 50% of the seeds in bird droppings and ca. one-third in the seed traps, despite both are single-seeded fruits. These two fruit species are known to support the frugivorous diet of wintering birds in the lowlands of the Mediterranean region (Herrera, 1984a; Jordano, 1984; González-Varo et al., 2017; Parejo-Farnés et al., 2020) and in olive groves and wild olive scrublands (Rey, 1992; Rey et al., 1997; Rey and Valera, 1999). Besides these species, some summer fruits (*F. carica*, *R. ulmifolius,* *Solanum* spp., and *R. lycioides*, the latter common in many habitat remnants) that typically serve as fruit sources for resident and migrant frugivores (Jordano, 1982, 1988; Herrera, 1984a) were abundantly dispersed locally during summer-autumn (**Tables 4**, **5**).

This bias in frugivory and seed dispersal is in part due to the lack of some frugivores in olive dominated landscapes, where for example, some migrant *Curruca* and *Sylvia* and *Oriolus* species or resident *Turdus viscivorus*, were virtually absent, while others like *Turdus merula* and *Erithacus rubecula* decay sharply with the loss of woodlands. In particular, the seed dispersal of all but one fruit species involves the Blackcap, even though some fruits were available mainly when this species has left most olive groves. Blackcap was by far the most abundant frugivore in winter, and the one most captured in mist nets, while large birds, even those that were relatively abundant, were rarely captured if any with mist-nets, meaning their contribution to frugivory and seed rains may be underestimated. Although Blackcap leaves olive groves during spring and summer, some individuals remain in olive groves in the piedmont of the mountain systems of the region during summer-autumn periods and consume summer-autumn fruits, which explains its ample fruit diet. Even though some frugivores, such as European robin, Song thrush, and European blackbird, are known to disperse many seed species in Mediterranean forests and scrublands (Herrera, 1984a; Jordano, 1984); they dispersed a relatively low number of fruit species in the olive grove landscapes. Although they were captured less than Blackcaps (compared to its abundance in censuses) their frequency of occurrence in seeds in droppings (proportion of droppings with seeds) was rather low in our study, for example, European robin (0%), Song thrush (9%) and European blackbird (7.4%) (see also Rey, 1992; Tarifa et al., 2021). It seems that these species are slower to consume fruits in olive grove landscapes than in natural habitats, perhaps because in many olive grove landscapes these fruits are too scarce to attract these birds or to make profitable the tracking of their availability.

In short, these results suggest that olive expansion and concomitant loss of forest/scrubland patches constrain local fruit and frugivore assemblages, limiting their fruit consumption, and disrupting the seed dispersal and seed rain of many plant species.

## Habitat and Landscape Effects: Woodland Habitat Loss and Olive Grove Expansion Filter Frugivore Assemblage, and Simplify Frugivory and Seed Deposition Patterns

We found an important pervasive effect of the type of habitat within the farm on frugivore abundance and diversity, frugivory, and the seed abundance and diversity in the seed rain. Avian frugivores tended to concentrate in forest/scrubland patches and their activity of fruit consumption was higher compared to those in the olive field, as revealed both by the number of seeds and species richness in droppings, which subsequently translated into the concentration of the seed deposition in the natural habitat remnants. This is related to the fact that within the olive fields most fruiting species are actively eliminated by agricultural practices, with only some species, such as *Asparagus* spp., *Solanum* spp., *Capparis spinosa*, persisting (Rey, 2011; Tarifa et al., 2021) in the olive fields of low-intensity farms. This is in sharp contrast to the availability of non-cultivated fruits in the remnant forest patches (Tarifa et al., 2021). This type of effect of patch quality on avian abundance and richness, and on frugivory and seed arrival, has been reported in other landscapes of the Iberian Peninsula, which were fragmented or structurally degraded by annual croplands and livestock grazing (Santos and Tellería, 1994; Alcántara et al., 2000; García and Chacoff, 2007; García et al., 2010; Herrera and García, 2010; Rey and Alcántara, 2014). Similar local or patch effects are also typical in tropical agroforest systems, such as cocoa and coffee plantations (for instance, Lozada et al., 2007) that, as olive groves, are permanent croplands. In these agroforest systems, the practice of growing the crop under a more or less diverse canopy of tropical trees (shade plantations), compared to the most common and productive sun plantations, conditions the farm for frugivores and affects their frugivory activity and seed deposition patterns, which have been shown to approach those reported on intact forests (Lozada et al., 2007; Araújo-Santos et al., 2021). As in other agricultural landscapes, isolated trees, that overtop olive tree canopy serve as perches for birds and provide alternative resources (food or mating and nesting sites) in the olive fields (e.g., Hoi-Leitner et al., 1999), introducing a source of heterogeneity for the patterns of frugivore activity and seed deposition. They typically have a focal effect, attracting frugivore activity within inhospitable matrices and generating seed nucleation (Herrera and García, 2009), especially if they bear fruit. We confirmed the nucleation effect of isolated perching trees, with findings revealing that, even if they did not bear fruit, they attracted seeds to the olive field. The probability of seed deposition under the canopy of isolated perching trees in the olive field, and the number and the diversity of arrived seeds, were to some extent comparable to those of habitat remnant patches and much higher than under the canopy of olive trees (**Figures 4A,D,G**).

Interestingly, the habitat effects in olive groves interact frequently with SNWH cover and/or olive grove cover (i.e., expansion) in landscapes. These types of interacting effects have been reported in literature on avian frugivores in landscapes fragmented by agroforest croplands (Araújo-Santos et al., 2021). While the abundance and diversity of frugivores in the seminatural patches of the olive farms varied in relation to olive grove cover in the landscape and/or across levels of SNWH (augmenting the abundance but decreasing the diversity as olive grove cover is higher and the patches of natural habitat smaller and infrequent), they remain invariantly low in the olive field (see **Figures 2A,C**). In turn, frugivory decreased with the olive grove cover in the landscape (**Figures 3B,E**). On the one hand, this means that as seminatural woodland habitat patches become smaller and infrequent in landscapes, they host fewer fruiting species (a typical patch cover effect, Arroyo-Rodríguez et al., 2020), and attract a less diverse group (fewer species) of frugivores that concentrate in the small patches still present and build less diverse fruit diets. On the other hand, these results suggest that independently of the landscape context in which olive farms are located, olive fields in their current state are of very low attractiveness, and are even inhospitable for many species of frugivores. This is unexpected since they supply them with a rewarding and abundant fruit (the cultivated olive) and their agroforest-like nature could be relatively suitable for forest/scrubland birds. These results also suggest that the spillover of avian frugivores and seeds of fruit-bearing plants from the remnant patches of natural habitat toward the olive field matrix is rare, with several species of frugivores concentrating their abundance and activity exclusively in the seminatural patches, this effect will become even more pronounced with the expansion of the olive groves and the homogenization of olive-dominated landscapes. Therefore, unlike the permeability typically found in tropical agroforest systems to animal-mediated seed dispersal (for instance, shade coffee or cocoa plantations, Lozada et al., 2007; Araújo-Santos et al., 2021), olive fields are less permeable than we initially presumed to frugivorous birds, and to their dispersal function.

In this scenario, the persistence of a diverse frugivore guild and its dispersal function in the olive grove landscapes strongly depends not only on the presence but also on the cover provided by seminatural woodland habitats. Maintenance of forest remnants has been shown to be fundamental to maintaining frugivory and seed dispersal services and potential forest regeneration in agricultural landscapes, both in temperate and Mediterranean regions and in the tropics. A number of forest remnants in such landscapes promotes local (within patch) and regional (between patches) seed dispersal by medium-sized and large avian frugivores capable of long-distance flight, which to some extent counteracts the effects of forest fragmentation on fruit consumption and removal from focal plant species (Breitbach et al., 2010; González-Varo, 2010; Parejo-Farnés et al., 2020). This ultimately should enable abundant and diverse seed rains and the persistence of species at landscape scale through long-distance seed dispersal (García et al., 2010; González-Varo et al., 2017; Araújo-Santos et al., 2021).

## Impact of Local Intensive Ground Herb Cover Removal on Frugivory and Seed Dispersal

Intensification of agricultural practices may affect the abundance and diversity of farmland birds (Donald et al., 2001) and birds thriving in woody and agroforest croplands (for instance, Assandri et al., 2016, 2017, for vineyards; Rey et al., 2019; Morgado et al., 2020 in olive groves; Bennet et al., 2021, in cocoa agroforest, Araújo-Santos et al., 2021 in coffee plantations). Among the most susceptible avian guilds to intensive agricultural practices in permanent croplands are insectivores and frugivores (Rey, 2011; Martínez-Núñez et al., 2020a; Bennet et al., 2021). It has been suggested that intensive agriculture affects pest biocontrol and seed dispersal services (Johnson et al., 2010; Maas et al., 2013; Araújo-Santos et al., 2021). The different methods adopted and levels of intensification that take place in local agriculture and how they affect avian frugivory and seed deposition patterns in these croplands have only been investigated in tropical agroforest systems (Lozada et al., 2007; Araújo-Santos et al., 2021) to date, and there is no previous information on olive groves. We found that the effects of intensification of local agricultural practices to be more frequent in olive groves than we initially expected.

Different from other woody and agroforest croplands, such as cocoa and coffee agroecosystems, where intensification mainly concerns the removal of a canopy of tropical forest tree species, the form of intensification in olive groves involves the persistent application of herbicide to remove ground herbaceous cover (which are considered weeds in olive fields) (Rey et al., 2019; Martínez-Núñez et al., 2020b; Tarifa et al., 2021). We confirmed that local intensive practices of ground cover removal notably impacted the frugivory. This practice has consistent negative effects on the proportion of frugivorous species dispersing seeds, and the seed number and diversity in their droppings. This effect eventually translated to decay in the probability of seed deposition and the diversity of seed rains, especially in the olive field. Active practices of ground cover removal in intensive farms much probably hampered the occurrence of fruit species in the olive fields, affecting the frugivory.

Frugivorous birds also use insects in their diets in olive groves (Rey, 1992; Rey et al., 1996; Rey and Valera, 1999). Intensive farms more frequently use pesticides against olive pests, which together with the removal of herbaceous cover substantially decreases insect availability in intensive farms (Carpio et al., 2019) and could contribute to making these farms inhospitable for some birds. This may explain our findings, which indicate the negative (although only marginally significant) effects of local intensification practices on avian frugivore richness.

## Synthesis and Applications

The olive agroecosystem plays an important role in the conservation of numerous frugivorous birds that winter in the circum-Mediterranean region and of their seed dispersal function (Rey, 2011). However, although information about their abundance and species composition is extensive, we still know very little about the consequences of agriculture on the seed dispersal service that frugivores provide. Our study was conducted at the farm, landscape, and regional levels, and suggests that olive cultivation threatens the seed dispersal service delivered by avian frugivores for many Mediterranean plant species. Importantly, all components of the seed dispersal function (abundance and diversity of avian frugivores, intensity of frugivory, and seed deposition) are threatened in most olive grove landscapes due to olive expansion and loss of woodland habitats. Moreover, this phenomenon is further aggravated by predominant intensive agricultural practices that remove ground herb covers with herbicides.

The increasing area occupied by olive grove-dominated landscapes not only threatens frugivorous/insectivorous bird communities, it also lessens their functional complementarity and diminishes the connectivity, and potential regeneration of, the persisting remnant patches of seminatural woodland habitat within these landscapes. There is a current expansion of olive groves, toward superintensive olive growing in hedges, which could further aggravate the ongoing loss of frugivore diversity and frugivory in olive groves (but see Morgado et al., 2021) due to the deep structural modification of this agroforest-like agroecosystem. These threats could be generalized to most olive growing areas of the Mediterranean Basin since land conversion to olive cultivation by intensive and superintensive practices is being pursued throughout the entire region.

Our findings have important applications for the management and recovery of the seed dispersal function. We have shown that the presence and cover of fruit-rich SNWH patches in olive grove landscapes are critical for the conservation of avian frugivores and their dispersal services and that intensive agriculture further impacts this function. Management actions should take these effects into account, and address this increase in SNWH cover, especially in fruit-rich woodlands, and undertake maintenance of ground herb cover, working at several spatial scales.

At a large scale, we recommend:

- Compulsory maintenance of woodland habitats within agricultural landscapes.
- Avoidance of property concentration (land consolidation), which frequently entails the elimination of edges, field margins, and hedgerow.

At the farm scale, the following should be prioritized:

- Maintenance of the still present woodland habitat patches within the farm, and further enriching them with native fleshy-fruited species, since many of them have already been internally cleared (through historical practices).
- Creation of new woodland patches that are rich in fleshy fruits and located in unproductive zones of the farm, especially in olive-dominated landscapes.
- Reforestation of the edges and field margins and installation of hedges rich in fleshy fruits.
- Conservation of isolated perching trees and promotion of new perches for birds by planting trees or installing artificial perches (e.g., wooden poles).
- Fomenting the maintenance of herbaceous ground cover, at least between rows of olive trees and in strips around woodland patches, and decreasing the levels of pesticide application.

These actions could be implemented within the frame of the new European Union Common Agriculture Policy (CAP post-2020), through its different environmental instruments, which include enhanced conditionality, eco-schemes, agri-environmental, and climate measures targeted specificaly and regionally to the agroecosystem (Díaz et al., 2021) of the Mediterranean region.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## ETHICS STATEMENT

This animal study was approved under licenses authorized by Bird Migration Center of Spain (dependent of SEO-BirdLife) and the Regional Government of Andalucía.

## AUTHOR CONTRIBUTIONS

PR and DG design this study and provided funding from their projects. FC, RT, CM-N, and AP conducted most field and lab work. RT, FC, and CM-N conducted the statistical analyses supervised by PR. TS carried out SIG data processing and analyses. PR, DG, RT, and FC wrote several drafts of this manuscript with feedback from CM-N. All authors critically revised the final version and give approval for its submission.

## FUNDING

This study was funded by the projects AGRABIES (CGL2015-68963-C2 funded by MCIN/AEI/10.13039/501100011033/ FEDER Una manera de hacer Europa) and RECOVECOS (PID2019-108332GB-I00, MICIN/AEI/10.13039/501100011033). It was conducted under the general framework and associated farms of the LIFE Program project OLIVARES VIVOS (ref. LIFE14 NAT/ES/001094). CM-N received a grant as part of the FPI fellowship (BES-2016-078736) of the National Research Plan. DG was awarded a grant by the project ClaveSER (044-2019; Fundación BBVA).

## ACKNOWLEDGMENTS

We are grateful to Gemma Calvo and Enrique Muñoz for their assistance in the field and our colleagues at the OLIVARES VIVOS LIFE project, Francisco Valera, J. Eugenio Gutiérrez, and Carlos Ruiz for continuous support in our research on olive groves. We are also in debt to the owners and workers at the olive farms that participated, who facilitated many aspects of the work.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.782462/full#supplementary-material

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Ecology and Evolution

ORIGINAL RESEARCH
published: 22 December 2021
doi: 10.3389/fevo.2021.789264

# Long Distance Seed Dispersal by Forest Elephants

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**Specialty section:**
This article was submitted to Population, Community, and Ecosystem Dynamics, a section of the journal Frontiers in Ecology and Evolution

**Received:** 04 October 2021
**Accepted:** 06 December 2021
**Published:** 22 December 2021

**Citation:**
Poulsen JR, Beirne C, Rundel C, Baldino M, Kim S, Knorr J, Minich T, Jin L, Núñez CL, Xiao S, Mbamy W, Obiang GN, Masseloux J, Nkoghe T, Ebanega MO, Clark CJ, Fay MJ, Morkel P, Okouyi J, White LJT and Wright JP (2021) Long Distance Seed Dispersal by Forest Elephants. Front. Ecol. Evol. 9:789264. doi: 10.3389/fevo.2021.789264

By dispersing seeds long distances, large, fruit-eating animals influence plant population spread and community dynamics. After fruit consumption, animal gut passage time and movement determine seed dispersal patterns and distances. These, in turn, are influenced by extrinsic, environmental variables and intrinsic, individual-level variables. We simulated seed dispersal by forest elephants (*Loxodonta cyclotis*) by integrating gut passage data from wild elephants with movement data from 96 individuals. On average, elephants dispersed seeds 5.3 km, with 89% of seeds dispersed farther than 1 km. The longest simulated seed dispersal distance was 101 km, with an average maximum dispersal distance of 40.1 km. Seed dispersal distances varied among national parks, perhaps due to unmeasured environmental differences such as habitat heterogeneity and configuration, but not with human disturbance or habitat openness. On average, male elephants dispersed seeds farther than females. Elephant behavioral traits strongly influenced dispersal distances, with bold, exploratory elephants dispersing seeds 1.1 km farther than shy, idler elephants. Protection of forest elephants, particularly males and highly mobile, exploratory individuals, is critical to maintaining long distance seed dispersal services that shape plant communities and tropical forest habitat.

**Keywords: seed dispersal, elephant, tropical forest, animal movement, central Africa, gut passage time, long distance dispersal**

## INTRODUCTION

Fruit-eating vertebrates play an important role in plant reproduction through seed dispersal (Levin et al., 2003). At local scales, seed dispersal increases plant fitness by potentially reducing competition between parent plants and their progeny and lowering the risk of density-dependent disease and predation (Janzen, 1970). Not only does seed dispersal promote community diversity (Harrison et al., 2013), it also reduces species aggregation (Wandrag et al., 2017). At landscape scales, long-distance dispersal (LDD) links local populations within a metapopulation, facilitates gene flow, and promotes migration and colonization of new habitats (Cain et al., 2000; Nathan, 2006). LDD by large vertebrates is of particular interest for biodiversity conservation because large-bodied vertebrates, past and present, are most at risk of human-related extinctions (Davidson et al., 2009). Under global climate change, the loss of large vertebrate seed dispersal services could compromise the survival of plant species that depend on range shifts driven by migration and colonization.

Vertebrate species affect plant community composition and population expansion by shaping the seed shadow – the spatial distribution of seeds relative to the parent plant. After fruit consumption, two key processes determine the seed shadow: gut passage time (GPT), the time between seed ingestion and defecation, and animal movement. Large, wide-ranging species with slow GPTs, therefore, disperse seeds farther on average than small-bodied species with fast GPTs (Vidal et al., 2013; Bunney et al., 2017). GPS technology can precisely measure animal movement (Kays et al., 2015), but measurements of GPT are less precisely known. GPT estimates have primarily depended on trials with captive animals owing to difficulties of relocating ingested seeds in the wild (Kinahan et al., 2007; Campos-Arceiz and Blake, 2011; Harich et al., 2016; Bunney et al., 2017). Captive animals, however, often have different diets and behaviors than wild animals, potentially introducing considerable error into the modeling of realistic seed shadows (Campos-Arceiz et al., 2008).

Most studies of vertebrate seed dispersal have focused on estimating species-level seed dispersal services (Holbrook et al., 2002; Russo et al., 2006; Blake et al., 2009), ignoring individual physiological traits (sex and size) and behavioral differences (boldness, aggressiveness, and sociability) (Zwolak and Sih, 2020). But individuals can vary consistently in behavior, with behaviors often covarying in behavioral syndromes, such as being more or less active (Sih et al., 2004). Behavioral syndromes could have direct ecological effects for seed dispersal (Sih et al., 2012). More exploratory, active foragers, for example, will likely have larger home ranges, higher encounter rates with high quality fruits, and consume more fruit because of higher energy needs (Zwolak and Sih, 2020). Thus, behavioral syndromes that control movement patterns of frugivores might affect seed dispersal distances and even the probability that seeds are deposited in suitable sites for recruitment.

To advance knowledge of seed dispersal for both theory and management, we need to understand the extrinsic, environmental and intrinsic, individual drivers of seed dispersal. Forest elephants (*Loxodonta cyclotis*) are an ideal model for studying variation in seed dispersal because they are intelligent, highly mobile, keystone seed dispersers in African tropical forests. Forest elephants consume seeds of more species than any other large vertebrate (Beirne et al., 2020) and are the sole disperser of many large or robustly encapsulated seed species (Guimarães et al., 2008; Beaune et al., 2013). With daily travel distances of 7.4 km per day (Mills et al., 2018) and annual home ranges of 195 km$^2$ (Beirne et al., 2021), they contribute to LDD. Using coarse GPT categories for small and large seeds, Blake et al. (2009) estimated that 88% of elephant-dispersed seeds were moved >1 km and 14% of seeds were moved >10 km from parent plants. Forest elephants, however, likely vary in the quality of their seed dispersal services with habitat use, sex and individual behavior. Male elephants, for example, move farther and have larger home range size and weaker site fidelity than females (Beirne et al., 2021). And, forest elephants exhibit distinct behavioral syndromes along an axis that varies from individuals with smaller home ranges, shorter movement distances and less exploratory behavior to those with larger ranges, longer movement distances and more exploratory behavior (Beirne et al., 2021). Finally, poaching and habitat loss have drastically reduced forest elephant populations in the last two decades (Maisels et al., 2013; Poulsen et al., 2017, 2018), thus we need to understand how the reduction or loss of seed dispersal services provided by elephants could affect forest species composition.

