ARTICLE

Divergent seed dispersal outcomes: Interactions between seed, disperser, and forest traits

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Abstract

Animals disperse seeds in various ways that affect seed deposition sites and seed survival, ultimately shaping plant species distribution, community composition, and ecosystem structure. Some animal species can disperse seeds through multiple pathways (e.g., defecation, regurgitation, epizoochory), each likely producing distinct seed dispersal outcomes. We studied how seed traits (size and toughness) interact with disperser species to influence seed dispersal pathway and how this ultimately shapes the proportion of seeds deposited in various habitat types. We focused on three frugivorous species of duikers (African forest antelopes) in the Dja Faunal Reserve, a tropical rainforest in southern Cameroon. Duikers can both defecate and regurgitate seeds, the latter predominantly occurring during rumination at their bedding sites (or "nests"). We located duiker nests and dungs along 18 linear 1-km-transects to assess: (1) how seed traits affect the likelihood of dispersal via defecation versus regurgitation, (2) if defecated versus regurgitated seeds are deposited at different rates in different forest types (assessed by indigenous Baka),

microhabitats, and forest structural attributes (measured by drone lidar), and (3) if these differ between three duiker species that vary in size and diel activity patterns. We found that duikers predominantly defecated small seeds (<3 mm length) and regurgitated larger and tougher seeds (>10 mm length), the latter including 25 different plant species. The three duiker species varied in their nesting habits, with nocturnal bay duikers (*Cephalophus dorsalis*) nesting in dense understory vegetation at proportions 3–4 times higher than Peter's and yellow-backed duikers (*Cephalophus callipygus* and *Cephalophus silvicultor*). As a result, bay duikers deposited larger regurgitated seeds at a higher rate in habitats with denser understory where lianas and palms predominate and near fallen trees. This directed regurgitation seed deposition likely plays an important and unique role in forest succession and structure. This study highlights the importance of ungulate seed dispersal by regurgitation, a vastly understudied process that could impact many ecosystems given the prevalence of ruminating ungulates worldwide.

INTRODUCTION

Many plants rely on animals for seed dispersal, making seed dispersers critical determinants of plant distribution, diversity, and vegetation structure (Willson & Traveset, 2000). However, not all seed dispersers are equal. Animals differ in the types and quantity of seeds they can disperse and in the spatial patterns of seed deposition (or "seed shadows") based on their movements, habitat preferences, and behaviors (Schupp et al., 2010; Van Leeuwen et al., 2022). While variations in dispersal functions have been linked to animal species or even to groups within species (e.g., sex or age), less is understood about how dispersal services can vary within species when they provide different dispersal pathways based on plant traits (Rehm et al., 2019; Zwolak, 2018). Some animals provide multiple pathways for seed dispersal (e.g., defecation, regurgitation, on fur) depending on fruit and seed traits like size, shape, and toughness. Ultimately, these pathways may result in distinct seed shadows and microhabitat deposition rates (Baltzinger et al., 2019; Delibes et al., 2019). Here we compare seed dispersal via defecation and regurgitation by ruminating ungulates and study how seed traits interact with animal behaviors to produce diverging dispersal outcomes.

There is substantial work linking animal ecology to dispersal rates, deposition sites, and seedling survival. Different disperser species create distinct seed shadows based on removal quantity, distance traveled, habitat selection, and behaviors (Martínez et al., 2008). For example, bellbirds are more likely to disperse seeds in forest gaps and farther distances than some other tropical bird species, resulting in lower rates of fungal infections to seedlings (Wenny & Levey, 1998). Scatter-hoarding agoutis move seeds to areas with lower densities of adult conspecific trees (Hirsch et al., 2012), and pinyon mice are more likely to disperse seeds in small-particle soils (Pearson & Theimer, 2004). Hornbills and communal fruit bats aggregate seeds under nests or roosts, causing an increase in competition (Deshpande & Kelkar, 2015; Kitamura et al., 2004). There are even individual-level variations within animal species based on physiological and behavioral differences between sexes, age groups, or individual personalities (Zwolak, 2018).