Here we quantify seed dispersal by forest elephants to improve understanding of their role in LDD. To do so, we use a novel experimental approach to: (1) estimate the GPT distribution of wild forest elephants and compare them to existing estimates; (2) model elephant-mediated seed dispersal patterns, using GPS tracking data from 96 elephants; and (3) evaluate the effects of human disturbance, habitat openness, elephant sex, and behavioral syndrome on seed dispersal distances. Filling gaps in the knowledge of a keystone seed disperser contributes to forecasting future forest composition in light of climate change, deforestation, and defaunation.

## MATERIALS AND METHODS

### Study Area and Subjects

Located on the Atlantic coast of central Africa, the Gabonese Republic (Gabon) holds 23 million ha of evergreen forest with 88% forest cover (Sannier et al., 2014) and high aboveground biomass stores in large trees (Poulsen et al., 2020; **Figure 1**). In part due to its low human population (∼ 1.5 million people), historic economic dependence on offshore oil, and conservation efforts (creation of 13 national parks in 2004), the country is renowned for its diversity of natural, intact ecosystems and the animal and plant species that inhabit them. The annual mean temperature of Gabon is about 25.0°C (1901–2015). On average, temperatures are highest in March (26.2°C) and lowest in July (23.1°C). Annual average precipitation is about 1800 mm, but it varies along a west-to-east gradient, declining from 2650 mm along the Atlantic coast to 1400 mm in the southeast of the country.

In 2015, the Gabon Parks Agency (ANPN) developed a GPS tracking program to guide elephant management and protection (Mills et al., 2018). Ninety-six elephants were collared between October 2017 and October 2019 in and around five protected areas (Ivindo, Loango, Moukoulaba-Doudou, Mwagna, and Minkébé National Parks and the Wonga Wongué Presidential Reserve) (**Figure 1**), which vary to some extent in habitat type and weather (**Table 1**). The field team attempted to collar a balanced sample of males and females; however, males were scarce in some locations. The proportion of collared males at each site was 33% (Ivindo), 47% (Loango), 8% (Minkébé), 20% Moukoulaba Doudou, 20% Mwagna, and 55% Wonga Wongué. For a full description of capture and collaring protocols see

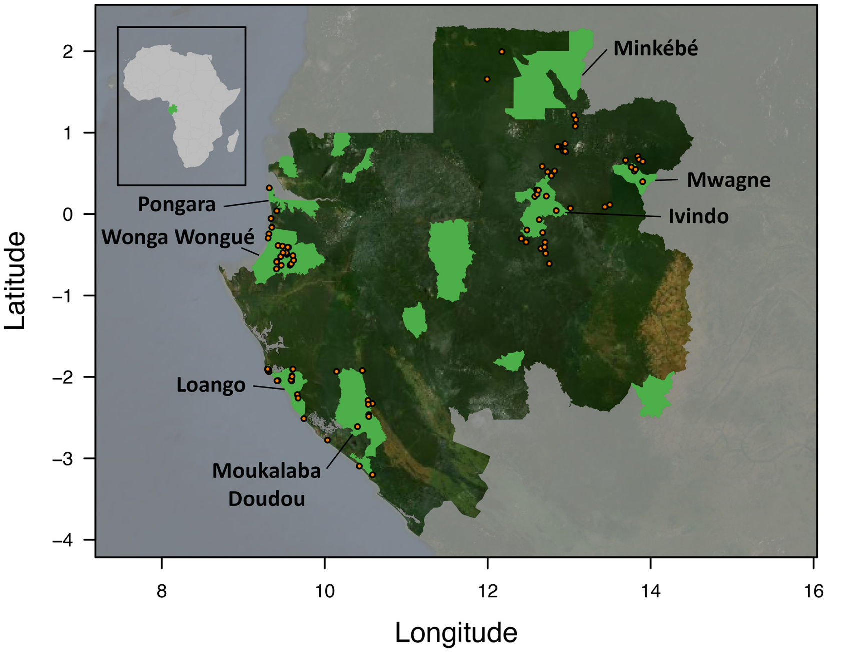

**FIGURE 1 |** The location of Gabon in Africa (inset) and the approximate locations of the 96 collared elephants (orange points) in and around 7 protected areas (light green polygons) in the country.

Mills et al. (2018). For a short time after deployment of the collars and after 2 years of functioning, a 4- or 12-h GPS fix interval was used, otherwise the collars were set to a 1-h interval. To preclude the possibility that fix interval influences the results, we only use data with fix intervals of 1 h.

## Gut Passage Times

To estimate GPTs of wild forest elephants, we conducted baiting trials and elephant follows between June and August 2018 in and around Ivindo National Park in northeastern Gabon [**Figure 1**; see Beirne et al. (2019) for details]. The trials consisted of baiting GPS-collared elephants with fruits embedded with experimental seeds, and then following the elephant using its GPS track to recover the seeds. The smallest experimental seeds (pellets) were colored plastic pellets (6 mm diameter Airsport Ammo, 0.11 g). Different colors of pellets were employed for each baiting trial to distinguish the origin of pellets recovered in dung. The second type of experimental seed (tin seeds) consisted of hollowed out *Chrysophyllum lacourtianum* seeds filled with aluminum foil and sealed with medical safe epoxy (Henkel Loctite Hysol M-31CL Medical Device Epoxy). Note that we also tested epoxy-sealed, iButtons as experimental seeds on captive elephants at the North Carolina Zoo and in the field (Beirne et al., 2019). At the zoo, the average GPT for three Savanna elephants (*Loxondonta africana)* was relatively slow (**Figure 2**, point 1); in the field, wild forest elephants spat out the iButtons, so they are not included in estimates of seed dispersal distances below (Beirne et al., 2019).

Based on GPS data, we located baiting stations at places likely to be visited by collared elephants. Baiting stations consisted of piles of wild *C. lacourtianum* fruits, a seasonally and locally abundant elephant-dispersed fruit, and market bought mangos (*Mangifera* spp.) and ripe plantains (*Musa* spp.). Each *C. lacourtianum* fruit was hollowed out, embedded with up to six tin seeds and stuffed with a mixture of *C. lacoutianum* and plantain fruit flesh and colored pellets. We washed prepared fruits with stream water to reduce the possibility that elephants rejected the seeds due to human handling. In total, each pile contained 250 g of pellets (∼2270 beads) and between 19 and 46 tin seeds. Additional fruit, such as mangos, undisturbed *C. lacourtianum* and plantains, were added to each pile to make them more attractive to elephants.

Although we attempted to bait multiple elephants, our GPT estimates are derived from a GPS-collared adult female (age 30–40) accompanied by two juveniles (ages 2–3 and 7–8) that traveled as a unit. After a feeding event, we followed the focal elephant for 10 days to record the location of all dung containing experimental seeds. In sum, we followed this group for three separate 10-day periods (see below). The difference in time from the initial feeding event to the timestamp of the GPS point from the elephant movement track closest to the dung was considered the GPT. Using this approach, we obtained 118 GPT observations over three separate elephant follows (Beirne et al., 2019).

## Modeling Seed Dispersal

To estimate a GPT distribution, we fit a Bayesian generalized linear model using a gamma distribution with a log link to estimate relevant parameters (mean, shape, $k$, and scale, $\Theta$; **Figure 2**). Additional models were fit that included the elephant follow and dung age class as potential explanatory predictors of both mean and shape; however, none of the

**TABLE 1 |** Description of primary sites where forest elephants were GPS collared, including the general precipitation patterns and habitat types at each site.

| Site and area | Precipitation | Habitat |
|---|---|---|
| Ivindo National Park 2990 km$^2$. | Annual mean: 1700 mm Bimodal rainfall with two relatively dry (January–March and June–August) and two rainy seasons (September–December and April–May). | Primary and secondary lowland forest, swamps, and a series of baïs [forest clearings maintained by animal activity Beirne et al. (2020)]. |
| Loango National Park 1550 km$^2$. | Annual mean: 2099 mm Bimodal rainfall with long rainy season (October–April), interrupted by a short dry season (December–January), and a long dry season (May–September Martínez-Íñigo et al., 2021). | Mosaic of rivers, swamps, coastal forests, mangroves, savannahs, and secondary and mature forests, bordered by the Atlantic Ocean and a lagoon. |
| Moukoulaba–Doudou National Park 4500 km$^2$. | Annual mean: 1176–2043 mm One dry season (May–September) and one rainy season (October–May Terada et al., 2021). | Mosaic of forest, savanna, and papyrus swamp; the park is divided, north to south, by the Doudou Mountain Range with elevations up to 900 m. |
| Mwagna National Park 1160 km$^2$. | Annual mean: 1700 mm Bimodal rainfall with two relatively dry (January–March and June–August) and two rainy seasons (September–December and April–May). | Primary and secondary lowland forest, swamps, and a series of baïs. |
| Minkébé National Park 7570 km$^2$. | Annual mean: 1500–1800 mm Bimodal rainfall with two relatively dry (January–March and June–August) and two rainy seasons (September–December and April–May). | Diversity of habitats including primary lowland forest, inselberg forest, herbaceous swamps, and seasonally flooded river valleys. Landscape includes hills and inselbergs, so that elevations range from 450–900 m Huijbregts et al., 2003. |
| Wonga Wongué Presidential Reserve 4250 km$^2$. | Annual mean 2600 mm Bimodal rainfall with two dry seasons (May–October and December–January) and two wet seasons (January–May and October–December Mills et al., 2018). | Variety of habitats, from white sand beaches and mangrove wetlands on the Atlantic coast to a mosaic of open grasslands and tropical forest in the interior made up of 85% lowland forest and 15% savanna. |

parameters from these models differed significantly from the combined model.

Simulations of possible seed shadows were constructed using a Monte Carlo simulation of seed dispersal by sampling time from the GPT distribution and then using these values to conditionally sample from an empirically determined distribution of elephant movement. This empirical distribution was constructed from timestamped GPS movement tracking data of forest elephants in Gabon. These data consist of hourly latitude and longitude coordinates for 96 different elephants collected between 2015 and 2019 (Beirne et al., 2021). Specifically, these hourly observations for each elephant observations were differenced with all subsequent observations to calculate $\Delta t$, change in time, $\Delta d$, change in distance, $\Delta x$, change in east/west distance, and $\Delta y$, change in north/south distance. Only pairs with a $\Delta t$ of less than 7 days were used as all observed GPTs were below 100 h. Kernel density estimates were then constructed of $\Delta t$, $\Delta d$, $\Delta x$, and $\Delta y$ separately for each elephant and for all differenced elephant data combined. These were then used to simulate the 1 dimensional seed distance distribution (**Figure 3**) and the 2 dimensional seed shadow distributions (**Figures 4**, **5**).

## Drivers of Variation in Seed Dispersal Distance

To evaluate drivers of variation in seed dispersal distances, we modeled median seed dispersal distance, $\Delta d$, for each elephant as a linear model incorporating extrinsic (site, human footprint index, and habitat) and intrinsic (sex, behavioral syndrome) factors as independent variables. Site was defined as the protected area in which the collared elephant occurred, or the closest protected area to its home range. We assessed the role of human disturbance on seed dispersal distances by including the mean annual human footprint index (HFI) for the home range of each elephant (Wildlife Conservation Society – WCS, and Center for International Earth Science Information Network – CIESIN – Columbia University, 2005). The HFI spatial raster is derived from nine data layers reflecting three key elements: human population (population density); human land use and infrastructure (built-up areas, nighttime lights, land use/land cover); and human access (coastlines, roads, railroads, and navigable rivers). Habitat was included by quantifying the proportional use of forest habitat by each elephant. Using discrete classifications of habitat types from the CGLS-LC100 Collection 3[1], we collapsed five habitat types into either “open” (grassland, savanna, and bare ground) or “closed” (forest) categories for each GPS fix, and then calculated the proportion of all GPS fixes in forest for each elephant. Sex was recorded during the collaring of each elephant. Behavioral syndrome was incorporated as the degree of exploratory behavior from Beirne et al. (2021), where negative values represent idlers (small home ranges, short movement distances, and low exploratory behavior) and positive values represent explorers (large home range, long movement distances, and low exploratory behavior).

We built a full linear model, including main effects for HFI, forest, and exploratory behavior, and an interaction between sex and site to account for the uneven ratios of males and females at each site. We then used backward model selection to reduce the model and verified model fit by inspecting model residuals for normality and homoscedasticity. All statistical analyses were conducted in R 4.0.0 (R Core Team, 2020).

# RESULTS

## Gut Passage Times

During three complete elephant follows, we encountered 437 elephant dungs, 118 of which contained experimental seeds, and recovered 845 experimental seeds (842 pellets and 3 tin seeds). No seeds were found after 4.7 days, suggesting that following

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[1] https://developers.google.com/earth-engine/datasets/catalog/COPERNICUS_Landcover_100m_Proba-V-C3_Global

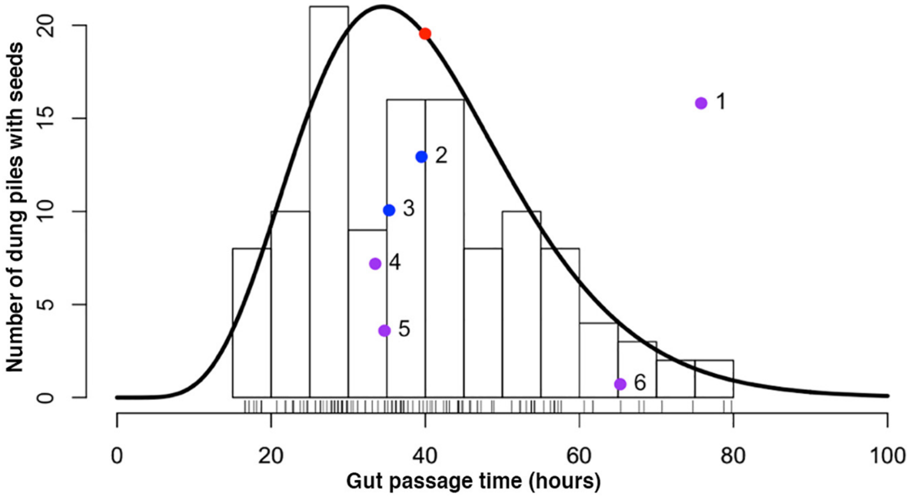

**FIGURE 2 |** Distribution of gut passage times from baiting trials on wild forest elephants in Gabon. The histogram shows the number of dung piles containing experimental seeds over time. The red point depicts the mean GPT from these observed data 39.5 h (sd = 14.5 h). The curve over the histogram is the best fitting gamma distribution [$\Gamma(k = 7.6, \Theta = 5.2)$], with a mean GPT of 39.5 h. Numbered points represent GPT estimates from studies of *L. africana* (purple) and *E. maximus* (blue) with the height of each point representing the number of dung piles with seeds from which the estimates were derived. The numbers by the points correspond to: (1) captive *L. africana* using iButtons (*n* = 3, 2 females, and 1 male) (Beirne et al., 2019); (2) captive *E. maximus* using beads (*n* = 4, 1 male, and 3 females) (Campos-Arceiz et al., 2008); (3) captive *E. maximus* using seeds of *Dillenia indica* (*n* = 6, all females) (Harich et al., 2016); (4) captive *L. africana* using melon seeds (*n* = 4, 2 males, and 2 females) (Bunney et al., 2017); (5) captive *L. africana* using mango seeds (*n* = 4, 2 males, and 2 females) (Bunney et al., 2017); and (6) captive *L. africana* using iButtons (*n* = 4, 1 male, and 3 females) (Kinahan et al., 2007).

elephants for 10 days was sufficient to recover most seeds. Of dung with recovered seeds, 47% of dung piles were attributed to the adult female, 21% to juveniles, and 32% of dung piles could not be reliably categorized as adult or juvenile dung. Mean GPT was 42.2 h [95% CI: 37.0, 47.4] for females, 34.8 h [28.2, 41.5] for juveniles, and 41.1 h [37.0, 45.2] for undefined dung piles. Because the dung pile categories did not differ significantly in GPT ($F_{2,106} = 1.30$, $p = 0.278$), we combined all dung to estimate mean GPT and its distribution.

From the field data, we estimated a mean GPT of 39.8 h, and a range of 16.6–113.7 h (Beirne et al., 2019). The mean GPT of tin seeds was 65.6 h (range 29.2–113.7 h). We fit the 118 observations of GPTs with a gamma distribution with shape parameter ($k = 7.6$) and scale parameter ($\Theta = 5.2$). The mean GPT from the gamma distribution was 39.5 h (95% CI: 16.6, 72.2) (**Figure 2**). Our estimates of mean GPT are similar to estimates from previous studies of captive Asian (*Elephas maximus*) and savanna (*Loxodonta africana*) elephants that include both male and female adult individuals (**Figure 2**).

## Seed Dispersal Distances

The median seed dispersal distance of all elephants combined was 5.3 km (mean = 6.5 km, 95% quantile = 16.4 km) (**Figures 3**, **4**). Forest elephants dispersed 89% of seeds farther than 1 km and 10% of seeds farther than 14.1 km. Evaluating dispersal across all 96 individual elephants, the median dispersal across all individual medians was 4.4 km and ranged from 2.3 to 6.0 km; average mean dispersal distance was 5.3 km and ranged from 2.7 to 7.1 km.

To investigate long distance dispersal by forest elephants, we estimated maximum dispersal distances. Using the mean GPT of 39.5 h from the gamma distribution (gamma distribution ranges from 0 to infinity, thus a maximum GPT cannot be defined) and the longest observed movements, we determined maximum seed dispersal distances of 101 km for a female and 87.4 km for a male elephant. Mean maximum dispersal for all 96 elephants was 40.1 km (95% CI: 20.0, 70.8).

Correlation between longitude and latitude of dispersal locations can assess the degree of asymmetry in dispersal. For several elephants, dispersal was strongly directional (max $r = 0.701$, min $r = 0.91$; **Figure 5**), but across all elephants the correlation was near 0 (mean $r = -0.09$; mean x = 0.001, mean $y = 0.005$), indicating that on average seed dispersal was symmetrical in distribution (e.g., **Figure 4**).

## Drivers of Variation in Seed Dispersal Distance

Neither human disturbance (HFI), habitat openness, nor interaction between sex and site significantly influenced median seed dispersal distances. Therefore, the final model included significant effects of exploratory behavior, sex, and site ($F_{8,87} = 5.37$, $R^2 = 0.269$, and $p < 0.001$). The measure of exploratory behavior ranged from −1.1 (idler) to 1.3 (explorer) with a median of 0. For every one-unit change in exploratory behavior, median seed dispersal increased by 0.45 km ($t = 2.41$, $p = 0.018$). Male elephants dispersed seeds 0.81 km farther than female elephants ($t = 3.98$, $p < 0.001$). Elephants at Mwagne ($t = 2.98$, $p = 0.004$), Moukalaba Doudou ($t = 2.09$, $p = 0.040$), and Wonga Wongué ($t = 2.77$, $p = 0.007$) dispersed seeds 1.0 km, 0.70 km, and 0.80 km farther than elephants at Ivindo (the base contrast).

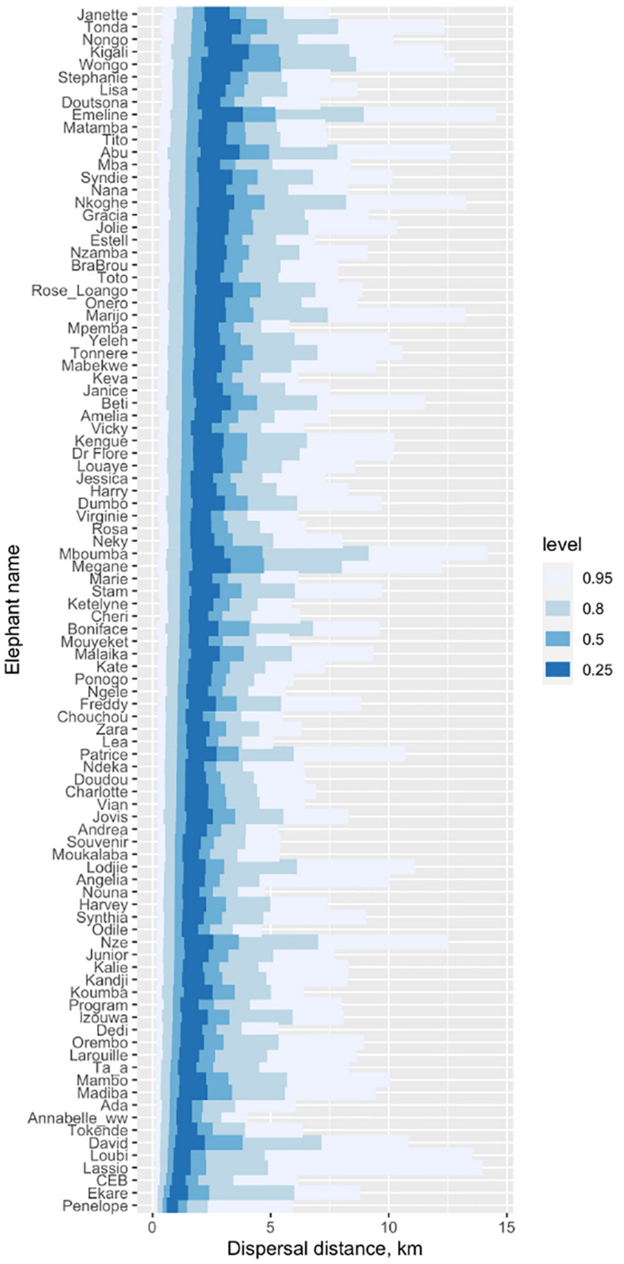

**FIGURE 3 |** Seed dispersal distances, km, for 96 individual forest elephants. Different shades of blue depict highest probability density intervals representing 25, 50, 80, and 95% of simulated dispersed seeds for each elephant.

To further assess the potential drivers of differences in seed dispersal distances among sites, we examined the proportional use of closed habitat by elephants. With approximately 99% of GPS fixes being in forest in Ivindo, Minkébé, and Mwagna NPs, compared to 81.2% in Moukoulaba Doudou, 62.6% in Loango, and 58.0% in Wonga Wongué, proportional use of forest differed significantly among sites, but not between sexes (beta regression, pseudo $R^2$ = 67.6%). The finding that males and females did not differ in their proportional use of forest lends support to our above result that differences in dispersal distances among sites are not due to the uneven sex ratios of collared elephants. However, the fact that seed dispersal distances did not vary by habitat given the large differences in use of forest among sites was surprising but indicates that other environmental differences among sites must be identified and measured.

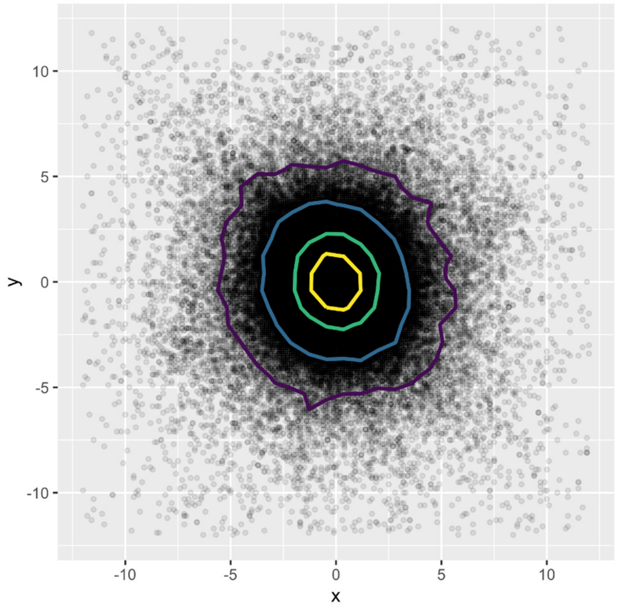

**FIGURE 4 |** Simulated seed shadow generated by forest elephants integrating GPT data and movement data from 96 elephants. x and y represent distances east-west and north-south in km. Colored lines show the location of 25, 50, 75, and 90% of dispersed seeds.

## DISCUSSION

By integrating gut passage data from wild forest elephants with movement patterns from 96 GPS-collared individuals, we support previous findings that forest elephants are important long-distance seed dispersers (Blake et al., 2009). Median seed dispersal distance was 5.3 km, with 89% of seeds dispersed farther than 1-km and 10% of seeds dispersed farther than 14-km. At average GPT, maximum simulated seed dispersal was 101 km and average maximum dispersal of all elephants was 40.1 km. These dispersal distances are well beyond the local neighborhood of parent trees, and likely contribute to gene flow between populations and colonization of new habitats. Seed dispersal distances, however, depend on both extrinsic and intrinsic variables. Median seed dispersal distances varied significantly across sites and with both the sex and exploratory behavior of elephants. Our study highlights the importance of intraspecific variation among seed dispersers; through the portfolio effect intraspecific variation in dispersal can increase the range of habitats

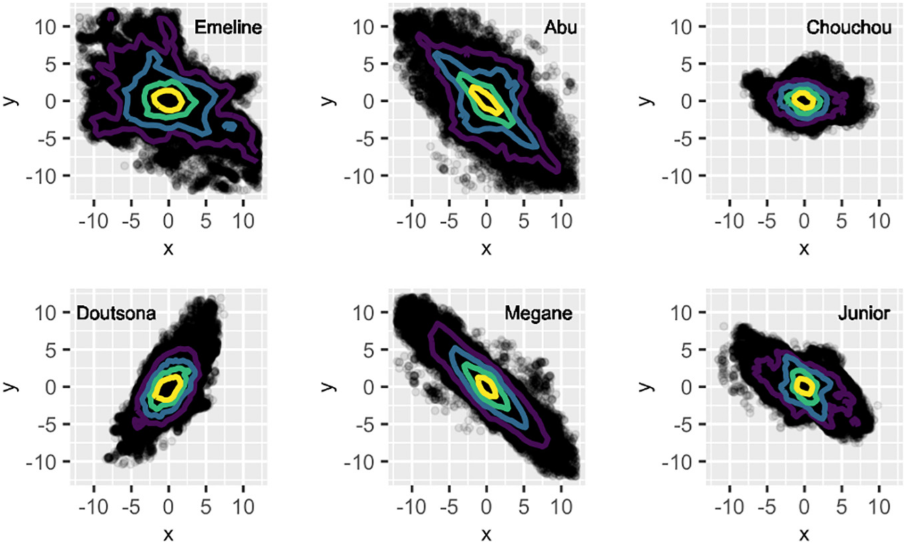

**FIGURE 5 |** Simulated seed shadows generated by individual forest elephants integrating GPT data and movement data. x and y represent distances east-west and north-south in km. Colored lines show the location of 25, 50, 75, and 90% of dispersed seeds. These individual elephants were selected to illustrate seed dispersal patterns that deviate from the overall symmetrical distribution (i.e., **Figure 4**): Emeline (*r* = –0.56), Abu (*r* = –0.71), Chouchou (*r* = –0.14), Doutsona (*r* = 0.58), Megane (*r* = –0.91), and Junior (*r* = –0.56).

and conditions where seeds are dispersed, improving the likelihood of population persistence under unfavorable events (Bolnick et al., 2011).

Among tropical animals, forest elephants play a keystone role as seed dispersers because of the high number of fruit species they consume, their slow GPTs, and relatively long dispersal distances (Blake et al., 2009; Beirne et al., 2020). Large, frugivorous birds like hornbills can fly nearly 300 miles (Holbrook et al., 2002), but their faster GPTs limit maximum seed dispersal distance to 7-km (Holbrook and Smith, 2000). Compared to other elephant species, forest elephants demonstrate longer median and maximum dispersal distances. Savanna elephants (*Loxodonta africana*) move 50% of ingested seeds over 2.5 km from the parent plant, with a maximum dispersal distance of 65 km (Bunney et al., 2017). Asian elephants (*Elephas maximus*) disperse 50% of seeds >1.2 km, with a maximum distance of 5.8 km (Campos-Arceiz et al., 2008).

The distances elephants disperse seeds vary across sites. Elephants in Wonga Wongué dispersed seeds 1-km farther on average than elephants in Ivindo. Surprisingly, even though forest elephants move faster through grasslands than other habitats (Mills et al., 2018) and two (Wonga Wongué and Moukoulaba Doudou) of the three sites with significantly longer seed dispersal than Ivindo contain extensive grasslands, seed dispersal distances were not associated with the greater presence and proportional use of open habitat by elephants. But site-level variation in dispersal distances could be caused by a multitude of factors influencing movement, including differences in landscape heterogeneity (Levey et al., 2008) and configuration (Uriarte et al., 2011), presence of clearings (baïs), topography, weather, and spatial patterns in food resources. For example, rainfall, and to a lesser extent fruit availability, predict the proportion of time forest elephants spend in long, directionally persistent movements (Beirne et al., 2020). Thus, site-specific environmental characteristics can significantly influence frugivore movement patterns, and consequently, dispersal patterns, potentially driving among population differences in plant distribution and abundance (Nuñez et al., 2019).

Despite strong effects of poaching and human activities on elephant populations and movement (Beirne et al., 2021), seed dispersal distances did not vary significantly with human disturbance. Like Markl et al. (2012), we found that dispersal distances were slightly, but not significantly, lower in disturbed areas. Failure to detect an effect might be due to our use of HFI – a relatively coarse metric of human disturbance – and because most of the focal elephants inhabited protected areas where human pressure should be weak. Our study also focused only on dispersal distance, whereas hunting and logging could affect seed dispersal by altering rates of tree visitation and fruit removal (Markl et al., 2012). Alternatively, rather than reducing movement, forest elephants might respond to human activities in other ways, such as moving nocturnally, that could have weaker effects on seed dispersal.

Seed dispersal distances of individual forest elephants vary with sex and behavior. Males tended to disperse seeds farther, consistent with their larger home ranges and lower site fidelity (Beirne et al., 2021). Sex-based effects are likely even stronger than our findings because males are large, have higher energy needs, and therefore likely consume more fruit and disperse more seeds than females. In addition, mammalian GPT tends to scale with mass (Abraham et al., 2021); thus, male GPTs should be slower than female GPTs.

Behavioral syndromes, specifically the degree to which elephants are idlers or explorers, significantly affect seed dispersal distances. The most exploratory elephants dispersed seeds 1.1 km farther than the least exploratory elephants and 0.6 km farther than elephants with balanced movement behavior. This is consistent with findings that colonization, invasion, and range expansion are often associated with bolder, more aggressive, or more exploratory animals (Duckworth and Badyaev, 2007; Chapple et al., 2012). Within-population variation in behavioral types of elephants can result in seeds being deposited in a more diverse array of places, including a greater range of distances away from parent plants. This will result in dispersal kernels with fatter tails. The same behavioral traits, like boldness and exploration, that positively correlate with dispersal distances might differentially affect other stages of seed dispersal. For example, fast explorers might be more likely to first find fruits that are larger, brighter or more abundant (Zwolak and Sih, 2020); alternatively, explorers can be poor dispersers if they collect fewer seeds before moving on (weaker area-concentrated search: Spiegel et al., 2017).

Our estimates of LDD for forest elephants are likely conservative for several reasons. First, our GPT estimates are largely derived from small pellets and large seeds likely have longer GPTs. In fact, the three large tin seeds recovered from our baiting trials had an average GPT of 65.6 h. Second, we based our estimates of maximum seed dispersal on median GPT, meaning that half of seeds would be passed more slowly and dispersed farther. Third, our GPT estimates were based on data from a single female elephant and two juveniles, which may not be fully reflective of larger, male elephants. Reassuringly, however, mean GPT from other studies and elephant species correspond closely to our estimate of mean GPT (**Figure 2**). This suggests that estimating GPT from captive elephants is an effective (and less laborious) alternative to following wild elephants in the field. Using captive elephants has the advantage of being able to control for elephant characteristics (e.g., sex, age, size, and species) and to manipulate diet items (e.g., seed size, fruit type), enabling the identification of potentially important factors that drive GPT and dispersal distances. For example, if large seeds are retained in the gut longer than small seeds (Kleyheeg et al., 2018), then elephants might provide two types of dispersal services.

Our study demonstrates the importance of forest elephants in LDD and the strong effects of extrinsic (site) and intrinsic (sex and behavioral type) variables on seed dispersal distances. It also reveals gaps in our knowledge of seed dispersal by large, frugivorous animals. The specific environmental characteristics of sites that influence disperser movements need to be investigated, particularly in the face of global climate change, habitat fragmentation, and diminishing intact wilderness. In addition, life history strategies and stages of individuals, such as phenotypic, size-, and age-related variation influence animal GPTs and movements (Byrne et al., 2019; Abraham et al., 2021). While we identified individual variation in elephant movement, individual variation in fruit consumption rates and seed deposition patterns could also strongly influence the patterns and outcomes of seed dispersal (Markl et al., 2012).

In a rapidly changing world, failure of plant species to reproduce could alter the species composition of tropical forests and reduce fruit resources to the detriment of fruit-dependent animals like forest elephants (Bush et al., 2020). Conservation of seed dispersal processes depends on protection of keystone disperser species. Thus, we need to know which habitats to conserve and possibly which individuals to protect. Male elephants, for example, are often targeted by poachers for their large tusks (Mondol et al., 2014); but bolder, more exploratory males may be more likely to disperse plant species into new or regenerating habitats. Understanding extrinsic and intrinsic drivers of seed dispersal can improve management of species critical to broader ecosystem and biosphere functioning.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

## ETHICS STATEMENT

The animal study was reviewed and approved by Duke Institutional Animal Care and Use Committee, Duke University.

## AUTHOR CONTRIBUTIONS

JP conceived the ideas and led the writing of the manuscript. CB, MB, SK, JK, WM, GO, JM, and TN collected the data. CR, JP, CB, TM, CN, LJ, and SX analyzed the data. All authors contributed critically to the drafts and gave final approval for publication and designed the methodology.

## FUNDING

The elephant collaring program was funded by a cooperative agreement from the United States Fish and Wildlife Service to ANPN. The Duke Bass Connections program generously funded the GPT research.

## ACKNOWLEDGMENTS

We thank the Gabonese Government, particularly the Agence Nationale des Parcs Nationaux (ANPN) for logistical support, permission to work in Ivindo National Park, and permission to use the elephant movement data. We also thank the Centre National de la Recherche Scientifique (CENAREST) for permission to conduct the research and Université Omar Bongo for its collaboration.

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
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ORIGINAL RESEARCH
published: 04 January 2022
doi: 10.3389/fevo.2021.761293

# Secondary Seed Ingestion in Snakes: Germination Frequency and Rate, Seedling Viability, and Implications for Dispersal in Nature

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OPEN ACCESS

***Edited by:***
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***Specialty section:***
*This article was submitted to Population, Community, and Ecosystem Dynamics, a section of the journal Frontiers in Ecology and Evolution*

***Received:*** *19 August 2021*
***Accepted:*** *24 November 2021*
***Published:*** *04 January 2022*

***Citation:***
*Schuett GW, Reiserer RS, Salywon AM, Blackwell S, Hodgson WC, Foster CD, Hall J, Zach R, Davis MA and Greene HW (2022) Secondary Seed Ingestion in Snakes: Germination Frequency and Rate, Seedling Viability, and Implications for Dispersal in Nature. Front. Ecol. Evol. 9:761293. doi: 10.3389/fevo.2021.761293*

The importance of vertebrate animals as seed dispersers (zoochory) has received increasing attention from researchers over the past 20 years, yet one category in particular, diploendozoochory, remains understudied. As the term implies, this is a two-phase seed dispersal system whereby a secondary seed predator (carnivorous vertebrate) consumes a primary seed predator or granivore (rodent and bird) with undamaged seeds in their digestive tract (mouth, cheek pouch, crop, stomach, or other organ), which are subsequently eliminated with feces. Surprisingly, although snakes are among the most abundant predators of granivorous vertebrates, they are the least studied group insofar as our knowledge of seed rescue and secondary dispersal in a diploendozoochorous system. Here, using live snake subjects of the Sonoran Desert (one viperid and two colubrid species) and seeds of the Foothill Palo Verde (*Parkinsonia microphylla*), a dominant tree of the same region, we experimentally tested germination frequency and rate, and seedling viability. Specifically, to mimic rodents with seed-laden cheek pouches, we tested whether wild-collected *P. microphylla* seeds placed in the abdomen of thawed laboratory mice and ingested by the snakes would retain their germination viability. Second, we examined whether seeds exposed to gut transit germinated at a greater frequency and rate than the controls. While we found strong statistical support for our first hypothesis, both aspects of the second one were not significant. Accordingly, we provide an explanation for these results based on specific life-history traits (dormant and non-dormant seeds) of *P. microphylla.* Our study provides support for the role of snakes as important agents of seed rescue and dispersal in nature, their potential as ecosystem engineers, and crucial evidence for the investment of field-based studies on diploendozoochorous systems in deserts and other ecosystems.

**Keywords: *Crotalus atrox*, Foothill Palo Verde, diploendozoochory, *Lampropeltis splendida*, *Pituophis catenifer*, reptiles, seed rescue, seed dispersal**

# INTRODUCTION

The significance of diverse seed dispersal systems to biotic communities, especially concerning the important role of vertebrate animals as dispersers (zoochory), has received increasing attention from researchers over the past several decades (Correa et al., 2007; Traveset et al., 2007, 2008, 2014; Schupp et al., 2010; Hämäläinen et al., 2017; Coughlan et al., 2019; Beckman et al., 2021). In the dispersal system termed endozoochory, seeds are directly consumed (e.g., by bears, primates, rodents, birds, or turtles) and later voided via regurgitation or defecation (Traveset et al., 2001, 2007, 2008; Steyaert et al., 2019). Endozoochory is prevalent in many vertebrate lineages, including freshwater fishes (Galetti, 2007), anuran amphibians (Silva and de Britto-Pereira, 2006; Hocking and Babbitt, 2014), a variety of nonavian reptiles (Blake et al., 2012; Reiserer et al., 2018; Valido and Olesen, 2019; Falcón et al., 2020), birds (Nathan et al., 2008; Heleno et al., 2011; Padilla et al., 2012; Baños-Villalba et al., 2017; Bartel et al., 2018; Blanco et al., 2018; Coughlan et al., 2019; Bravo et al., 2021), and both placental and marsupial mammals (Traveset et al., 2014; Jaganathan et al., 2016; Hämäläinen et al., 2017).

Diploendozoochory, possibly first documented by Darwin (1859), differs from endozoochory in that it is a two-phase seed dispersal system with several key players. In this system, a secondary (P2) predator (e.g., carnivorous vertebrate) consumes a primary (P1) seed predator (granivore) such as a rodent or bird with seeds in its digestive tract (e.g., mouth, cheek pouch, crop, and stomach) which are subsequently eliminated in the feces of P2 (Hämäläinen et al., 2017). In diploendozoochorous systems, when a secondary predator (P2) such as a rattlesnake subjugates and consumes a primary seed predator (P1) that has intact seeds in its cheek pouches (e.g., in our system, a heteromyid and geomyid rodent), the seeds are transported to the digestive tract of P2 and ultimately excreted. Consequently, some or all excreted seeds from P2 potentially survive this journey and germinate; thus, they can be viewed as "rescued" from P1 (post-dispersal seed predation, see Gong et al., 2015). Unlike endozoochory, our present knowledge of seed germination and the fate of dispersed seeds by P2 in diploendozoochorous systems is limited (Vander Wall and Longland, 2004; Hämäläinen et al., 2017; van Leeuwen et al., 2017). Nonetheless, with increased knowledge of the trophic behavior and ecology of carnivorous vertebrates that feed on granivores, the role of seed rescue and secondary dispersal appears to be non-trivial (Vander Wall and Longland, 2004; Hämäläinen et al., 2017; van Leeuwen et al., 2017; Pérez-Méndez and Rodríguez, 2018; Reiserer et al., 2018). Importantly, diploendozoochory can influence plant fitness in a number of ways including (a) seed transport, (b) altering the viability of transported seeds, and (c) changing the quantity of seeds that are dispersed (Hämäläinen et al., 2017; Saldaña-Vázquez et al., 2019; Rubalcava-Castillo et al., 2020). Accordingly, numerous avenues of inquiry remain to be investigated in diploendozoochorous systems which include diversity of species as agents of seed rescue and dispersal (Hämäläinen et al., 2017; Reiserer et al., 2018).

Among the terrestrial vertebrates, snakes are the least studied group with respect to seed rescue and secondary dispersal (Engel, 1997; Reiserer et al., 2018). This deficiency is somewhat perplexing and lacks a clear explanation given that they can be among the most abundant predators (high population densities and biomass per hectare) of seed-eating mammals (e.g., rodents) and birds in temperate and tropical regions (Klauber, 1972; Greene, 1997; Bonnet et al., 2002; King et al., 2018; Reiserer et al., 2018; Martins and Lillywhite, 2019; Henderson et al., 2021). And recent work on rattlesnakes indicates their potential importance as agents in diploendozoochorous systems (Reiserer et al., 2018). Specifically, in a museum study of 50 preserved rattlesnake specimens, nearly 1000 seeds were found to be indirectly ingested by way of consuming rodents possessing seed pouches, particularly heteromyids and geomyids. Careful examinations of entire digestive tracts revealed that not only were rodent-derived seeds abundant, but that numerous seeds germinated in the snakes' colons (Reiserer et al., 2018).

In North America, geomyid, heteromyid and some sciurid rodents have specialized cheek pouches for transporting seeds from plant source to cache sites, where they are often eliminated from the pool of plant propagules by consumption (Price et al., 2000; Kaufman et al., 2004; Hope and Parmenter, 2007). However, in some cases, seeds stored in these caches will germinate after a rainy season. Distances for seed dispersal in scatter-hoarding mammals (Morris, 1962) vary greatly and depend on species, size of seeds, nutrition value, season, and a host of other factors. For example, kangaroo rats, species with cheek pouches (heteromyids), typically predate, and cache seeds close to their home burrow systems (Jones, 1989; Daly et al., 1992; see Price et al., 2000; Lichti et al., 2017; Wang and Cortlett, 2017). Lifetime dispersal distances in Merriam's kangaroo rat (*Dipodomys merriami*), a common rodent in the Sonoran Desert, range from 0 to 265 m in males, and from 0 to 158 m in females (Jones, 1989).