Less is known about the variation in dispersal services from single animal species offering different dispersal modes. There are various mechanisms for animal-mediated seed dispersal, including (1) defecation: Seeds are ingested and defecated intact, (2) regurgitation: Seeds are fully ingested and later moved back up and spat out, and (3) epizoochory: Seeds are carried on body surfaces like fur or hooves. Interestingly, individual animals of certain species can facilitate multiple dispersal pathways (Baltzinger et al., 2019). Fruit and seed traits (e.g., size, shape, toughness) interact with animal traits (e.g., size, behaviors, digestive system) to influence the probability of seeds being dispersed via a specific pathway (Figure 1A; Albert, Mårell, et al., 2015). For example, an

A. Conceptual flowchart for seed pathway
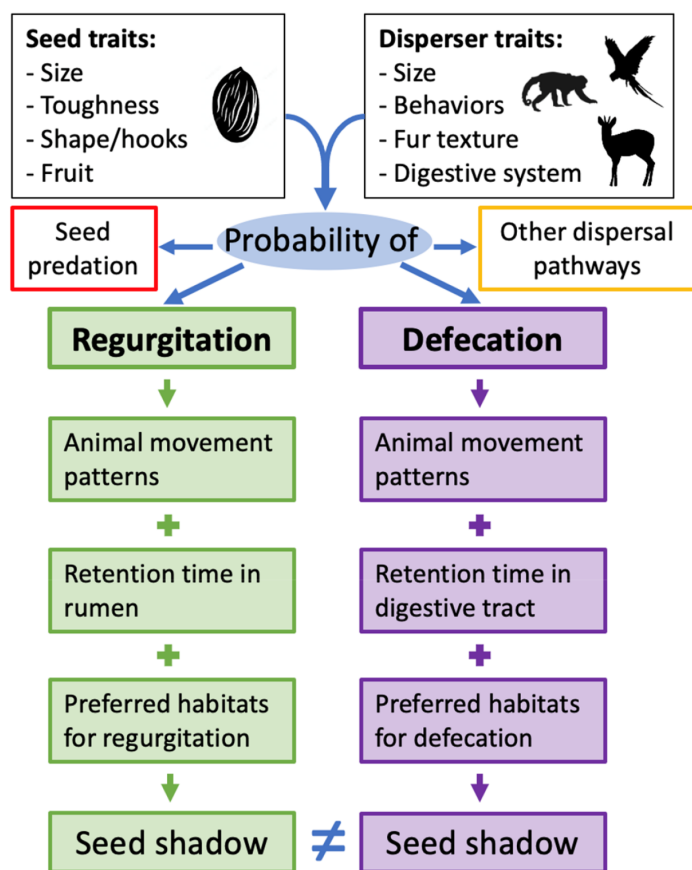
B. Hypothesis for duiker divergent dispersal
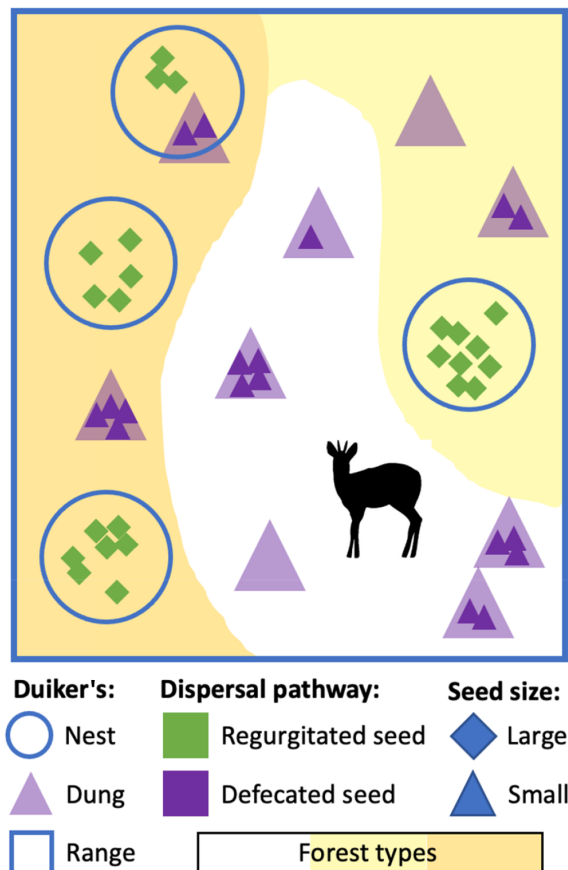
FIGURE 1 Seed and disperser traits interact to shape seed deposition sites. (A) Flowchart describing how seed and disperser traits affect seeds' pathways, resulting in different seed shadows. Green coloring indicates regurgitation pathway, while purple indicates defecation pathway (coloring is consistent in all figures). (B) Hypothesis for distribution of seeds deposited by duikers based on dispersal pathway, and how it may be non-random in relation to forest types.

animal may regurgitate large hard seeds at resting sites and also defecate small seeds while on the move. If these behaviors occur in different proportions across habitats, then each dispersal pathway may produce unique seed shadows, even if through the same individual disperser.