Seed-laden rodents are commonly consumed by snakes as they forage, but unlike raptors, coyotes, bobcats, and other endothermic predators which eat rodents and are known (or implicated) to be secondary seed dispersers (Sarasola et al., 2016), the role of snakes in seed dispersal in nature remains unexplored (Reiserer et al., 2018). Nevertheless, desert-dwelling rattlesnakes and other vipers can be abundant (e.g., western diamond-backed rattlesnake, *Crotalus atrox*, more than 50 adults per km$^2$), and individuals are capable of consuming 12–20 rodent meals and potentially hundreds of seeds (Vander Wall et al., 1998) during an active season lasting 25–30 weeks. Moreover, individuals occupy large home ranges in which they sometimes travel more than 2 km within several days (Beaupre, 2016; Schuett et al., 2016; DeSantis et al., 2020). Consequently, vast numbers of seeds may potentially achieve exceptionally long secondary dispersal distances, perhaps several times greater than those distributed by the rodents themselves (Nathan et al., 2008; Wang and Cortlett, 2017). Despite the importance of the findings by Reiserer and colleagues (Reiserer et al., 2018) and the potential of snakes as seed dispersers outlined above, the most crucial ecological and evolutionary corollaries of this system hinge on the survival of voided (excreted) seeds and viability of seedlings (Hämäläinen et al., 2017).

Here, using live snake subjects, we experimentally tested germination properties in seeds of the Foothill Palo Verde (*Parkinsonia microphylla*), an ecologically important and

**FIGURE 1 |** Western Diamond-backed Rattlesnake (*Crotalus atrox*). New Mexico SR 9, near Animas, Hidalgo County, New Mexico. Photograph courtesy of William Wells.

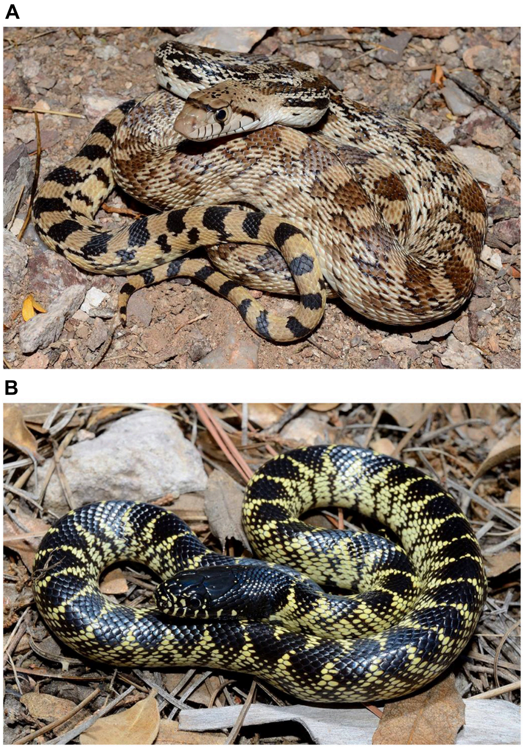

**FIGURE 2 | (A)** Sonoran Gophersnake (*Pituophis catenifer*). Vulture Mine Road, Maricopa County, Arizona. **(B)** Desert Kingsnake (*Lampropeltis splendida*). Animas Road, Hidalgo County, New Mexico. Photographs courtesy of William Wells.

dominant tree of the Sonoran Desert (Bowers, 1994, 2004). We first tested whether wild-collected seeds of *P. microphylla* placed in the abdomen of thawed laboratory mice and ingested by viperid and colubrid snakes would germinate and produce viable seedlings. Specifically, in our primary study, we tested the Western-Diamond-backed Rattlesnake, *Crotalus atrox* (**Figure 1**), a pitviper (viperid) species, based on results of a previous study (Reiserer et al., 2018). In a secondary study, we tested two species of sympatric colubrid snakes in which seed rescue and consumption are unknown but predicted to be present based on their diets, the Desert Kingsnake, *Lampropeltis splendida*, and the Sonoran Gopher Snake, *Pituophis catenifer affinis* (**Figure 2**). Second, in the primary study only, we tested whether the seeds exposed to gut transit germinated at a greater rate and frequency than those of the controls, viz. the accelerated germination hypothesis (Castilla, 2000; Traveset et al., 2001; Hämäläinen et al., 2017; Hanish et al., 2020; Salazar-Rivera et al., 2020; Yang et al., 2021).

# MATERIALS AND METHODS

## Selection of Plant Species

The Foothill Palo Verde (*Parkinsonia microphylla*), the State Tree of Arizona[1], was selected for this study for several reasons. First, detailed information on its biology and life-history is widely available, including details on germination under natural and artificial conditions (Benson and Darrow, 1981; McAuliffe, 1986, 1990; Bowers, 1994, 2004; Bowers and Turner, 2002; Medeiros and Drezner, 2012). Second, the seeds are reasonably large (several mm across) and easily manipulated for the experiments we executed. Third, wild heteromyid and geomyid rodents (seed predators) are known to harvest the seeds of *P. microphylla*, temporarily storing them undamaged in their external cheek pouches and subsequently cache them below the soil surface, such as in burrows (McAuliffe, 1990; G. Schuett, pers. observ.). Furthermore, both of these rodent groups are common prey species of snakes from the Sonoran Desert, including the rattlesnake species (*Crotalus atrox*) and colubrid species (*Lampropeltis splendida* and *Pituophis catenifer affinis*) used in this study (Schuett et al., 2016; Reiserer et al., 2018; see **Supplementary Figure 1**). Last, Foothill Palo Verde is one of the most widely distributed and abundant trees of the Sonoran Desert (Arizona and northern Mexico) and its seeds are easily harvested (Bowers, 2004).

## Experimental Protocol

Though there are many approaches to reveal the impacts of digestion on seed germination and seedling viability (e.g., Guillen et al., 2009; Benítez-Malvido et al., 2014; Gonzalez-Di Pierro et al., 2021), few studies have assessed these metrics in diploendozoochorous systems, and none have examined them in snake species. We, therefore, developed a novel approach in which seeds of *P. microphylla* for this study were collected locally (Phoenix, Maricopa County, Arizona) from mature pods of a single tree in late June 2019. In most seeds, both germination frequency and rate are dependent on numerous factors (Mitchell et al., 2017), including genetic diversity within (Hantsch et al., 2013) and among populations (Ginwal and Gera, 2000; Donohue et al., 2005). Accordingly, to explicitly decouple these aspects of germination from potential genetic effects, we chose to

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[1] http:aznps.com/arizona-state-tree

select seeds from a single source tree. The study was divided into two treatments conducted at two different locations. The primary treatment group was conducted at the Chiricahua Desert Museum (Rodeo, Hidalgo County, New Mexico) and involved using the Western Diamond-backed Rattlesnake, *Crotalus atrox,* Trials involving the secondary group were conducted at the Desert Botanical Garden. The Phoenix Zoo and involved using the Desert Kingsnake (*Lampropeltis splendida*) and Sonoran Gopher Snake (*Pituophis catenifer affinis*).

The primary study (Chiricahua Desert Museum) used two Western Diamond-backed Rattlesnake, *Crotalus atrox* (**Figure 1**), collected as juveniles in Cochise County, Arizona as neonates (summer 2017), and reared individually to adulthood (1 m in total length at time of testing, 2019–2020). We followed standard institutional protocols for housing and husbandry for these two venomous snake species (e.g., Warwick et al., 1995; Smith, 2005). Five trials per snake (10 trials in total for both snakes) were carried out on the same day from December 2019 to April 2020. In each trial the two snake subjects were fed thawed (frozen) laboratory-reared mice (40 g) obtained from a common source that were impregnated with seeds of *P. microphylla*. Specifically, seeds for the treatment and control groups were randomly selected from a common source (∼1000 seeds). For each of the treatment trials, a total of 10 seeds (5–7 mm length) were used; five seeds were placed in the abdominal cavity in each of two adult commercial white laboratory mice (40 g each) that had been thawed. Owing to restrictions and difficulties of using wild heteromyid or geomyid rodents to feed snakes at our respective institutions (e.g., potential of disease transmission; availability of appropriately sized rodents), we lacked a rodent model with cheek pouches as vehicle for intact seeds. To mitigate this problem, we used a simple method to act as surrogate to the rodent cheek pouch: a small (1 cm) incision was made at the mid-venter of each thawed lab-reared mouse; five randomly selected seeds were inserted by hand and pushed gently just beneath the skin in the abdominal cavity; the incision was then lightly pinched to prevent seeds from being expelled during ingestion by the snakes. Both snake subjects were then promptly hand-fed two seed-impregnated mice. Each snake was observed during feeding until both mice were consumed. Hence, by coupling intact seeds with the body of the rodent, we experimentally mimicked the way seeds are handled by mice in nature. Since our main goal was to test for germination viability (performance) of snake-ingested foothill palo verde seeds (*P. microphylla*), the rodent was the natural vehicle for the seeds. Thus, whether test seeds were located in cheek pouches or just beneath the abdominal skin (abdominal cavity) of the ingested rodent, the fate of the seeds remains the same in being readily exposed to the digestive processes of the test snakes. No meal was refused by the snakes during the 5-month period of testing (December 18, 2019 to April 31, 2020).

Typically, the snakes had their first defecation within 5–7 days after ingesting the two mice implanted with seeds; a second defecation occurred up to 14 days after the treatment meal. Snakes were checked 3–4 times daily to obtain seeds as quickly as possible after defecation. Wastes were carefully removed from the cages and placed into a fine metal wire strainer. Tap water was gently run to help separate the seeds from the wastes (feces and urates). All harvested seeds were placed onto damp paper toweling before being incubated for germination tests. Five control trials, each with a total of 10 seeds (total of 50 seeds), were set up the same day (within 60 min) as the snakes were fed their treatment meal. None of the control seeds were scarified or altered in any way.

## Seed Germination Protocol – Treatment and Control Groups

The *P. microphylla* seeds harvested from the feces of *C. atrox* were placed on damp paper toweling inside a plastic germination box. Each box was 28 L × 18 W × 13 H cm and had three small (7 mm) holes drilled on each side for air circulation. Furthermore, the seeds were covered with a single layer of wet (damp) white paper toweling (no dyes). The paper toweling and seeds were never allowed to become dry. The germination boxes were placed on a commercial rack equipped with 5 cm wide heat tape at one end. Each rack could hold 10 boxes. One end of each germination box was exposed to heat tape (28–30°C), which was set by an electric timer for 12D:12N. The seeds were never exposed directly to the heat tape. Air temperature inside the box during the day (12 h) was 26–30°C. During night, when the heat tape was off, air and substrate temperature was 21–24°C. Seeds used for the control trials were set up in different boxes, but were identical in all other respects to the treatment group.

The treatment and control groups of the primary study were checked 3–4 times daily for evidence of germination. Once seeds became imbibed (e.g., larger and paler in color than their original state), germination was imminent (hours). A seed was scored as germinated when a radicle was visible. Although the overall study was conducted from December 18, 2019 to August 31, 2020, a total of 257 days, each trial was allowed to run for approximately 4 months (128 days) for balance. Owing to diversified germination behavior (dormant and non-dormant seeds) in this species (Bowers and Turner, 2002; Bowers, 2004), this amount of time allotted for germination was deemed to be sufficient. For the treatment group, germination (days) was calculated as the period from the day of feeding until the appearance of the radicle.

## Seedling Viability

To determine whether the germinated seeds of *P. microphylla* in the primary study would show seedling viability, multiple germinated seeds from each of the five treatment (snake-1: $n = 19$; snake-2: $n = 12$) and control trials ($n = 14$) were sown in small pots with commercial soil and allowed to grow to approximately 13–15 cm in height. The remaining germinated seeds were preserved in 95% EtOH. Germinated seeds in the secondary study were not tested for seedling viability.

## Statistical Analyses

To test hypothesis 1, we used a binomial linear regression with germination (0 = no germination and 1 = germination) as the response variable, and treatment (snake digested and control), trial (first or second), snake (1 or 2), and defecation as explanatory variables. To test hypothesis 2, we used the non-parametric Mann-Whitney *U* test, as data were non-normally distributed, with time to germination (in days) as the response variable and treatment (as above) as the explanatory variable. The first analysis was conducted in R (R Core Team, 2021) in R Studio (R Studio Team, 2019), and the second using the open-source project JASP (Love et al., 2019)[2].

## Secondary Study

Our secondary study using *P. microphylla* seeds was executed using two species of nonvenomous colubrid (colubrine) snakes, the Sonoran Gophersnake, *Pituophis catenifer,* and the Desert Kingsnake, *Lampropeltis splendida* (**Figure 2**). Both species are inhabitants of the Sonoran Desert and sympatric with the Western Diamond-backed Rattlesnake, *Crotalus atrox* (**Figure 1**). To our knowledge, there are no publications on seed rescue and germination in colubrid snakes. Importantly, as adults, both colubrid species consume similar mammalian prey (e.g., heteromyid and geomyid rodents) as the Western Diamond-backed Rattlesnake, *C. atrox* (Holycross and Mitchell, 2021).

Trials consisted of using two species of the two colubrid snakes (as above) and the same set of seeds collected for the primary study. Snakes were maintained at The Phoenix Zoo (PZ) as part of their public outreach collections. Owing to close proximity (1.6 km) seed germination was overseen at the Desert Botanical Garden (DBG). Three trials were run: the first was from August 23 to September 03, 2019 (11 days); the second was from October 1–28, 2019 (27 days); and the third was from March 1 to June 4, 2020 (95 days) Feeding of snakes at PZ was conducted as in the primary study, but only five seeds per feeding were used instead of 10. Snakes were checked once daily for voided excreta (feces and urates) and seeds. Excreta were placed in small plastic tubes (**Supplementary Figure 2**) and transferred to the DBG (1.6 km) for germination trials. In cases where the germination tests could not be started immediately, the samples were refrigerated at 1°C until use with 24–48 h. In trials 1 and 2, the seeds were not removed from the excreta before initiating germination tests. This was done to determine if any compounds in the excreta itself inhibit or promote germination and subsequent growth. The seeds used in the third trial were gently removed from excreta and briefly washed with tap water, similar to the trials in the primary study.

All seeds (in excreta or washed) were placed on Whatman® qualitative filter paper, Grade 1, 90 mm (Sigma-Aldrich, Inc., St. Louis, MO, United States) inside a plastic petri dish and moistened with distilled water. The petri dishes were then placed into a Percival 36-L germination chamber, model GR36L (Percival Scientific, Inc., Perry, IA, United States) with a schedule of 12 h day/12 h night, 25°C day /15°C night, and 75 micromole light intensity.

# RESULTS

## Primary Study (Chiricahua Desert Museum)

In the primary study, each trial in the treatment and control groups was 128 days. Excreted seeds of *P. microphylla* successfully germinated (**Figure 3**). Specifically, in the treatment group (five trials for each of the two rattlesnake subjects), 94 of 100 seeds (94%) were recovered from the feces; 78 (83%) of those successfully germinated (**Table 1**). Mean gut-passage time of seeds was 6 to 14 days (9.1 ± 2.5 days), and in eight instances germinated seeds were recovered in snake feces, implicating germination occurred during gut transit (sensu

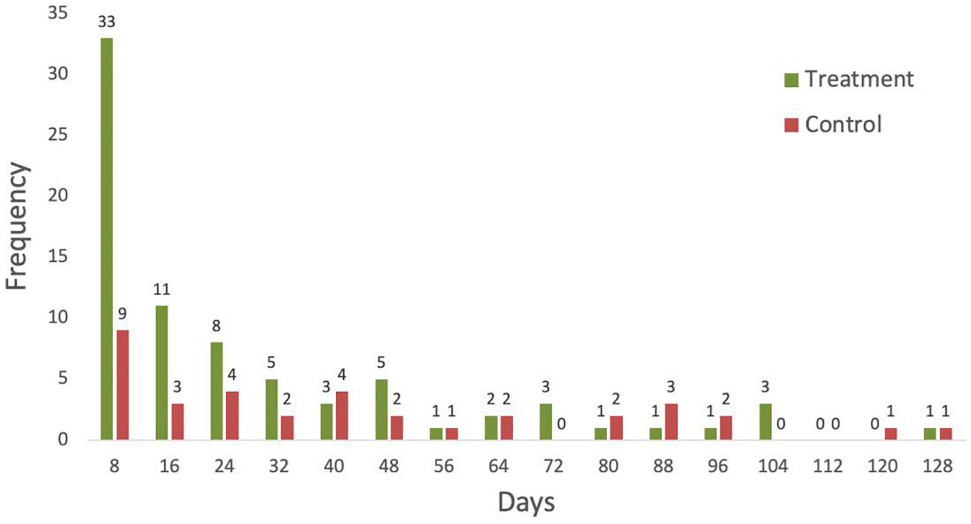

**FIGURE 3 |** Summary of germination frequency of *P. microphylla* seeds in the primary study involving the rattlesnakes (*Crotalus atrox*). Five trials were conducted and each one lasted 128 days. Germination (days) was calculated as the period from the day of feeding until the appearance of the radicle. The histogram was generated using the Histogram function in Excel version 16.48 Data Analysis Tool.

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[2] jasp-stats.org

**TABLE 1 |** Seed counts of *P. microphylla* in trial conditions of the primary study involving the Western Diamond-backed Rattlesnakes (*C. atrox*).

| Trials | Initial sample | Unrecovered from scat | Ungerminated | Germination frequency |
|---|---|---|---|---|
| Control | 50 | – | 14 | 36/50 (72%) |
| Treatment | 100 | 6 | 16 | 78/94 (83%) |

Reiserer et al., 2018). All seeds in preparation of germination (i.e., appearance of the radicle) became imbibed, swelling to twice their original size (11–13 mm length) and were noticeably paler in coloration (**Figure 4**). Of the 16 treatment seeds that did not germinate, 2 developed mold, yet the remaining 14 were normal in appearance and thus likely were viable (Bowers, 2004). In the control group (five trials), 36 of 50 seeds (72%) successfully germinated; 2 of the 14 ungerminated seeds developed mold yet the remaining 12 appeared to be viable. No significant differences were found in germination frequency with respect to any of the explanatory variables (i.e., treatment, trial, snake, and/or defecation) (**Table 2**).

Over the full duration of the experiment (128 days), mean germination time (GT) for treatment seeds was 31.9 days (SD: ± 27.2 days; min-max: 6–112 days) and 40.56 days (SD: ± 36.3 days; min-max: 2–123 days) for control seeds. No significant difference in GT was found between the treatment and control groups (Mann-Whitney $U$ test: $U = 1465.5$, $p = 0.626$, ns) (**Figure 5**). All germinated seeds ($n = 45$) that were planted (treatment: $n = 31$; control: $n = 14$) from the primary treatment group survived and were grown to a height of 13–15 cm (**Figure 6**).

**FIGURE 4 |** Seeds of *P. microphylla* post-gut transit and recovered from feces of a rattlesnake (*C. atrox*) in the primary study. The seed on the left has undergone imbibition (imbibed and swelled) and germinated (presence of the radicle) while the other seed shows no outward change in appearance from its original state despite having been exposed to the digestive tract.

**TABLE 2 |** Logistic regression results from primary study.

| Coefficients | Estimate | Std. Error | *Z*-value | *a* |
|---|---|---|---|---|
| Intercept | 2.56E+00 | 1.38E+00 | 1.853 | 0.0639 |
| Treatment | −9.42E-01 | 1.34E+00 | −0.705 | 0.4808 |
| Trial | −7.52E-02 | 1.59E-01 | −0.474 | 0.6356 |
| Snake | 1.15E-15 | 5.78E-01 | 0.000 | 1 |
| Defecation | −3.35E-01 | 5.82E-01 | −0.575 | 0.5652 |

*Testing whether or not seeds germinated with Treatment (snake digested or control), Trial (1 or 2), and Snake (1 or 2) as explanatory variables. With an alpha level of 0.05, all factors were non-significant, suggesting that snake-digested seeds were as viable as non-digested seeds.*

## Secondary Study (Desert Botanical Garden, the Phoenix Zoo)

The three trials yielded 22, 23, and 17 excreted seeds, respectively, from both snake species, and tests for germination ran for 11, 27, and 95 days, respectively. Five untreated seeds were used as controls for each of the three trials. A total of 16 seeds germinated after passing through the digestive tracts of the Sonoran Gophersnake (4 of 17 = 17.4%) and Desert Kingsnake (12 of 29 = 41.4%). Comparing trials 1 and 2, in which the feces containing the seeds were placed in the petri dishes, vs. trial 3, where seeds were removed from the fecal bolus and washed with tap water, revealed a large difference in germination frequency. Germination for the combined trials 1 and 2 were 15.6% (7/45) vs. 53% (9/17). Mold was an issue in many cases in the combined trials (**Supplementary Figure 3**), but far less so in trial 3 where the seeds were gently washed in tap water. Mold was rarely observed in the primary trials (see main text). The combined results of the three secondary trials revealed that the germination rate of the treatment seeds (16/62 = 25.8%) was not significantly different (7/25 = 28%) (Z-test, two-tailed: $Z = -0.210$, $p > 0.05$, ns) from the control seeds.