Ruminating ungulates are particularly likely to disperse seeds via both defecation and regurgitation. Ruminants are diverse, ubiquitous across most terrestrial ecosystems, and often exert strong ecological impacts on the local flora (Hackmann & Spain, 2010). Ruminant species like wild cattle, goats, antelopes, and deer swallow food without much chewing, ferment it in their stomach chamber, and then regurgitate it back to their mouth for additional chewing of the cud. While chewing the cud, they can spit out hard particles such as seeds. However, ungulate seed regurgitation is vastly understudied and often overlooked in seed dispersal reviews (Albert, Auffret, et al., 2015; Delibes et al., 2019; Parolin et al., 2013).

We studied seed dispersal by ruminating ungulates in the Congo Basin rainforest. Duikers are a common and

diverse group of frugivorous forest antelopes widely distributed in Africa. Duikers are important seed dispersers in Afrotropical forests, where up to 90% of tree species are dispersed by animals (Houngbégnon et al., 2023; Osuri et al., 2016). Their diets comprise a wide range of fallen fruits supplemented with other items like softer chewable seeds and foliage (Feer, 1989b). Duikers ruminate at their bedding sites (henceforth "nests"), where regurgitated seed piles have been observed (Feer, 1995). Duiker seed dispersal depends on which seeds are regurgitated versus defecated, and on where these behaviors occur.

We tested how seed traits (size and toughness) influence the probability of regurgitation versus defecation and whether these pathways affect the distribution of seeds deposited in different forest types, microhabitats, and forest structure. We conducted linear 1 km transects to locate duiker nests and dungs and collected data on seeds, duiker species, forest type (indigenous Baka classifications), and microhabitat. We also used drone

lidar technology for forest structure. We predicted that: (1) Larger, tougher seeds would be regurgitated in nests, while smaller seeds would be defecated; (2) Duiker nests would be located in the forest type with denser understory or near specific microhabitats (e.g., liana thicket, fallen tree) at a higher rate than dungs, which would be present at a higher rate in more open understory forest types and microhabitat in comparison with nests; (3) The proportion of nests in different forest types would be highly dependent on the duiker species, with the nocturnal (day-nesting) species opting for thicker understory for concealment and safety.

METHODS

Study site

The Dja Faunal Reserve in southeast Cameroon covers 526,000 ha of tropical rainforest classified as a UNESCO World Heritage Site (Figure 2B). The reserve consists predominately of primary lowland tropical rainforest, raffia palm swamps, and is interspersed by rocky grassy inselbergs. It harbors over 100 mammal species, 350 bird species, and 270 tree species (Manfred & Oum Ndjock, 2020). Notably, it contains multiple endangered megafaunas including African forest elephants (*Loxodonta cyclotis*), western lowland gorillas (*Gorilla gorilla gorilla*), and central chimpanzees (*Pan troglodytes troglodytes*). The Dja Faunal Reserve is surrounded by villages inhabited by Bantu and Baka communities. Although hunting in the Reserve is prohibited, uncontrolled hunting and other illegal activities remain. The forest in the reserve is relatively intact but encroaching agriculture and logging are increasingly putting pressure on habitats and animals (Amin et al., 2023). We conducted our sampling around the Bouamir Research Station, which is accessible by a 26-km footpath.

The indigenous Baka people describe the forest around Bouamir station with five forest type categories, which we recorded for each nest and dung: (1) Mandja = forest with closed canopy and relatively open understory due to the limited light passing through the canopy, making it easier to walk through. (2) Woundo = forest with semi-closed canopy and semi-closed understory. (3) Étobé = dense understory often composed of lianas and/or palms, making it difficult to walk through. This forest type often has a sparser tall tree canopy.
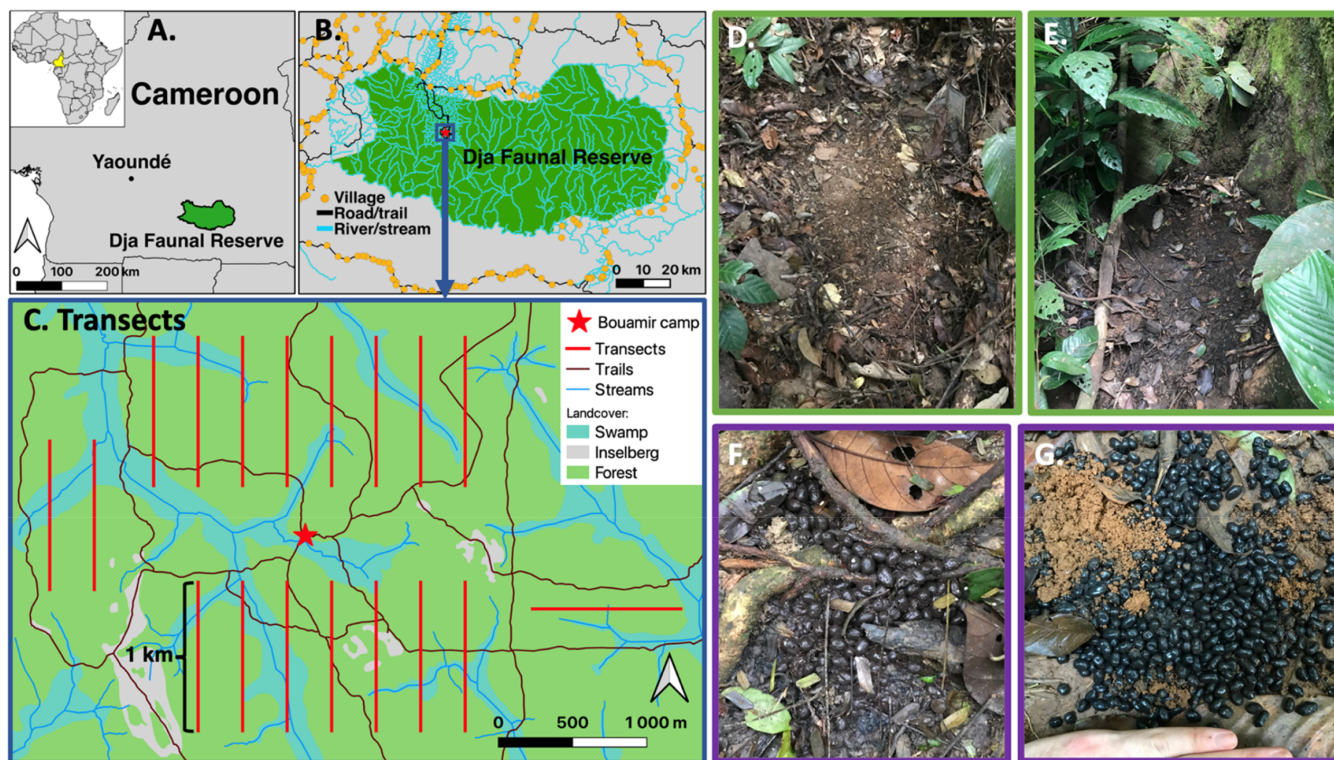
FIGURE 2 (A, B) The Dja Faunal Reserve in southeastern Cameroon is part of the Congo Basin rainforest (MINFOF, 2017). (C) Study area and the 18 transects we conducted to monitor duiker seed dispersal. (D) Peter's duiker bedding site, or "nest," in an "open" microhabitat. (E) Yellow-backed duiker nest in "buttress roots" microhabitat. Duiker nests often contain seed piles because it is where much of their rumination and seed regurgitation occur. (F) Red duiker (Peter's or bay) dung. (G) Yellow-backed duiker dung (dirt mound is a sign of beetles burying some of the dung). Photo credit: Bastien Dehaudt.

(4) Njambo = swamp with *Raphia* palms, and (5) ndoumbo = swamp edges without *Raphia* palms. To simplify, we combined njambo and ndoumbo into one category we named "swamp."