# DISCUSSION

The most important outcome in all diploendozoochorous systems, from ecological and evolutionary perspectives, is that seeds survive all steps of the process—harvested or swallowed by a seed predator, swallowed whole or stored undamaged (e.g., cheek pouches and crop), swallowed undamaged by a secondary predator, and retention of germination viability after elimination from the gut of the secondary predator (Traveset et al., 2008, 2019; Hämäläinen et al., 2017). In a study involving preserved museum specimens of rattlesnakes (Reiserer et al., 2018), seeds found in the guts of snakes were derived from heteromyid and geomyid rodents, which were likely harvested and stored unharmed in their seed pouches. Uniquely, rattlesnakes and most other snake species subjugate and consume these rodents whole and do not chew (crush); consequently, most seeds transmitted through the GI tracts of snakes from these sources do not appear to be mechanically damaged. Furthermore, owing to long retention times in the digestive tracts of rattlesnakes, some seeds are capable of germination during gut transit (Reiserer et al., 2018; see Cabral et al., 2019; Carbajal-Márquez et al., 2020).

In support of our main hypothesis, we provide the first experimental evidence, to our knowledge, that seeds of the Foothill Palo Verde (*P. microphylla*) retain germination performance (e.g., viability) when indirectly consumed by

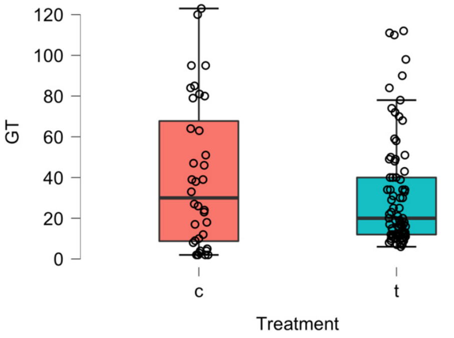

**FIGURE 5 |** Results of the primary study involving Western Diamond-backed Rattlesnakes (*C. atrox*). Box plots of mean germination times (GT - days) of *P. microphylla* seeds for control (c) and treatment (t) groups for 128 days. Horizontal line in boxes = median. Mann-Whitney *U* test: $U = 1465.5$, $P = 0.626$, ns.

**FIGURE 6 |** Examples of *P. microphylla* seedlings derived from the treatment group (snake gut transit) in the primary study involving Western Diamond-backed Rattlesnakes (*C. atrox*).

snakes—one species of rattlesnake and two species of colubrids—and subsequently recovered from their excreta (**Figures 1**, **2** and **Table 1**). Importantly, seeds found to be imbibed and germinated in excreta (feces/urates) produced healthy seedlings (**Figure 6**). In the primary study, all germinated seeds subsequently tested for seedling viability grew normally and developed into healthy plants.

Under the conditions of the primary study, both aspects of our second hypothesis (accelerated germination) were not statistically supported. The mature seeds of *P. microphylla* (located in pods) are dry and have physical dormancy ("hardseededness") resulting from densely packed layers of palisade cells impregnated with water-repellent substances which constrains imbibition of water (Baskin et al., 2000; Baskin and Baskin, 2014), a necessary step for germination. Thus, based on studies of other organisms (reviewed in Hämäläinen et al., 2017), we anticipated that gut-passage in the snakes would aid in chemical and mechanical scarification and accelerate germination frequency and rate. In other studies involving reptiles, the effects of gut passage on germination rate and frequency are mixed; in some cases both germination rate and frequency are accelerated (e.g., Yang et al., 2021), yet in others neither of these effects are observed (e.g., Castilla, 2000). Clearly, for reptiles, studies of diploendozoochory are in their infancy and it would be premature to make hard conclusions at this time based on the little available information (Hämäläinen et al., 2017; Reiserer et al., 2018). Nonetheless, the importance of positive effects of gut passage, such as described in other studies (Hämäläinen et al., 2017), cannot be overlooked and should encourage further research in this understudied area of ecology.

We provide several possible explanations for our results with respect to the second hypothesis tested in the primary study. Foothill Palo Verde (*P. microphylla*) is a species in which the seed crop is comprised of both non-dormant and dormant seeds and thus diversified germination behavior is exhibited (Bowers and Turner, 2002; Bowers, 2004). Consequently, a variable proportion (e.g., 20 to 34%) of them can germinate shortly after dispersal from their pods and, importantly, without scarification (Bowers, 2004). The dormant seeds can persist into a second season (or even longer) and require exposure to environmental scarification (high heat, rain, wind, and abrasion) for germination to occur (Bowers and Turner, 2002; Bowers, 2004). Second, in germination studies of *P. microphylla*, we suspect capturing subtle differences in germination rate and frequency will likely require a much larger sample size of seeds to achieve statistical significance for a potentially small effect. Perhaps a 10-fold increase of seeds (e.g., 1000) would be required to overcome the abovementioned problems associated with dormant and non-dormant seed types (see **Figure 5**). Finally, because wet heat appears to be an important release of dormancy in a closely related species of tree from Mexico (*Parkinsonia aculeate*), and thus likely others (Van Klinken et al., 2006), subjecting *P. microphylla* seeds to warmer conditions (e.g., 38–45°C) during germination tests in future studies is warranted.

Similar to the results of the primary study involving Western Diamond-backed Rattlesnakes (*C. atrox*) mice impregnated with seeds of *P. microphylla* were consumed by the Sonoran Gophersnake (*P. catenifer*) and Desert Kingsnake (*L. splendida*). Some of those seeds that transited the digestive tracts retained viability and germinated (see **Supplementary Figure 3**). Nonetheless, when compared to the primary study, germination frequency was significantly greater in the primary study (z-test, two-tailed: $z = 7.140$, $p < 0.001$). This result was likely due to greater trial duration in the primary study (trials were run for 128 days) and germination conditions were likely superior (e.g., less moisture, warmer, and better air circulation) for *P. microphylla*. Mold was commonly associated with seeds kept in their feces in trials 1 and 2 of the secondary study. However, we suspect that the highly moist conditions and lack of air flow in petri dishes contributed to this issue. In the Sonoran Desert, similar condition would rarely be encountered. Clearly, in the secondary study, better results were obtained when seeds were removed from the feces and urates. Germination conditions that more closely mimic natural situations (e.g., drier and warmer) are warranted in future studies, which we envision to include performing tests under a range of natural conditions (e.g., outdoor plots).

We provide herein support for the role of viperid and colubrid snakes as important agents of seed rescue and dispersal in nature, highlight their importance as potential ecosystem engineers, and offer sound justification for future field-based studies (Reiserer et al., 2018). Clearly, more research is needed, especially studies that are field-based, to provide context and to better understand the ecological and evolutionary ramifications of this fascinating, yet mostly understudied, diploendozoochorous system (Franklin et al., 2016). Although our current focus has been on organisms of North American deserts, clearly other regions (e.g., tropics) should be explored (Reiserer et al., 2018; Dugger et al., 2019). Furthermore, other plant and snake species would be desirable to study purely for inclusion of greater biodiversity and exploration of potential variation (Hämäläinen et al., 2017).

## CONCLUSION

Topics closely related to seed dispersal and their fates will need to be investigated in field-based studies of diploendozoochory in snakes. For example, despite an abundance of research on defecation sites of lizards, the closest extant relatives of snakes, similar detailed studies are nonexistent, to the best of our knowledge, for wild snakes. Unquestionably, the fate of eliminated seeds in instances of diploendozoochory by snakes and other vertebrates may be further influenced, both positively and negatively, by abiotic (e.g., rain and temperature) and biotic factors (Vander Wall and Longland, 2004). Invertebrates, for instance, such as ants (Pascov et al., 2015; Luna et al., 2018; Anjos et al., 2020) and dung beetles (D'hondt et al., 2007; Midgley et al., 2015), are common vectors in several ecosystems that disperse and alter ground-based seeds both mechanically and chemically (Franklin et al., 2016). Given that only anecdotal data are available, the ecology of defecation sites in snakes and the fate of seeds present in their feces remain open research questions. Indeed, as stated by Anni Hämäläinen and her colleagues (Hämäläinen et al., 2017, p. 13), "It is currently unknown how important the phenomenon (of diploendozoochory) is ecologically, but given its potentially vast prevalence and the possible implications, it is possible that ignoring it could impair the interpretation of broad ecological patterns or hinder conservation efforts." Importantly, it is incumbent upon us to comment that many of the vipers and other snake species that are candidate ecosystem engineers are themselves endangered for various reasons, including from direct persecution by humans (Maritz et al., 2016; Birskis-Barros et al., 2019; Fathinia et al., 2020). It is hoped that highlighting their potential new role as agents of seed rescue and secondary dispersal for deserts and other ecosystems will encourage both academic and public involvement (e.g., citizen scientists) in generating interest and legislature for their protection and long-term conservation.

## DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: Dryad Digital Repository: https://doi.org/10.5061/dryad.65gr2.

## ETHICS STATEMENT

Ethical review and approval was not required for the animal study because our study involved collecting seeds from the fecal wastes of museum and zoo snakes. No animals were harmed in this study. Written informed consent was obtained from the owners for the participation of their animals in this study.

## AUTHOR CONTRIBUTIONS

GS and RR conceived the study. GS, AS, WH, SB, JH, RZ, and CF collected the data. RR, GS, AS, and MD analyzed data. GS, RR, AS, WH, MD, and HG wrote the manuscript. All authors contributed to the article and approved the submitted version.

## FUNDING

GS was supported by the Chiricahua Desert Museum and Georgia State University. AS, SB, and WH were supported by the Desert Botanical Garden. Support for JH, RZ, and CF was by The Phoenix Zoo. HG has been most recently supported by the Lichen Fund and US National Science Foundation (OPUS 1354156).

## ACKNOWLEDGMENTS

We are grateful to our respective institutions (Chiricahua Desert Museum, Georgia State University, Desert Botanical Garden, and The Phoenix Zoo) for access to animals, plants, laboratory space, and other courtesies. Brandon La Forrest provided the photo of the snake scat sample (**Supplementary Figure 1**). Two reviewers provided helpful suggestions that improved an earlier version of the manuscript.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.761293/full#supplementary-material

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers
in Ecology and Evolution

REVIEW
published: 22 February 2022
doi: 10.3389/fevo.2022.794481

# Drivers of Ecological and Evolutionary Disruptions in the Seed Dispersal Process: Research Trends and Biases

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**Specialty section:**
*This article was submitted to Population, Community, and Ecosystem Dynamics, a section of the journal Frontiers in Ecology and Evolution*

**Received:** *13 October 2021*
**Accepted:** *18 January 2022*
**Published:** *22 February 2022*

**Citation:**
*Donoso I, Fricke EC, Hervías-Parejo S, Rogers HS and Traveset A (2022) Drivers of Ecological and Evolutionary Disruptions in the Seed Dispersal Process: Research Trends and Biases. Front. Ecol. Evol. 10:794481. doi: 10.3389/fevo.2022.794481*

As the sole opportunity for most plants to move, seed dispersal influences the biodiversity and functioning of plant communities. Global change drivers have the potential to disrupt seed dispersal processes, affecting plant communities and ecosystem functions. Even though much information is available on the effects of seed dispersal disruption (SDD), we still lack a comprehensive understanding of its main causes at a global scale, as well as the potential knowledge gaps derived from research biases. Here we present a systematic review of biotic and abiotic SDDs to ascertain the global change drivers addressed, dispersal modes impacted, plant processes affected, and spatial focus of existing research on this topic up-to-date. Although there are many modes of dispersal and global change drivers in temperate and tropical ecosystems worldwide, research efforts have predominantly addressed the effect of alien species for biotic seed dispersal in temperate systems and oceanic islands as well as how defaunation of bird or mammal dispersers has affected seed removal in the Neotropics. SDD studies were also biased toward forest ecosystems, with few in shrublands or grasslands. Finally, the effects of climate change, ecological consequences at the whole community level, and evolutionary changes were largely unrepresented in SDD studies. These trends are likely due to a combination of true geographic and ecological patterns in seed dispersal and global change and bias in research focus. We conclude that increased research investment in the less-studied systems and a better understanding of potential synergies and feedback between multiple global change drivers will be important to forecast the threats to plant biodiversity and those ecosystem functions derived from seed dispersal in the Anthropocene.

**Keywords: dispersal failure, global change, anthropogenic disturbance, non-native species, climate change, defaunation, habitat loss, fragmentation**

## INTRODUCTION

The movement of seeds away from the mother plant allows them to colonize specific microsites and new areas, reduces sibling competition and attack by natural enemies (e.g., herbivores, pathogens), and determines the potential area of recruitment, acting as a template for the subsequent stages of plant growth as well as the plant spatial patterns (Howe and Smallwood, 1982; Howe and Miriti, 2004; Jordano, 2014; Traveset et al., 2014; Rogers et al., 2021a). The dispersal vectors can be both biotic (i.e., transported in the digestive tracts, fur, plumage or feet of animals) or abiotic (i.e., transported by wind, water, or gravity). Besides moving seeds across the landscape, animals that ingest fruits and pass viable seeds through their digestive tracts can further play an important role in plant establishment as they can modify the rate of seed germination and seedling growth (Traveset and Verdú, 2002; Rogers et al., 2021b). Biotic and abiotic movement of seeds supports plant communities, the biodiversity they contain, and the ecosystem services they provide, including fruit, wood and non-timber products, in addition to enhanced carbon sequestration, at no cost to humans (Leverkus and Castro, 2017; Egerer et al., 2018; Chagas et al., 2021).

Animals play an important role as seed dispersers for more than half of the plant species around the globe (Levey et al., 2002; Dennis et al., 2007; Rogers et al., 2021a), thus contributing to numerous ecosystem functions. Birds, mammals, lizards, fish and invertebrates are common dispersers, although their relative importance varies across habitat types (Fleming and John Kress, 2011; Albert et al., 2015; Correa et al., 2015; Gómez et al., 2019; Anjos et al., 2020; Araujo et al., 2021; Rogers et al., 2021a). Within studies involving avian frugivores, most research has focused on those species dispersing fleshy-fruited plants, but many birds can serve as dispersers including waterbirds which move many wetland species (Green et al., 2016, 2021). Among mammals, key dispersers include frugivores like primates and bats which disperse seeds primarily through endozoochory (Fuzessy et al., 2018), herbivores like deer and sheep which disperse non-fleshy fruits both through ectozoochory and endozoochory (Albert et al., 2015), and rodents which typically disperse seeds through scatter-hoarding (Gómez et al., 2019).

Multiple drivers of global change have been documented to cause pervasive disruptions in the seed dispersal process (SDD, hereafter), with poorly understood consequences for the functioning of communities and ecosystems. In the case of animal seed dispersal, plants are susceptible to dispersal failure when the animal populations on which they depend decline or even become extinct (Traveset and Richardson, 2006; Traveset et al., 2012; Pérez-Méndez et al., 2016; Rogers et al., 2017; Tucker et al., 2021). Habitat loss, land use change, overhunting, and invasive species are key threats underlying animal seed disperser population declines, extinctions, and range reductions (McConkey et al., 2012; Dirzo et al., 2014). The disruption of plant-seed disperser interactions have shown to have serious consequences for plants, which can cascade to affect other species, as well as the whole community and ecosystem (reviewed in Rogers et al., 2021a). Widespread rapid changes in seed disperser assemblages—which likely translate into changes in the seed dispersal process—have occurred since prehistoric times, when humans started colonizing new regions, strongly modifying landscapes and defaunating ecosystems by hunting mainly large-body animals, leading to many megafaunal extinctions in the Pleistocene (Janzen and Martin, 1982; Malhi et al., 2016; Galetti et al., 2018). Megafauna frugivores and herbivores are still the most susceptible seed dispersers to defaunation in tropical forests (Young et al., 2016) and globally (Ripple et al., 2015; Atwood et al., 2020). Islands mostly lack megafauna, but the pattern of greater threat to larger-bodied animals holds true on islands as well, where large-bodied birds, bats, and lizards are those suffering the largest declines (Traveset et al., 2012; Pérez-Méndez et al., 2016; Kingston et al., 2021). It is important to recognize that small-bodied bird and mammal dispersers tend to be less threatened although they also have experienced declines (Davidson et al., 2009; Rosenberg et al., 2019), which could cause imperceptible but pervasive impacts on seed dispersal (Rogers et al., 2021a).

Seeds dispersed by abiotic vectors may also be disrupted by human activities (Zhang et al., 2011; Teller et al., 2015). For instance, the construction of dams influences the connectivity of riparian plant communities along rivers by affecting seed dispersal distances of hydrochorous species and strongly altering community composition (Jansson et al., 2000; Merritt and Wohl, 2006). In general, landscape structure can have strong effects on the distance that seeds travel (San-José et al., 2019) and, thus, any kind of disturbance (e.g., habitat loss, fragmentation, defaunation, or modification by a non-native species) is likely to change the patterns of seed movement and recruitment, as well as the genetic structure of plant populations. Wind-dispersed seeds, for instance, can travel much further in open landscapes than in dense forests, owing to differences in the shape of the wind profile (San-José et al., 2019). Seed dispersal disruptions (SDDs), in fact, might either increase or decrease dispersal distance depending both on the dispersal mode of the species, the global change driver, and the specific context in which the disruption occurs.

Despite the large amount of information dealing with the effects of SDD, biases in scientific research can limit our understanding of the actual main patterns of SDD at a global scale. Moreover, most studies focus on just one global change driver, when it is widely accepted that such drivers act synergistically on many processes (Tylianakis et al., 2008; González-Varo et al., 2013; Maciel et al., 2021). A previous study by McConkey et al. (2012) pointed out that such synergistic interactions between different types of disturbances could exacerbate the negative impacts on seed dispersal, although they were rarely examined (but see Portela and Dirzo, 2020). Since then, there has been a number of studies that have simultaneously evaluated the effects of more than one driver on SDD, but they remain scarce (e.g., Pejchar, 2015; Rotllan-Puig and Traveset, 2016; Brocardo et al., 2018; de Assis Bomfim et al., 2018; Qie et al., 2019; Kiel et al., 2020; Bello et al., 2021; Marone and Pol, 2021; Sales et al., 2021). Assessing regional trends in research could thus help in understanding the main patterns in the drivers and synergisms between processes that could operate at different scales.

In this study, we review and synthesize the most up-to-date evidence of different drivers of global change causing SDD. Using a comprehensive dataset of 184 studies encompassing all biogeographical regions, our goal is to: (1) identify the realms and ecosystems in which SDD are more frequently documented. Given that a higher proportion of animal-dispersed species is found in tropical areas than at higher latitudes, and given also the high rates of habitat loss in the tropics, we might expect more studies on SDD in the tropics; (2) assess which are the most commonly studied drivers behind SDD, and (3) in the case of animal-seed dispersed species, evaluate what disperser taxa are the most often studied. In addition, we aim to (4) identify general mechanisms by which the different drivers of global change interfere with the seed dispersal process. For this, we evaluate which are the response variables most often measured in SDD studies. Here we move beyond previous reviews by (i) considering both abiotic and biotic SDD, (ii) quantifying research trends accounting not only for ecological consequences but also for evolutionary disruptions and (iii) capturing the latest studies addressing potential synergistic effects among drivers. We conclude by pointing out research gaps and frontiers in the study of global change that can help mitigate SDD and thus avoid their cascading impacts.

## MATERIALS AND METHODS

We performed a thorough literature search using Web of Science (WOS)[1] and included all records from 1980 (oldest record found) until May 2021. We used the following search string "seed dispers*" AND "disrupt*" OR "seed dispers*" AND "failure*" resulting in a total of 884 records published in English-language journals. We first read titles and abstracts to exclude papers which were clearly not relevant, and then inspected 489 full texts searching for evidence of SDD. Out of these scientific publications, we developed a final database which retained a total of 184 original papers reporting at least one driver causing SDD from unique case studies (i.e., we exclude review papers). PRISMA guidelines were followed for this systematic revision (Page et al., 2021, see **Supplementary Figure 1**). Although most of the scientific publications were based on empirical data, we also considered theoretical studies aiming at modeling the effects of human impacts on seed dispersal (see e.g., Jones et al., 2017). We realize that the resulting database is not fully comprehensive since there are some articles not captured by our search terms (e.g., Donoso et al., 2020). However, we believe this compilation represents a largely unbiased sampling of the literature which allows us to identify patterns in seed dispersal research. As noted above, our search was mostly limited to English language publications, except for two studies published in Portuguese and one in Spanish that appeared in our search because their abstract was published in English. Therefore, research from regions where publications are commonly in other languages are likely under-represented in our database. Because we aim to provide a broad understanding of SDD, we focused on both anthropogenic and non-anthropogenic drivers, which were classified according to the following eight categories: (*i*) *non-native species*; (*ii*) *climate change*; (*iii*) *defaunation*; (i*v*) *habitat loss/degradation*; (*v*) *fragmentation*; (*vi*) other *anthropogenic disturbances;* (*vii*) *non-anthropogenic abiotic factors*; and (*viii*) *non-anthropogenic biotic factors*. The criteria used to classify each driver within each category is shown in **Table 1**.