Study species

We targeted three duiker species present in the Dja Faunal Reserve: Peter's duikers (*Cephalophus callipygus*), bay duikers (*Cephalophus dorsalis*), and yellow-backed duikers (*Cephalophus silvicultor*). The bay and Peter's duikers are similar in size, weighing 15–25 kg, while the yellow-backed duiker is larger, weighing 60–80 kg. We did not include the abundant blue duiker (*Philantomba monticola*; ~4–5 kg) because we had low confidence in identifying its nests. Peter's are diurnal and nest at night, while bay duikers are nocturnal and nest during the day. Yellow-backed duikers can be active day and night. Peter's typically create a new nest daily, while bay and yellow-backed duikers often reuse the same nest several times (Feer, 1989a). Peter's and bay duikers are relatively abundant, while yellow-backed duikers are less common. The white-bellied (*Cephalophus leucogaster*) and black-fronted duikers (*Cephalophus nigrifrons*) are exceedingly rare in this reserve (Amin et al., 2022), so we ignored these species in the nest and dung analyses and recognize this may slightly affect corresponding estimates on similar-sized bay and Peter's duikers. Forest antelopes such as duikers are a major source of local bushmeat and are the most frequently hunted species in the region (Avila Martin et al., 2020). As a result, some populations have suffered steep declines (Newing, 2001; Van Vliet et al., 2007).

Transects for duiker nests and dungs

We conducted systematic linear transects to sample duiker nests and dungs from November to December 2022, at the end of the long wet season and beginning of the long dry season. Transects were 1000 m in length and approximately 15 m wide (three observers were spaced 5 m apart). Transects were spaced evenly and sought to sample habitats randomly and in proportion to their frequency in the landscape (Figure 2). For each nest and dung encountered, the duiker species was identified by indigenous Baka research assistants based on nearby tracks in combination with nest and dung traits. We grouped dungs of Peter's and bay duikers as "red duiker dungs" due to difficulties identifying them at the species level (Van Vliet et al., 2008) and assumed no differences in defecation between the two species. In line with our objectives, we recorded (1) nest and dungs coordinates, duiker species, and forest type, (2) nest microhabitat (relatively open, fallen tree, buttress root, or liana thicket) and dungs microhabitat (on or off animal trails), and (3) we collected all seeds from nests and a portion of dungs for further data collection on seed quantity, traits, and species. We noted any signs of germination at time of initial collection. In addition to transects, we also conducted opportunistic surveys (non-linear, filling gaps in sampling areas), noting the same observations. These surveys increased the sample size for seed traits and germination experiments, but not for habitat analyses.

Seed collection and dung processing

We collected all regurgitated seeds from duiker nests. We also collected some duiker dungs, based on available time for daily processing, to look for defecated seeds within them. First, we measured pellet size and counted pellet number per dung to estimate an average for each duiker species. For each collected dung, we washed a three-pellet sample through 0.5×0.5 mm mesh to look for small seeds. For the remaining dung pellets, we either sieved them through a larger mesh (3×1 mm) to look only for larger seeds or we used them for dung germination trials.

Measuring seed dispersal and seed traits

We counted intact seeds in nests and in dung samples, which we considered dispersed. We recorded the following traits for all intact seeds found in nests and dungs: dispersal pathway (regurgitated or defecated), seed size (length, long-width, and short-width), seed coat (too tough to manually break vs. not), and species or morpho-species if identification was impossible. We did not attempt to identify species, toughness, or short-width for seeds smaller than 2 mm. For each nest, we recorded the total number of regurgitated seeds per plant species and calculated the average size of seeds it contained. For dung, we recorded the average size of seeds found and we extrapolated total seed number based on the number of seeds in three-pellet samples and the average total number of pellets counted for each duiker species. To calculate the estimated means and 95% confidence intervals of seed quantities and sizes we used linear modeling and the predict() function in R and ANOVA followed by a Tukey test for comparisons (R Core Team, 2023). To test for differences in locations, we used Pearson chi-square testing on contingency tables containing the number of dungs and nests in each forest type for each duiker species.