In addition, we extracted the following information. First, the *bioregion* was assigned according to the 11 broad realms identified by Holt et al. (2013), with the exception of oceanic islands that were treated as a separate region (following Fricke and Svenning, 2020). Second, the *ecosystem* in which the disruption was reported was categorized according to the IUCN Habitats Classification Scheme (Version 3.1) into: forest, savanna, shrubland, grassland, wetland, desert and artificial habitat (i.e., agricultural, gardens, pastures and urban areas). We established two additional categories to consider a few cases in which we were unable to assign one of the above-mentioned-categories. These categories corresponded to agroforests (e.g., agricultural crops such as coffee or cacao under canopy of shade trees) and others, in which we included gypsum soils and dunes. Third, in those studies focused on biotic seed dispersal, we identified the *seed dispersers* under study, which were assigned according to the following eight functional groups: bats, birds, fishes, invertebrates (mainly ants, beetles and gastropods), primates, reptiles, rodents, and other mammals (e.g., carnivores, herbivores, marsupials). Mammals were classified into four functional groups given their heterogeneity in terms of functional traits and handling

**TABLE 1 |** Categorization of the main drivers causing SDD.

| Categories | Definitions |
|---|---|
| 1. Non-native species (*n* = 54) | Studies reporting introduced plant or/and animal species |
| 2. Climate change (*n* = 7) | Studies considering climate change projections, involving human-induced global warming and extreme weather events |
| 3. Defaunation (*n* = 46) | Defined as seed disperser loss, mainly due to hunting/poaching activities |
| 4. Habitat loss/degradation (*n* = 40) | This category encompasses those studies reporting deforestation, logging, reduced patch size or/and land use intensification |
| 5. Fragmentation (*n* = 39) | Studies reporting fragmentation *per se*, involving changes in habitat spatial configuration |
| 6. *Anthropogenic disturbances* (*n* = 25) | Any other disturbance derived from human activities which were not considered in categories 1–5. This category includes road constructions, urbanization and pollution |
| 7. Non-anthropogenic abiotic factors (*n* = 14) | Any abiotic factor not derived from human activity, such as wildfires |
| 8. Non-anthropogenic biotic factors (*n* = 9) | Any biotic factor not related to human activities such as herbivory, natural changes in plant spatial distribution or behavioral changes of seed dispersers |

*All global change drivers reported in the literature were classified according to eight different categories defined above. The number of studies within each category (n) is shown between brackets. Note that a single study may fall in more than one category if it reported more than one driver.*

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[1] www.webofscience.com

behavior (e.g., flying vs. non-fly ability; diurnal vs. nocturnal habits, frugivory vs. scatter-hoarding) compared to other taxa. Lastly, to explore the general mechanisms by which the different drivers interfered with the seed dispersal process, we identified the *response variables* that were assessed in each publication. To homogenize the broad terminology used among studies, we established 10 categories trying to represent the main stages of the seed dispersal cycle (Wang and Smith, 2002; Carlo and Yang, 2011; see classification in **Table 2**). This information was further interpreted based on the seed dispersal effectiveness framework proposed by Schupp (1993), and re-classified into four main categories to identify whether SDD interfered with the seed dispersal process by causing: *(i)* community changes, (*ii*) disruptions in the quantity component of seed dispersal, *(iii)* disruptions in the quality component of seed dispersal, or/and (*iv*) by triggering evolutionary changes (see **Table 2**).

We completed our dataset by including the following information: (*i*) in the case of abiotic dispersal, the main seed dispersal vector (e.g., gravity, wind, water), (*ii*) whether SDD occurred on an island, and if so, the type of island (i.e., continental vs. oceanic); (*iii*) if the study considered SDD at a species- or at community- level; and (*iv*) whether the main results were based on empirical data, simulations, or the combination of these two approaches.

To identify general trends in the study of SDD, we estimated the percentage of scientific publications (out of the 184 studies) reporting each category per variable in our dataset. However, for some of the studies we identified more than one case per variable; i.e., the authors considered more than one bioregion, ecosystem, driver, disperser or response variable within the same study. Thus, percentages can add up to >100% because each paper could contain more than one category per variable.

# RESULTS

Overall, we found studies addressing SDD in all biogeographical regions (**Figure 1A**) but they were unevenly distributed worldwide. Disruptions have been more frequently documented in the Neotropics, with almost a quarter of the publications reporting SDD in this realm (**Figure 1B**). Studies in the Paleartic are also highly represented (21%), followed by almost 15% of the studies carried out in the Nearctic. For the rest of bioregions, including the oceanic islands, disruptions in the seed dispersal process are reported in less than 10% of the publications.

Regarding the main drivers of SDD, the introduction of non-native species is the anthropogenic factor most frequently studied, with almost 30% of the publications, followed by defaunation (25%), habitat loss (∼22%) and fragmentation (∼21%) (**Figure 2**). On the contrary, the effect of non-anthropogenic abiotic and biotic factors, as well as the impact of climate change on SDD have been rarely documented, with < 8% of the publications in all three cases. Only about one fourth (26%) of the publications reported more than one driver of SDD.

Most SDD studies (75%) have been documented in forest ecosystems. By contrast, only 16 and 4% of the studies have been performed in shrublands and grasslands, respectively (**Figure 3A**). Among the functional groups assessed, disruptions involving birds were addressed in 45.6% of the studies, followed by mammals such as herbivores and carnivores (19%), rodents (16%), and primates (14%) (**Figure 3B**). While we detected that seed dispersal failure in interactions involving invertebrates occurred in almost 12% of the studies, only 4 and 1% of the publications considered bats and fishes, respectively.

The response variables most frequently measured in SDD studies were seed removal and seedling recruitment, reported in ∼30% of the publications in each case (**Figure 4**). Overall,

**TABLE 2 |** Categorization of the main mechanisms by which the different drivers of global change interfere with the seed dispersal process.

| Categories | Response variables | |
|---|---|---|
| Community changes | 1. Disperser species composition (*n* = 42) | Abundance, density of seed disperser, or functional diversity based on animal species' traits |
| | 2. Plant species composition (*n* = 30) | Abundance (including fruit production), taxonomic diversity and functional diversity based on plant species' traits |
| | 3. Network structure (*n* = 9) | Structural properties of seed dispersal networks (e.g., network specialization, nestedness, etc.) |
| Quantity component of seed dispersal | 4. Seed removal (*n* = 57) | Seed dispersal interactions, including interaction frequency, visitation or fruit removal rates |
| | 5. Dispersed seeds (*n* = 30) | Number of seeds that arrive at a site (e.g., seed rain/deposition) |
| Quality component of seed dispersal | 6. Dispersal distance (*n* = 21) | Seed dispersal kernels |
| | 7. Dispersal site (*n* = 24) | Where seeds end up or the spatial pattern of where plants grow (e.g., spatial organization, plant distribution, etc.) |
| | 8. Seed fate (*n* = 38) | Studies measuring seed germination and/or seed predation |
| | 9. Seedling recruitment (*n* = 55) | Seedling emergence and survival (e.g., seedling/sapling abundance and diversity) |
| Evolutionary changes | 10. Evolutionary changes (*n* = 21) | Genetic diversity and structure, including also changes in species' (plant or disperser) traits |

*Ten main categories were defined to account for the large heterogeneity used in the literature when describing the response variables under study. The number of studies within each category (n) is shown between brackets. Note that a single study may fall in more than one category if it evaluated more than one response variable.*

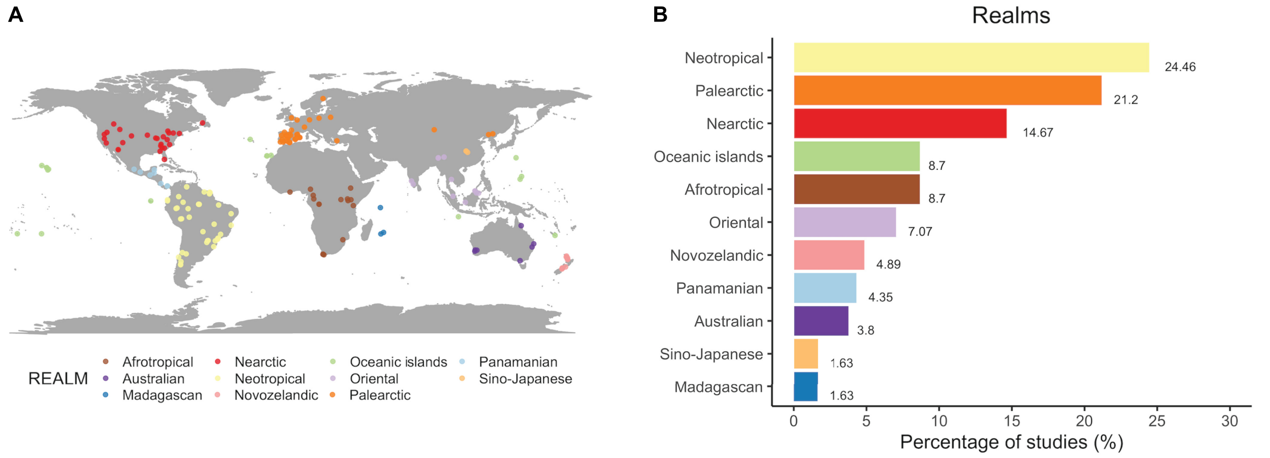

**FIGURE 1 |** The global extent of seed dispersal disruptions. **(A)** Map of the study locations, with each point representing an individual case (*N* = 186 realms identified in 184 articles). **(B)** Bar chart representing the percentage of studies addressing seed dispersal disruptions in each of the biogeographical regions.

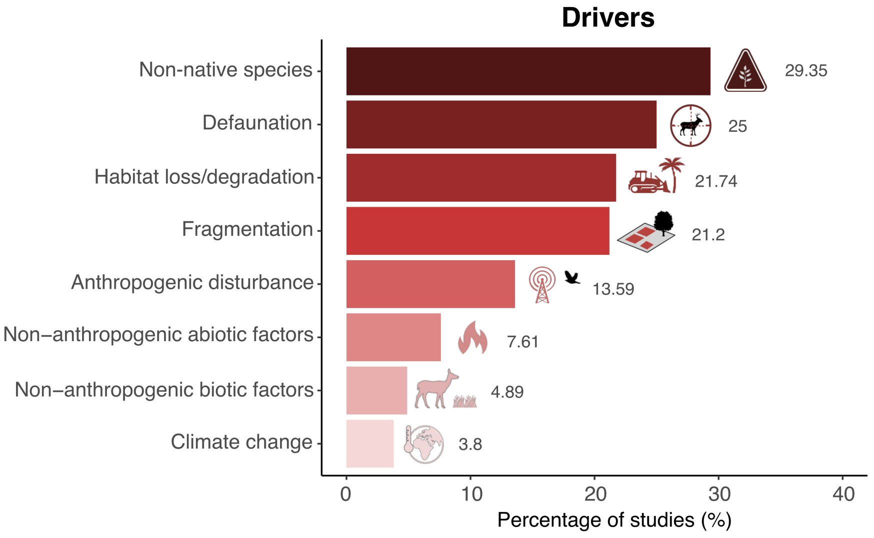

**FIGURE 2 |** Main causes of seed dispersal disruptions. Shown are the percentages of studies reporting each anthropogenic or non-anthropogenic impact as a driver of SDD. Note that the total sum of percentages exceeds 100% because some studies reported more than one driver (*N* = 237 records identified in the 184 original articles).

however, the quality component of SDD has received more attention than the quantitative component. Impacts on plant and disperser species composition were also often documented, while only a few studies (<5%) evaluated community changes in terms of network structure. Finally, a small fraction of the studies (11%) has assessed whether there are evolutionary changes involved in SDD, either from the plant or the disperser's perspective.

Although most of the studies reported biotic SDD, abiotically dispersed species have been the focus of ∼ 19% of the publications (see **Supplementary Figure 2**). In addition, regardless of the bioregion, SDD has been especially reported in mainland areas (∼70%), in studies carried out at a species-level (70%), and on empirical studies (87%) (see **Supplementary Figure 2**).

## DISCUSSION

Our review identified broad trends and research biases on SDD. The studies documented disruptions in biotically and abiotically dispersed plants, on every continent and many oceanic and continental islands, on every major biome, and involving all major groups of animal dispersers in the case of animal-dispersed plants. About two-thirds of studies have focused on the initial stages of the seed dispersal cycle—seed removal and seedling recruitment—while far fewer have dealt with the longer-term patterns demonstrated through plant community composition and evolutionary changes. Overall, about 70% of the studies focused on individual plant species, and only 30% on plant communities. We foresee that given the widespread nature of

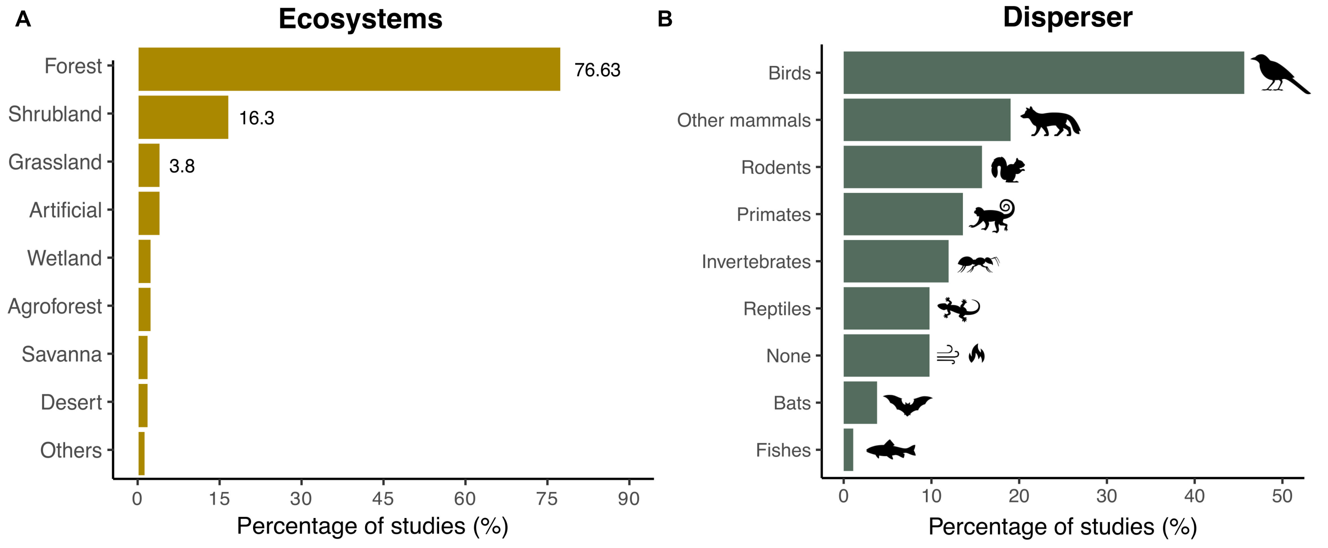

**FIGURE 3 |** Ecosystems and dispersers documented in SDD studies. Shown are the percentage of studies reporting SDD **(A)** in each type of ecosystem (*N* = 196 records) and **(B)** for each seed disperser functional group (*N* = 237 records). Note that the scale of the x-axes differs between **(A,B)**.

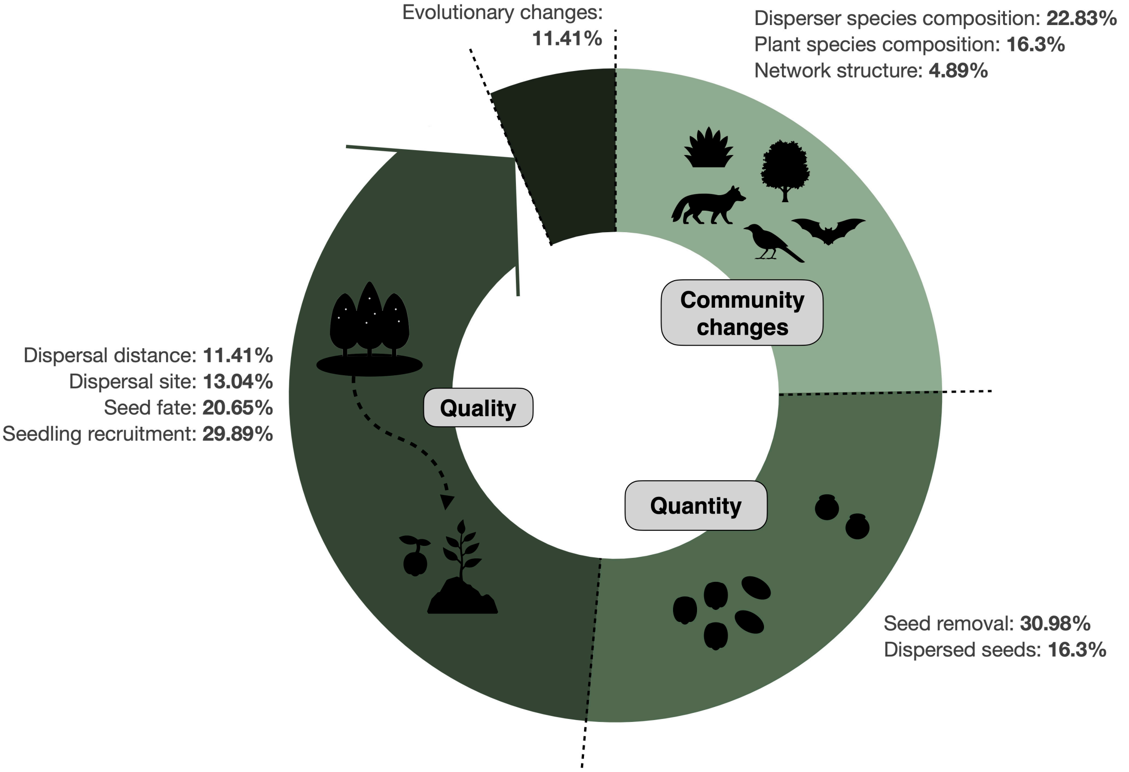

**FIGURE 4 |** Response variables measured in SDD studies. Doughnut chart illustrates how SDD occurred along the seed dispersal process (represented in the direction that happens in nature) by causing community changes, disruptions in the quantity and in the quality components of seed dispersal, as well as evolutionary changes. Shown are the percentage of studies evaluating disruptions for each individual response variable (*N* = 327 records). See **Table 2** for further details on the definition of these categories.

SDD and its impacts on the early life history stages in plant populations, there will be increasingly more evidence for changes in plant community composition and evolution.

The trends we identified in SDD research result from a combination of geographic and ecological patterns in seed dispersal and in global change, as well as bias in research focus. For example, islands have experienced more alien species invasions and native species extinctions than mainlands (Sax and Gaines, 2008; Turvey and Fritz, 2011; Blackburn et al., 2019). As such, this global change driver dominates island studies, with mainly birds and reptiles involved in such disruptions (see e.g., Traveset and Riera, 2005; Rumeu et al., 2011; Calviño-Cancela et al., 2012; Rogers et al., 2017; Fricke et al., 2018). Similarly, we found that SDD in the Neotropics is more frequently documented than in other regions. This could be due to Neotropical systems being more disrupted or to more research effort in this realm where biotic seed dispersal is prevalent (Jordano, 2014; Rogers et al., 2021a). The latter seemed to be the most likely reason given the following research evidence up-to-date. First, literature about global biodiversity sampling biases and biodiversity-ecosystem studies have found disproportionally poor tropical sampling overall (Clarke et al., 2017; Hughes et al., 2021). Second, results derived from the IPBES Global Report regarding the temporal trend of each main driver showed that neotropical systems were not under more threat than other high biodiversity regions (Balvanera et al., 2019). In contrast to the Neotropic, the Afrotropic and Oriental regions are understudied, especially given the commonness of biotic dispersal and the frequency of threatened species in these regions (Dirzo et al., 2014; Sridhara et al., 2016; Rogers et al., 2021a). This biogeographic bias mirrors the bias found in research on forest fragmentation and biological invasions, which has been attributed primarily to limited financial resources and political instability (Deikumah et al., 2014; Bellard and Jeschke, 2016; Chong et al., 2021). On the other hand, many studies on abiotic SDD have been carried out in the Palearctic and Nearctic (e.g., De hert et al., 2013; Labatore et al., 2017), probably because abiotic dispersal is more prevalent at higher latitudes (Kling and Ackerly, 2020; Rogers et al., 2021a) and because a good fraction of researchers investigating seed dispersal have home institutions in the temperate realm.

Although forests cover ∼30% of the world's terrestrial landmass (FAO and UNEP, 2020), they are by far the most common ecosystem studied with respect to SDD. This likely reflects the increased prevalence of animal-mediated dispersal in forest systems, as well as a bias toward studying biodiversity, and seed dispersal in particular, in forest systems compared to, for example, grassland systems (Hughes et al., 2021). Indeed, these results might also reflect the large body of scientific research into endozoochory by frugivores addressing how fragmentation could affect seed dispersal in multiple types of forests (e.g., Herrera and Garcia, 2010; Deikumah et al., 2014; Emer et al., 2018; Lander et al., 2019). However, grasslands cover around 40% of the Earth's surface (Bardgett et al., 2021). In grasslands, as well as in arid and wetland systems, many plants also benefit from dispersal by grazing herbivores and ants (Janzen, 1984; Wills and Landis, 2018; Anjos et al., 2020; Green et al., 2021; Hyvarinen et al., 2021). The dearth of research in non-forest ecosystems could be related to a higher proportion of abiotically (e.g., wind) dispersed species, assuming this kind of dispersal might be less limited than biotically dispersed species. Alternatively, it could also be related to the availability of suitable sites to conduct studies. In this sense, research activities could be facilitated in forest ecosystems, which usually fall within or nearby protected areas. Non-forested ecosystems, however, could be more complicated to sample, which can thus limit our global understanding of SDD.