Lidar-derived forest structural attributes

We calculated 3D vegetation structure data using UAV-LiDAR measurements of the Bouamir Research Site conducted in March 2022. We normalized the lidar 3D point cloud by removing the effect of topography, and we generated 10-m-resolution maps of structural attributes of interest: canopy height, leaf area index (LAI), and plant area volume density in the understory (from ground to 5 m up; henceforth "understory plant density"). Because we recorded the Baka-defined forest type at each nest and dung site, we compared vegetation structural attributes between Baka-defined forest types using one-way ANOVA followed by a Tukey test.

Duiker habitat selection for nests and dungs

We conducted a Resource Selection Analysis (similar to Davies et al., 2019) to determine whether each duiker species selects for 3D vegetation structural attributes (from lidar) when nesting or defecating. We selected variables likely to affect ground-dwelling animals or seed germination (elevation, canopy height, LAI, understory plant density). We generated 100 evenly spaced points for each transect and extracted structural attributes at each point using the "amt" package in R (Signer et al., 2019). We scaled and centered all variables and used generalized linear models with a binomial response to compare habitat characteristics of observed versus generated points. Duikers' habitat use may change between wet and dry seasons, but we were unable to investigate this.

RESULTS

## Duiker bedding sites and dungs

We found clear evidence that all three duiker species disperse seeds via regurgitation and defecation. We located a total of 78 nests and 124 dungs across both transect and opportunistic surveys. Of the 78 nests, 54 (69%) possessed at least one regurgitated seed, averaging 11.4 seeds per nest. There were 25 seed species regurgitated across all nests, with each nest containing an average of 1.2 different seed species. Per duiker species, we found 24 Peter's nests (15 with seeds = 63%; 3.3 average seeds per nest; seven seed species), 45 bay nests (30 with seeds = 67%; 10.4 average seeds per nest; 18 seed species), and 9 yellow-backed duiker nests (9 with seeds = 100%; 38 average seeds per nest; 13 seed species).

Yellow-backed deposited significantly more seeds in their nests than the other two species (ANOVA: $F_{2,75} = 5.78$, $p = 0.005$). Duikers dispersed more seeds of certain fruit species over others. The most abundant regurgitated seed species in nests (genus: *Vitex*) accounted for 47% of all regurgitated seeds (Figure 3). We found one *Ricinodendron heudelotii* seed germinating within a nest.

We located 95 red duiker (Peter's + bay) and 29 yellow-backed duiker dungs. On average, red duiker dungs contained 239 pellets of 10.2×6.1 mm, while yellow-backed dungs contained 458 pellets of 12.7×8.0 mm. We were able to collect 65 of these dungs (47 red duiker and 18 yellow-backed) to search for seeds. In all 65 three-pellet samples, we found 60 defecated intact seeds (38 from red duikers and 22 from yellow-backs). Although 63% of samples did not contain viable seeds, it averaged to 0.9 intact defecated seeds per three-pellet sample (0.8 for red duikers and 1.2 for yellow-backed). When multiplying with the average pellet number in dung, we estimated a total average of 93 seeds per duiker dung (64 for red duikers and 183 for yellow-backed). Using the larger sieve on remaining dung material, we found 16 defecated seeds >3 mm in three out of 41 dungs. Of these, 14 came from a single yellow-backed duiker dung (largest: 9.8×4.7 mm) and two from two different red duiker dungs (largest: 6.5×6.3 mm). See Appendix S1: Table S1 for a complete summary of the data.