Non-native species and defaunation were the most frequently studied global change drivers of SDD. Research efforts addressing the impact of alien species focused either on the introduction of non-native dispersers to a novel system or the loss of native dispersers caused by the introduction of non-native predators (e.g., Pejchar, 2015; Rogers et al., 2017). Biotic and abiotic seed dispersal have also been acknowledged as ecological processes that are vulnerable to climate change (Mokany et al., 2014; Kling and Ackerly, 2020). However, to date climate change has received much less attention. Given that seed dispersal is critical for species to shift ranges with climate changes (Dawson et al., 2011; Corlett and Westcott, 2013; González-Varo et al., 2021; Fricke et al., 2022), the relative lack of theoretical or empirical studies on this topic suggests SDD disruption due to climate change is an area requiring additional focus. We posit that advancing knowledge in this regard is particularly urgent given the omnipresent impact of climate change, which operates at a higher (i.e., global) scale and could thus worsen the consequences of any other driver of SDD (McConkey et al., 2012; Zhou et al., 2013; Maciel et al., 2021). For instance, one of the latest studies showed that the loss of seed dispersal interactions may impair the potential of a palm species to track climate change, especially in combination with fragmentation and forest loss (Sales et al., 2021). The handful of SDD studies found in our literature review mainly derived from empirical data concerning extreme climatic events (e.g., Zhou et al., 2013; Standish et al., 2018) or the combination of empirical data with simulations based on future climate projections and species distribution models (see e.g., Bello et al., 2021; Sales et al., 2021). More research following such an approach would thus be a promising way to provide insights into SDD under climate change, synergistic effects among drivers, and to guide management interventions for conservation and restoration.

Animal seed dispersal has been the focus of most SDD studies, although abiotic dispersal has also faced major disruptions due to habitat fragmentation, transformation, and destruction, as well as climate change. Among animal dispersers, birds and non-flying mammals are the most frequently studied groups, probably due to research bias on endozoochorous vertebrate dispersers, whereas fishes and bats are rarely studied. Only very recently, a few studies have demonstrated how overfishing may negatively affect seed dispersal, suggesting potential functional homogenization in floodplain ecosystems (Costa-Pereira et al., 2018; Araujo et al., 2021). Similarly, only a few studies showed dispersal limitation for bat-dispersed plants due to habitat loss and defaunation (Henry and Jouard, 2007; Valiente-Banuet and Verdú, 2013). This lack of SDD studies likely reflects the scant research attention of seed dispersers with nocturnal habits (Mello et al., 2011; Seltzer et al., 2013), as well as the limited knowledge on the impact of disturbances on bat's role as seed dispersers, especially in the Old World (see Aziz et al., 2021 and references therein). These results might be particularly worrisome given the non-redundant resource use and unique ecological role of bats compared to other seed dispersers (Fleming, 1987). For abiotic dispersal, on the other hand, most available information deals with wind-dispersed species that are affected by habitat loss and fragmentation (e.g., Alados et al., 2010; Bagchi et al., 2011), non-anthropogenic abiotic factors such as wildfires (e.g., Ziegenhagen and Miller, 2009; Legras et al., 2010) or how the dispersal of hydrochorous plant species is interfered with dam constructions (e.g., Merritt and Wohl, 2006).

We found that existing research addresses processes at the earliest life stages of plants to quantify outcomes of dispersal disruption on plant populations, by studying processes such as seed removal and seedling recruitment. Few studies track the effects of dispersal long enough to determine the true cost to fitness, and relatively few explore the community-level consequences of SDD. The seed dispersal mutualism can be challenging to study either observationally or experimentally, because seeds can move long distances, few seeds successfully establish and survive to reproduce, and the influence of dispersal on seed fate can take years to discern (Beckman et al., 2020). As a result, few studies comprehensively measure the impacts of SDD. Some of the best examples of population and community-level effects come from places that have experienced widespread defaunation, such as the Canary Islands (Pérez-Méndez et al., 2016), New Zealand (Bombaci et al., 2021), the Mascarenes (Albert et al., 2020, 2021), and Guam (Wandrag et al., 2017). Furthermore, few studies are able to capture evolutionary changes as a result of SDD (Galetti et al., 2013; Carvalho et al., 2016; Traveset et al., 2019). To capture the cumulative ecological and evolutionary effects of SDD on plant species and communities, research designs that match the spatial or temporal scales at which these processes operate are necessary. These may include long-term monitoring at study sites impacted by disruption to monitor change from historical baselines (Harrison et al., 2013), experimental disruption of seed dispersal and comparisons to appropriate controls (Wandrag et al., 2017; Albert et al., 2020), and observations across gradients of SDD through remote sensing or biodiversity monitoring networks (Terborgh et al., 2008).

## FUTURE PERSPECTIVES

Seed dispersal is a critical mechanism by which plants respond to environmental change (Nathan et al., 2008). This systematic review captures the latest studies addressing SDD and quantified research gaps in terms of, not only global change drivers, but also different realms, habitats, seed dispersers and response variables less often recorded. It also highlights the potential synergistic effects among drives in both biotic and abiotic seed dispersal process, which could be impacted by one or various global change drivers. Yet, feedbacks between different processes are still rarely investigated and represent an important frontier for forecasting the threats to plant biodiversity and ecosystem services in the Anthropocene. We caution that, as we enter a period of increasing environmental change due to climate change, the accrued impacts of SDD may only now manifest. Ecosystem tipping points may occur as the pace of environmental change exceeds plants' capacity to respond to these changes (Lenton, 2011). This prioritizes research to understand how seed dispersal function changes in human-modified environments that are impacted by multiple global change drivers. Quantifying the interactions among these drivers would be critical to avoid overestimating or underestimating threats to seed dispersal. A potential way forward to improve the predictability of multiple anthropogenic stressors could be the application of recently developed modeling frameworks based on the distribution of driver effects across targets and ecological scales (Simmons et al., 2021).

We further see the potential for significant advances in understanding the ecosystem services that seed dispersers provide as agents of biotic connectivity (e.g., enabling plant species to track climate change, González-Varo et al., 2021) and for their role in enhancing carbon storage in regenerating or intact forests (Bello et al., 2015). Although benefits of seed dispersal are well-studied and evidence for SDD is widespread, less research effort has focused on building an evidence base for the effective use of habitat corridors (Levey, 2005) or protected areas (Bombaci et al., 2021) to facilitate plant connectivity via seed dispersal. In addition, studying how dispersal mode influences which plant species experience SDD will help forecast biodiversity and ecosystem services. We anticipate that species that are abiotically dispersed or where humans commonly intentionally or unintentionally facilitate dispersal will be winners, to the detriment of many other plant species.

## DATA AVAILABILITY STATEMENT

The dataset generated for this study is based on the published literature and is available in Zenodo with the identifier https://doi.org/10.5281/zenodo.5794532.

## AUTHOR CONTRIBUTIONS

ID, EF, SH-P, HR, and AT assembled the data from published literature. ID prepared the figures and took the lead in writing the manuscript with substantial contributions from AT, EF, and HR and comments provided by SH-P. All authors contributed to the article and approved the submitted version.

## FUNDING

This work was supported by the Balearic Government to ID and SH-P, and the Spanish Ministry of Science to AT (project CGL2017-88122-P and PID2020-114324GB-C21).

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.794481/full#supplementary-material

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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BRIEF RESEARCH REPORT
published: 04 May 2022
doi: 10.3389/fevo.2022.794723

# Frugivore Population Biomass, but Not Density, Affect Seed Dispersal Interactions in a Hyper-Diverse Frugivory Network

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**Specialty section:**
*This article was submitted to Population, Community, and Ecosystem Dynamics, a section of the journal Frontiers in Ecology and Evolution*

**Received:** *14 October 2021*
**Accepted:** *21 March 2022*
**Published:** *04 May 2022*

**Citation:**
*Genes L, Losapio G, Donatti CI, Guimarães PR Jr and Dirzo R (2022) Frugivore Population Biomass, but Not Density, Affect Seed Dispersal Interactions in a Hyper-Diverse Frugivory Network. Front. Ecol. Evol. 10:794723. doi: 10.3389/fevo.2022.794723*

Mutualistic interactions are regulated by plant and animal traits, including animal body size and population density. In seed dispersal networks, frugivore body size determines the interaction outcome, and species population density determines interaction probability through encounter rates. To date, most studies examining the relative role of body size and population density in seed dispersal networks have examined animal guilds encompassing a narrow range of body sizes (e.g., birds only). Given non-random, body-size dependent defaunation, understanding the relative role of these traits is important to predict and, ideally, mitigate the effects of defaunation. We analyzed a hyper-diverse seed dispersal network composed of birds and mammals that cover a wide range of body sizes and population densities in the Brazilian Pantanal. Animal density *per se* did not significantly explain interaction patterns. Instead, population biomass, which represents the combination of body size and population density, was the most important predictor for most interaction network metrics. Population biomass was strongly correlated with body size, but not with density. Thus, larger frugivore species dispersed more plant species and were involved in more unique pairwise interactions than smaller species. Moreover, species with larger population biomass had the strongest influence (i.e., as indicated by measures of centrality) on other species in the network and were more generalist, interacting with a broader set of species, compared to species with lower population biomass. We posit that the increased abundance of small-sized frugivores resulting from the pervasive defaunation of large vertebrates would not compensate for the loss-of-function of the latter and the inherent disruption of seed dispersal networks.

**Keywords: frugivory, Pantanal, plant-animal interaction, seed dispersal, mutualism**

# INTRODUCTION

The Anthropocene defaunation resulting from global environmental change constitutes a driver of further biodiversity change (Dirzo et al., 2014). Large vertebrate species are more frequently affected by anthropogenic change because they are more intensively overexploited and more sensitive to disturbance than small vertebrates (Peres, 1990; Young et al., 2016). Large frugivores are potentially more important for seed dispersal networks than smaller frugivores in connecting different groups of species (Vidal et al., 2014; Guimarães, 2020), and their disappearance can lead to changes in plant communities due to disruptions in animal and plant trait-matching (Schleuning et al., 2015; Donoso et al., 2020).

Vertebrate population density decline leads to local extinctions of plant-animal interactions, including seed dispersal, even before species extinctions take place (Janzen, 1974; Redford, 1992; Säterberg et al., 2013; Valiente-Banuet et al., 2015). Extinction of interactions may be differentially affected by traits and population characteristics of the vanishing vertebrates. For example, large mammal defaunation and its associated changes in plant-animal interactions can cascade to long-term changes in plant communities, changing the structure and diversity of regeneration understories (Dirzo and Miranda, 1990; Villar et al., 2020; Villar and Medici, 2021; Souza et al., 2022), and reducing tropical forest aboveground biomass and carbon stocks (Peres et al., 2016). However, large frugivore defaunation can also lead to ecological release of smaller frugivores that could, potentially, compensate for the lack of large vertebrates (Jansen et al., 2012; but see McConkey and Drake, 2015). If seed dispersal interaction patterns are structured by animal population density, then one would expect an ecological release to compensate for such functional roles. On the other hand, if interactions are more structured by animal body size, then compensation is unlikely. Understanding to what extent smaller-sized species may compensate for the loss of larger ones is critical to predict the effects of differential defaunation (sensu Dirzo et al., 2014) on seed dispersal networks.

Vertebrate body size regulates seed dispersal interactions mainly through energy requirements, movement, and trait-matching. Larger-sized animals generally require more fruits than smaller animals to fulfill their energy requirements (Martin, 1985), which leads to foraging on a wider number of plant species, or consuming more fruits per plant (Fleming, 1991; Palacio et al., 2016). Furthermore, large-sized frugivores can consume a wider range of plant species due to their ability of swallowing both large and small-sized seeds (Jordano, 2000; Burns, 2013). For example, small-gaped birds may only be able to disperse small seeds (e.g., Galetti et al., 2013). Moreover, larger animals can forage across larger distances, having access to a wider range of fruit species (Jordano et al., 2007). In turn, animal density is expected to affect the frequency of interactions, such that more abundant species can interact more frequently and with more plant species (Vázquez et al., 2009, 2007). Furthermore, seed dispersal interactions may also be modulated by a combination of animal population density and body size (hereafter, population biomass). Populations with high biomass may represent very abundant species with small body size, non-abundant species with large body size, or abundant species with large body size. In non-hunted sites within the Brazilian Amazon, frugivore species with highest body size had stronger contributions to local population biomass, and declines in frugivore population biomass were caused by a disproportionate impact on larger-bodied frugivores, with possible consequences on seed dispersal and forest aboveground biomass (Peres et al., 2016).

Isolating the role of species density from body size in determining dispersal interaction outcomes requires observation of seed dispersal in a community that shows a natural range of density and traits, including size. However, interaction patterns have been mostly studied in communities with a low range in body size, such as bats or birds (Rezende et al., 2007; Laurindo et al., 2020). There are three potential explanations for the lack of studies in systems with a broader range in body size: (1) studies mostly take place in areas subjected to human disturbances that truncate the natural variation in body size within a community (Vidal et al., 2013); (2) a methodological focus on specific functional guilds (such as birds and bats) due to researchers' expertise or preference (Vidal et al., 2013; but see Timóteo et al., 2018); and (3) to record seed dispersal interactions from a more diverse functional guild, a suite of different methods may be required (e.g., Quintero et al., 2022).

To address this knowledge gap, we analyzed a hyper-diverse tropical seed dispersal network that comprises a frugivore assemblage of birds and mammals with a wide range of body size and population density (**Supplementary Table 1**) to assess the role of animal population density, body size and biomass in determining seed dispersal interaction patterns. In particular, we asked whether and to what extent animal body size, population density or population biomass, explain the role of species in this interaction network.

# METHODS

## Study Site and Data Collection

Data used in this study were collected in two non-defaunated sites within the Brazilian Pantanal, the largest wetland ecosystem in the world: Rio Negro (19°34′15′′S, 56°14′43′′W) and Barranco Alto farms (19°34′40′′S, 56°09′08′′W), totaling 18,500 ha. The vegetation consists of seasonally flooded savannas, semi-deciduous and gallery forests (Prance and Schaller, 1982).

Donatti et al. (2011) used various methods to detect seed dispersers and seed dispersal, interactions mediated by the vertebrate frugivore assemblage at both sites. These methods included plant focal observations to record bird seed dispersal, camera trapping below fruiting trees to record mammal and bird seed dispersal and analysis of mammalian scats (Donatti et al., 2011). Vertebrate occurrence was surveyed independently from interaction observations, using line-transects, as described in Peres (2000), that totaled 196 km (see Donatti, 2011 for a detailed description). Density estimates were obtained for ten mammal and six bird species (**Supplementary Table 1**)

**TABLE 1 |** Summary of generalized linear models for predicting the effect of population density, body size, and population biomass on seed dispersal network patterns.

| Response variables | Model | Predictors | β (Estimate) | Standard error | *z*-value (or *t*-value) | *P*-value |
|---|---|---|---|---|---|---|
| *Interaction richness* | *null* | Intercept | 2.24 | 0.15 | 14.92 | <0.001 |
| | *density* | Intercept | 2.19 | 0.18 | 11.93 | <0.001 |
| | | density | 0.09 | 0.23 | 0.42 | 0.6 |
| | *body size* | Intercept | 2 | 0.16 | 11.83 | <0.001 |
| | | **body size** | 0.33 | 0.15 | 2.15 | 0.03 |
| | *biomass* | Intercept | 1.71 | 0.23 | 7.24 | <0.001 |
| | | **biomass** | 0.38 | 0.14 | 2.67 | 0.007 |
| | *density + body size* | Intercept | 1.75 | 0.21 | 8.17 | <0.001 |
| | | density | 0.35 | 0.2 | 1.75 | 0.08 |
| | | **body size** | 0.44 | 0.15 | 2.82 | 0.004 |
| *Interaction diversity* | *null* | Intercept | 0.58 | 0.05 | 10.91 | <0.001 |
| | *density* | Intercept | 0.62 | 0.07 | 8.42 | <0.001 |
| | | density | −0.06 | 0.08 | −0.69 | 0.5 |
| | *density + body size* | Intercept | 0.78 | 0.1 | 7.45 | <0.001 |
| | | density | −0.13 | 0.08 | −1.59 | 0.1 |
| | | **body size** | −0.16 | 0.06 | −2.4 | 0.03 |
| | *biomass* | Intercept | 0.8 | 0.1 | 7.58 | <0.001 |
| | | **biomass** | −0.15 | 0.05 | −2.59 | 0.02 |
| | *body size* | Intercept | 0.67 | 0.07 | 8.94 | <0.001 |
| | | body size | −0.11 | 0.05 | −1.98 | 0.06 |
| *Interaction records* | *null* | Intercept | 3.95 | 0.27 | 14.28 | <0.001 |
| | *density* | Intercept | 3.95 | 0.33 | 11.72 | <0.001 |
| | | density | −0.001 | 0.42 | −0.004 | 0.9 |
| | *body size* | Intercept | 3.71 | 0.32 | 11.47 | <0.001 |
| | | body size | 0.32 | 0.3 | 1.05 | 0.2 |
| | *biomass* | Intercept | 3.46 | 0.43 | 7.9 | <0.001 |
| | | biomass | 0.36 | 0.28 | 1.28 | 0.2 |
| | *density + body size* | Intercept | | 0.4 | 8.33 | <0.001 |
| | | density | 3.41 | 0.42 | 0.96 | 0.3 |
| | | body size | 0.47 | 0.32 | 1.47 | 0.1 |
| *Closeness centrality* | *null* | Intercept | −1.97 | 0.04 | −46.05 | <0.001 |
| | *density* | Intercept | −2.03 | 0.04 | −42.91 | <0.001 |
| | | density | 0.12 | 0.05 | 2.07 | 0.05 |
| | *body size* | Intercept | 95.49 | 9.09 | 10.5 | <0.001 |
| | | body size | −9.4 | 7.9 | −1.19 | 0.2 |
| | *biomass* | Intercept | 114.67 | 12.93 | 8.86 | <0.001 |
| | | **biomass** | −18.39 | 7.37 | −2.49 | 0.02 |
| | *density + body size* | Intercept | 115.16 | 12.5 | 9.21 | <0.001 |
| | | **density** | −26.73 | 10.78 | −2.47 | 0.02 |
| | | body size | −17.11 | 8.5 | −2.01 | 0.06 |
| *C-score* | *null* | Intercept | 0.41 | 0.05 | 7.55 | <0.001 |
| | *density* | Intercept | 0.34 | 0.06 | 5.61 | <0.001 |
| | | density | 0.13 | 0.07 | 1.74 | 0.1 |
| | *body size* | Intercept | 0.38 | 0.06 | 5.73 | <0.001 |
| | | body size | 0.05 | 0.06 | 0.82 | 0.4 |
| | *biomass* | Intercept | 0.79 | 0.04 | 17.79 | <0.001 |
| | | **biomass** | −0.06 | 0.02 | −2.41 | 0.02 |
| | *density + body size* | Intercept | 0.78 | 0.04 | 18 | <0.001 |
| | | **density** | −0.09 | 0.04 | −2.16 | 0.04 |
| | | body size | −0.04 | 0.03 | −1.54 | 0.1 |
| *Z-score* | *null* | Intercept | 0.35 | 0.3 | 1.17 | 0.2 |
| | *density* | Intercept | 0.49 | 0.32 | 1.49 | 0.1 |
| | | density | −0.38 | 0.41 | −0.93 | 0.3 |

*(Continued)*

**TABLE 1 |** (Continued)

| Response variables | Model | Predictors | β (Estimate) | Standard error | *z*-value (or *t*-value) | *P*-value |
|---|---|---|---|---|---|---|
| | body size | Intercept | −0.46 | 0.34 | −1.37 | 0.1 |
| | | **body size** | 0.91 | 0.32 | 2.8 | 0.01 |
| | *biomass* | Intercept | −0.98 | 0.49 | −2 | 0.06 |
| | | **biomass** | 0.93 | 0.31 | 2.92 | 0.01 |
| | *density + body size* | Intercept | −0.66 | 0.47 | −1.4 | 0.1 |
| | | density | 0.29 | 0.49 | 0.59 | 0.5 |
| | | body size | 1.00 | 0.37 | 2.69 | 0.01 |

*Significant variables are shown in bold, and models selected based on ΔAICc and significance are shaded.*

by dividing the number of animals from a particular species observed by the surveyed area. For each animal species, the total area surveyed was computed by multiplying the distance surveyed by the average distance of animals of that particular species from the transect (Sutherland, 1996). Animal species body size was obtained from Fonseca et al. (1996), Mata et al. (2006), and Van Perlo (2015), and species population biomass was calculated as a product of each species body size and its local density. As we only had density estimates for 16 animal species, we used a subset of the original network presented in Donatti et al. (2011) when analyzing the relationship between animal traits and network metrics. However, we calculated network metrics based on the full seed dispersal network (see section "Data Analysis").

## Data Analysis

To assess the relative importance of animal population density, body size and biomass in structuring seed dispersal networks, we first calculated species-level metrics based on the entire Donatti et al. (2011) network comprising 46 animal species of birds, mammals, reptile and fish (excluding only the exotic feral pig *Sus scrofa*), and 46 plant species. We computed the following species-level interaction metrics: interaction richness (i.e., the number of plant species each frugivore consumed), interaction records (i.e., interaction events, or the total number of feeding records for each animal species), interaction diversity (i.e., the diversity of plant species consumed by each animal species), closeness centrality (i.e., how connected – through direct or indirect pathways – each animal species is to other species in the network), c-score (i.e., how evenly distributed are the interactions of a given species across all modules in the network, in which a module is a semi-independent cohesive group of interacting species) and z-score (i.e., a standardized measure of the number of interactions each species has within its own module). For these computations we used the *bipartite* package (Dormann, 2011) in R version 4.1.1 (R Core Team, 2021).

We then used these species-level interaction metrics as response variables. We used log-transformed population density, body size and biomass as explanatory variables. We applied log-transformation to predictor variables due to data skewness and ecologically meaningful outliers. First, we assessed the correlations between predictor variables using Pearson's correlation coefficient. Population density and species body size ($r = -0.32$, $t = -1.29$, df = 14, $p$-value = 0.2), and population density and population biomass ($r = 0.35$, $t = 1.41$, df = 14, $p$-value = 0.1) are not significantly correlated. Population biomass and species body size are correlated ($r = 0.74$, $t = 4.15$, df = 14, $p$-value $< 0.001$), but these two predictor variables were not simultaneously included in any model. Because metrics were calculated using the full network in Donatti et al. (2011), species roles were investigated in the context of the entire sampled seed dispersal interacting community.

We used generalized linear models (GLMs) with Negative Binomial error distribution for interaction richness and interaction records due to overdispersion, and Gamma error distribution for interaction diversity and z-score (continuous, non-negative, and positively skewed data), and Gaussian error distribution for closeness centrality and c-score. We built models with increasing complexity, from null models with only the intercept, to models testing the interaction between body size and density. Models were fitted using R version 4.1.1 (R Core Team, 2021), and selected based on the ΔAICc < 2.0 threshold.

# RESULTS

Within the studied seed dispersal network, frugivore species showed a wide range of population densities (0.1–36 individuals/km$^2$), body sizes (0.07–240 kg), and population biomasses (0.1–337 kg/km$^2$) (**Figure 1**). The set of 16 bird and mammal species included in this study dispersed 31 plant species (**Figure 2**) and, notably, large-bodied species such as the tapir (*Tapirus terrestris*) and the white-lipped peccary (*Tayassu pecari*) showed more frequent interactions than small-bodied species. Indeed, according to our models, body size and population biomass were better predictors of seed dispersal interaction patterns than population density (**Supplementary Figure 1**) in this non-defaunated frugivore community (**Figure 3**). However, relatively smaller-sized species, such as the toco toucan (*Ramphastos toco*) also interacted with a high number of plant species.

Body size (GLM, $p = 0.03$, $z = 2.154$, $n = 16$, $R^2 = 0.227$; AICc = 101.58) and population biomass ($p = 0.007$, $z = 2.674$, $n = 16$, $R^2 = 0.303$; AICc = 99.93) were equally good predictors of interaction richness (ΔAICc = 1.65; **Table 1**; **Supplementary Table 2**), although population biomass explained a slightly larger proportion of the variance in the response variable. Population biomass was the best predictor of interaction diversity (GLM, $p = 0.02$, $t = 0.05$, $n = 16$, $R^2 = 0.415$) and the

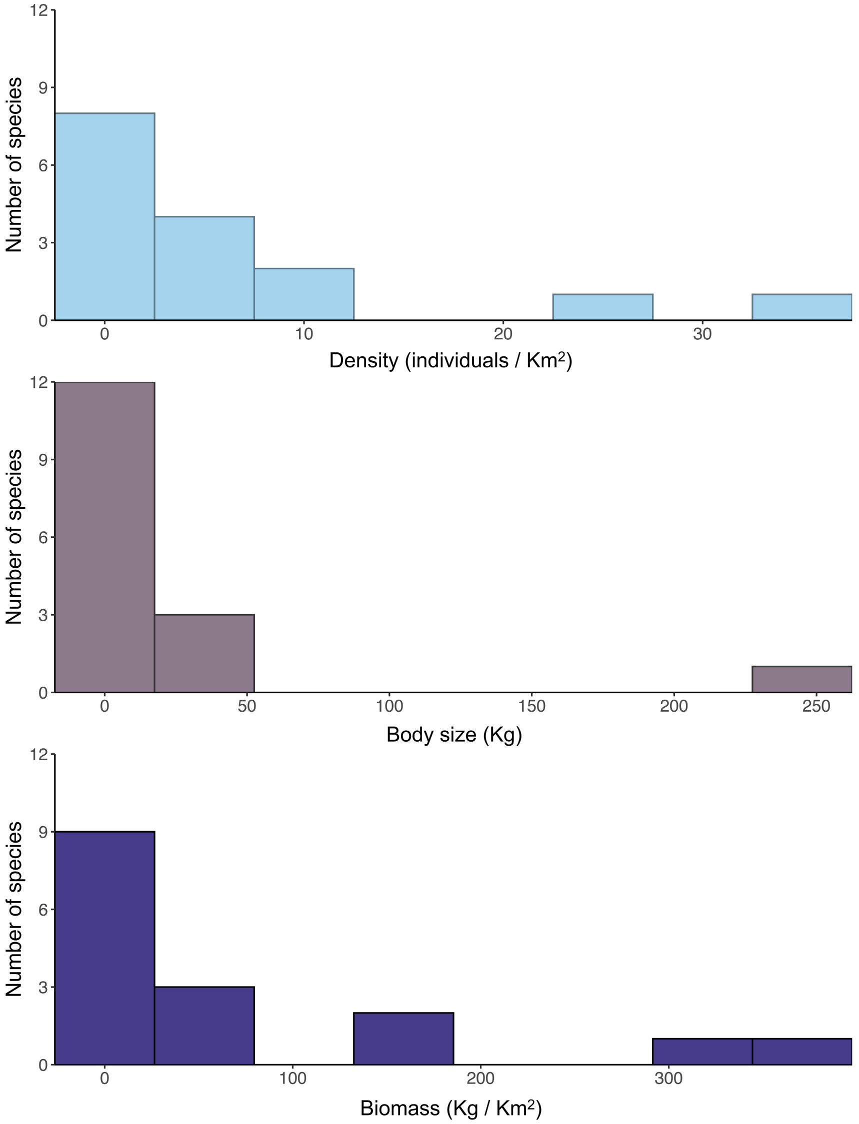

**FIGURE 1 |** Frequency distribution of studied frugivore population density, species body size and population biomass of frugivores from the Brazilian Pantanal, including ten mammal and six bird species.

c-score (GLM, $p = 0.02$, $t = 0.04$, $n = 16$, $R^2 = 0.264$), that represents the participation of each species in a network. A model with only population biomass (LM, $p = 0.03$, $t = 2.29$, $n = 16$, $R^2 = 0.221$) and a model that included both body size and density (LM, $p = 0.03$, $n = 16$, $R^2 = 0.309$) equally predicted closeness centrality, that is how connected each species is to other species in the network. Thus, as population biomass is a function of body size and density, these results support the notion that total biomass is a key factor structuring the role of frugivorous species in the network. Finally, population biomass (GLM, $p = 0.01$, $t = 0.31$, $n = 16$, $R^2 = 0.238$) and body size (GLM, $p = 0.01$, $t = 0.32$, $n = 16$, $R^2 = 0.326$; AICc = 40.56) were equally good predictors of the z-score.

## DISCUSSION

Recent studies highlight the role of frugivores in ecosystem functioning, but little is known about whether and how differences in animals body size and population density explain the structure of seed dispersal networks and compensate for the defaunation of large vertebrates. In a non-defaunated landscape from the Brazilian Pantanal we examined a frugivory community that comprises a wide range of population densities and body sizes across 16 native vertebrate species. We found that frugivore population density itself is not a good predictor of seed dispersal patterns and seed dispersal network structure. In contrast, both body size, and population biomass (which is a combination of density and body size) were

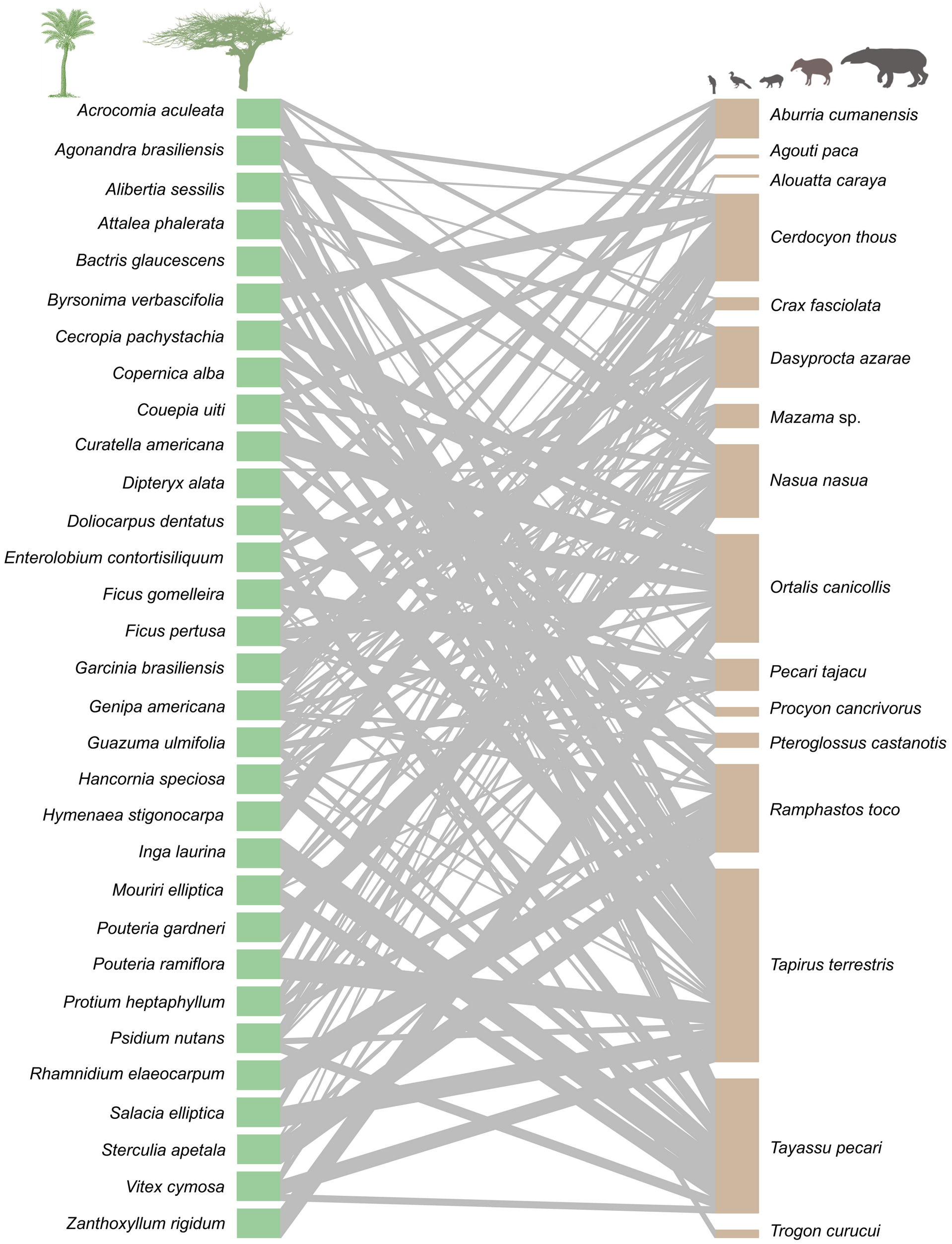

**FIGURE 2 |** Seed dispersal network at two nearby sites in the Brazilian Pantanal. Each green rectangle represents one plant species, and each brown rectangle represents an animal species. Gray lines represent links between species, meaning that fruits from the plant species on the left were consumed by the linked animal species on the right, and the width of the lines indicates the strength of the interaction. This network includes bird and mammal species for which population density was estimated. Species are sorted in alphabetic order.

found to significantly influence the role of frugivores in the seed dispersal network (**Figure 2**). In this same community, there is a strong association between species body size and population biomass, whereas the correlation between species density and population biomass is weak. More broadly, our results indicate that large frugivores are more important for the structure of this seed dispersal network, even if these species are not abundant. In general, these species consumed more fruit species (interaction richness), consumed more fruit species in a higher frequency (interaction diversity), were more central in the interaction network (closeness centrality) and were more generalist (c- and z-score). However, besides this general pattern, smaller-sized frugivores, such as the Chaco chachalaca (*Ortalis canicollis*) were found amongst the top-three frugivores for each of the metrics described above.

Other studies have pointed to the importance of functional traits, such as body size (e.g., Vidal et al., 2013) or neutral-based processes, such as species density (Krishna et al., 2008; Vázquez et al., 2009; Laurindo et al., 2020) in structuring species

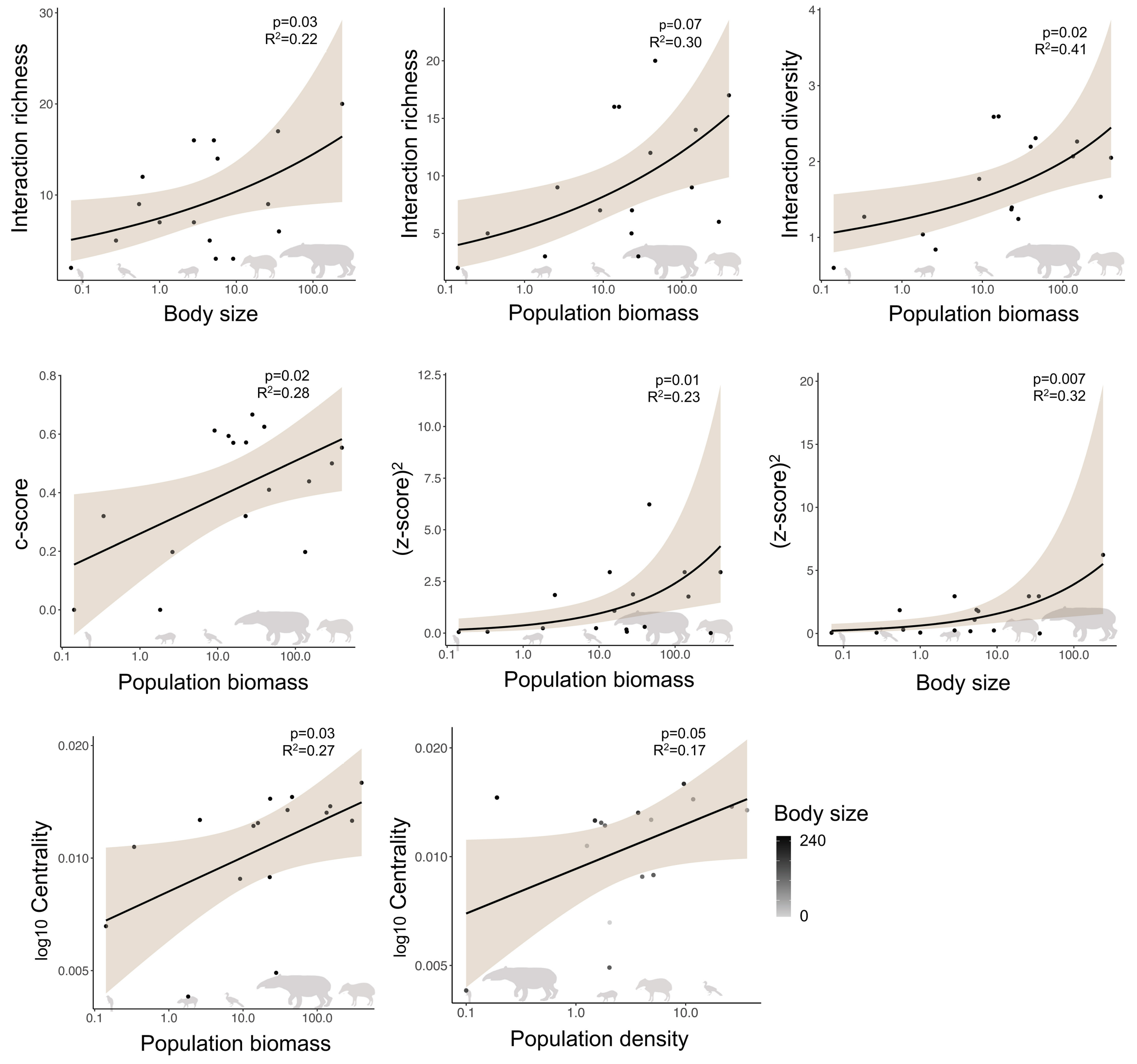

**FIGURE 3 |** Relationship for significant predictors of the structure of a seed dispersal network in the Brazilian Pantanal, according to generalized linear models. Shaded areas depict 95% confidence intervals. Body size is measured in kg, population biomass is measured in kg/km$^2$, and population density is measured by individuals/km$^2$.

interactions. In the context of defaunation, where large-sized, and often rarer animals, tend to be extirpated in higher frequencies (Dirzo et al., 2014; Bogoni et al., 2020), and smaller-sized animals tend to become more abundant (Peres, 1990), addressing this size-biased defaunation may provide relevant insights into the potential compensatory effect of smaller animals in seed dispersal networks. In the studied non-defaunated seed dispersal network, there are many large-seeded plant species which could not be dispersed by smaller frugivores if larger frugivores were extirpated (Donatti et al., 2007). Elsewhere, studies have found that compensation may also be unlikely due to frugivore and seed size matching (Donoso et al., 2017) and when seed dispersal compensation occurs, it may not translate into plant recruitment being comparable to before defaunation levels (Culot et al., 2017). The fact that density was not as important as body size or biomass in structuring interactions implies a low potential for compensatory effects. This low possibility for compensating the loss-of-function arises as abundant animals *per se* were not found to be as important to the seed dispersal patterns as species with high population biomass and high body size. In other words, even if numerous, small frugivores will not be enough to replace the functions once accomplished by larger frugivores. This finding is of conservation significance as the lack of potential for compensatory effects in defaunated communities could cause lower dispersal and regeneration of several plant species. Examining the consistency of this finding across multiple seed dispersal networks (in conserved and differentially defaunated sites) is an aspect that warrants further research.

Out of the top three most important frugivores in each interaction pattern, only the Chaco chachalaca bird is a Least Concern species according to the IUCN Red List. All other species are listed as Near Threatened, Vulnerable, or Critically Endangered. For example, population declines of large mammals, such as IUCN Vulnerable, specifically species that show larger population biomass such as peccaries (*Tayassu pecari*) and lowland tapir (*Tapirus terrestris*), are unlikely to be compensated for in their seed dispersal roles by abundant, but smaller animals, such as blue-crowned trogons (*Trogon curucui*). On the other hand, it would be important to examine if other types of compensatory effects might occur where some of these large mammals experience population declines (e.g., Williams et al., 2021). Many of them are also seed predators and herbivores (e.g., Dracxler and Kissling, 2021; Mittelman et al., 2021) and some plant species may be released from these pressures, with subsequent cascading effects on the overall plant community (Martínez-Ramos et al., 2016). In addition to that, seed dispersal effectiveness is a complex process that depends on quantity and quality of dispersal and includes many interaction steps, such as the number of seeds dispersed, number of seeds dispersed per visit, gut passage effect on germination, and deposition site suitability, among others (Schupp et al., 2010). The interaction network depicted in this study only considers the quantity component of seed dispersal effectiveness. Future studies that examine compensatory effects in seed dispersal networks should consider the wide range of quality components of seed dispersal effectiveness to better assess the potential for functional replacements. However, our results support the notion that smaller seed dispersers cannot compensate the loss of larger-bodied vertebrate seed dispersers.

Given the low support for compensatory effects in this seed dispersal network, we posit that conservation actions should target areas where a wide range of frugivore functional traits is extant and promote such species' reintroductions where they have been extirpated. Clearly, the relative role of species density and body size in structuring interaction networks continues to be a fundamental research agenda in ecology (Guimarães, 2020). The results we uncovered in this work suggest that similar studies, comparing defaunated and non-defaunated contexts considering a wide-range of disperser densities and body sizes, may provide further insights on the consequences of the omnipresent size-biased defaunation that characterizes the Anthropocene.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

## ETHICS STATEMENT

Ethical review and approval was not required for the animal study because we used data previously collected and available.

## AUTHOR CONTRIBUTIONS

LG, PRG, and RD conceptualized this study. CD conducted the fieldwork and participated in former discussions. LG and GL conducted the analyses and wrote the first draft. All authors contributed to the writing of the article and approved the submitted version.

## FUNDING

This work was supported by the Gabilan Stanford Graduate Fellowship (LG), Dirzo Lab's unrestricted funds (RD), GL was supported by the Swiss National Science Foundation (PZ00P3_202127), CD fieldwork and research was supported by FAPESP (2004/00810-3 and 2008/10154-7), Earthwatch Institute and Conservation International. PRG received funding from CNPq (307134/2017-2), FAPESP (2018/14809-0), and the Royal Society, London (CHL/R1/180156).

## ACKNOWLEDGMENTS

Members of the Dirzo Lab, and attendees at the 2020 Species Interactions Workshop (Stanford University/University of California, Santa Cruz) provided discussion and feedback that helped develop this manuscript.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.794723/full#supplementary-material

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