Regurgitated and defecated seed traits

Regurgitated seeds were significantly larger than defecated seeds for all three duiker species (average size: $18.4 \times 14.0 \times 11.1$ mm vs. 1.4×0.9 mm respectively; linear model: effect size $= -31.88$ mm, SE $= 1.52$, $t_{83} = -21.01$, $R^2 = 0.84$, $p < 0.0001$; Figure 4A). Average seed size did not differ significantly between our three duiker species for regurgitation nor for defecation (two-way ANOVA; Figure 4B). All the regurgitated seeds we found in duiker nests had tough seed coats and were predominantly of drupe fruiting type, a.k.a. stone-fruits, except for a couple of leguminous seeds and 63 tiny (2.2×1.2 mm) berry-type seeds with a watery membrane found inside one bay duiker nest. We found the largest regurgitated seed (by length + width), a slender *Canarium schweinfurthii* seed measuring $41.1 \times 13.8 \times 13.5$ mm, in a bay duiker nest. The largest regurgitated seeds for Peter's and yellow-backed duikers were $29.6 \times 18.6 \times 10.0$ mm and $35.2 \times 15.3 \times 15.1$ mm, respectively. The seed with the largest width (important for swallowing) was an *Antrocaryon klaineanum* seed measuring $25.7 \times 23.4 \times 13.1$ mm found in a yellow-backed's nest (largest duiker species). Most defecated seeds measured under 2.5 mm
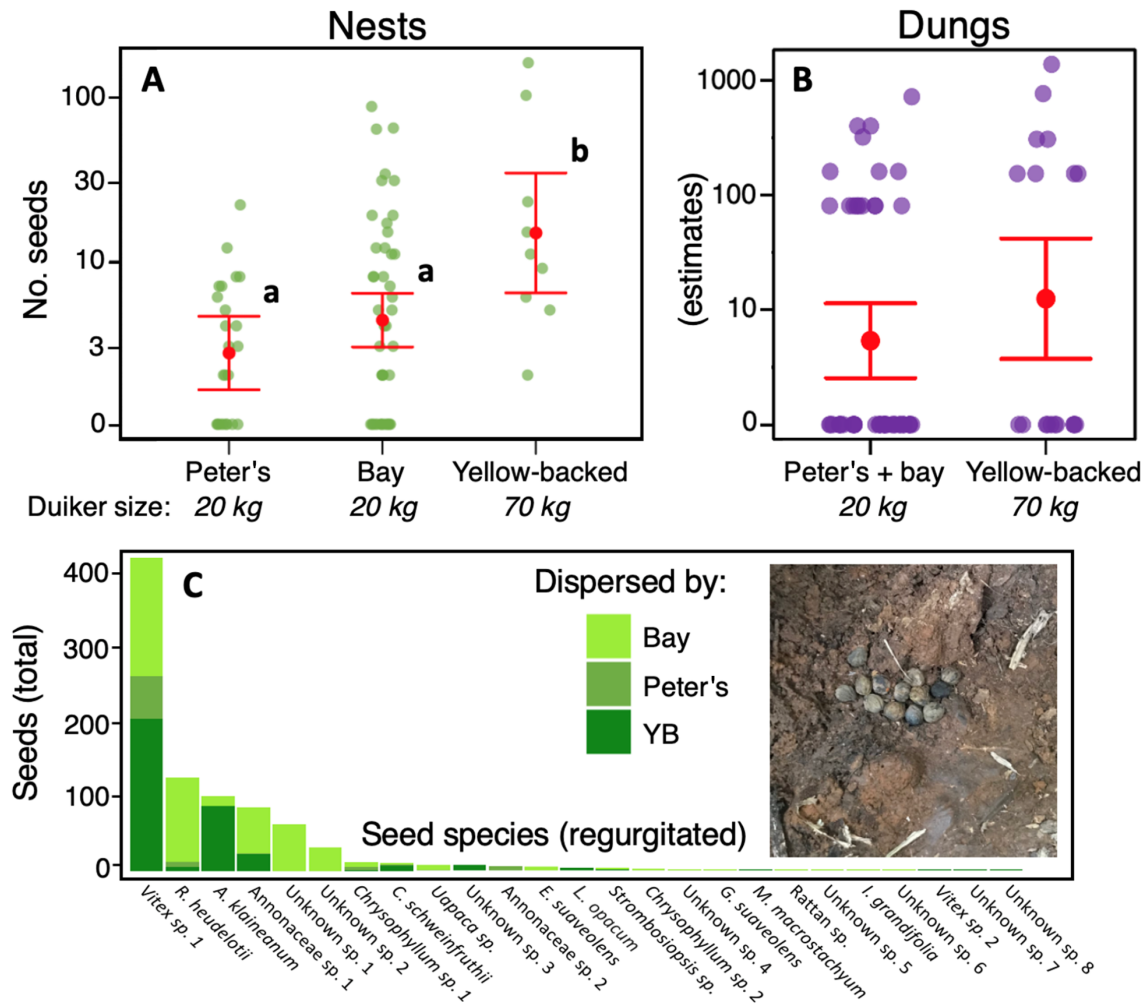
FIGURE 3 (A) Variation in the quantity of regurgitated seeds per nest among each duiker species (log-transformed; letters denote statistical differences among species pairs of $p < 0.05$; red dot shows the mean and whiskers show 95% CI). (B) Estimated quantity of defecated seeds in dungs, extrapolated from seed numbers in three-pellet samples (red duikers include both bay and Peter's). (C) Regurgitated seeds species found in nests, shaded by duiker species. The inset photo shows regurgitated seeds of *Ricinodendron heudelotii* in a bay duiker nest. YB, yellow-backed duiker. Photo credit: Bastien Dehaudt.

in length. The largest defecated seed came from a yellow-backed duiker dung and measured 9.8×4.7 mm, while for red duikers it measured 6.5×6.3 mm (Appendix S1: Table S1). For identification and photos of regurgitated seeds see Appendix S1: Table S2 and Figure S1.

Transects and duiker seed dispersal habitat/microhabitat patterns

We conducted 18 transects of 1000 m length and 15 m width, covering an estimated total of 270,000 m², and we found 41 duiker nests and 89 duiker dungs (Figure 5A). This equates to densities of 1.52 nest/hectare and 3.30 dung/ha. Per species, we found 17 Peter's duiker nests (0.63/ha), 17 bay duiker nests (0.63/ha), and 7 yellow-backed duiker nests (0.26/ha), while we found 71 red duiker dungs (2.63/ha) and 18 yellow-backed duiker dungs (0.67/ha; Appendix S1: Table S1).

We also evaluated variation in forest structure metrics within the indigenous Baka forest type categories and found that mandja and woundo had significantly higher canopy height than étobé and swamp (ANOVA: $F_{3,125} = 15.24$, $p < 0.0001$) and that étobé had significantly higher understory plant density (ANOVA: $F_{3,125} = 11.77$, $p < 0.0001$). We did not find a significant difference in LAI between the forest types (Figure 5B–D).

We found both nests and dungs in all four different forest types classified in this study. However, the dispersal pathway affected the proportion of seeds deposited in these habitats. We found that bay duikers nest and
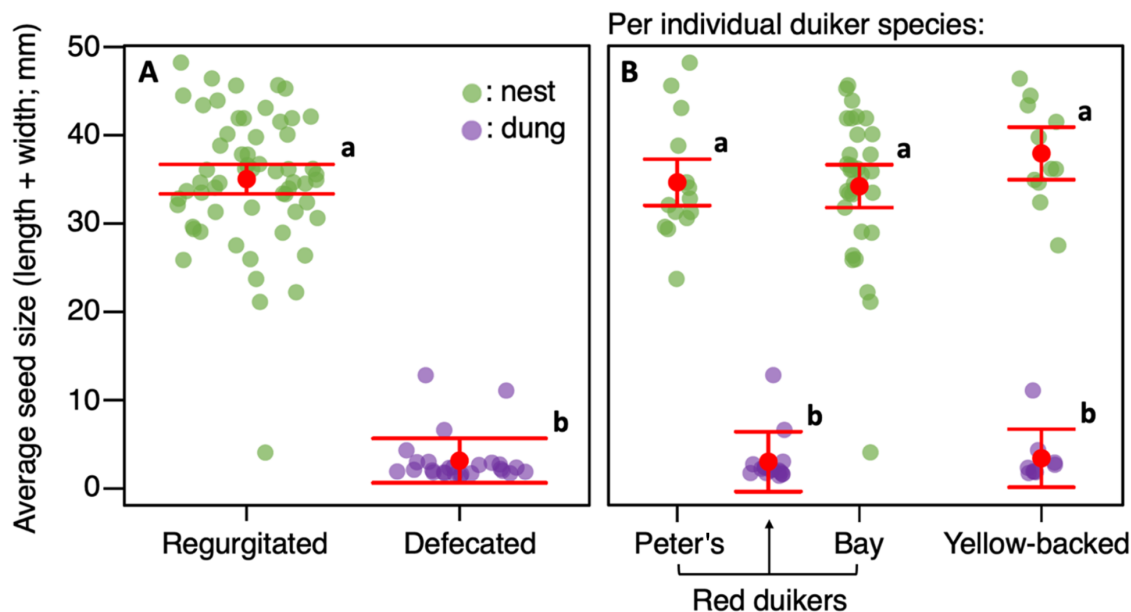
FIGURE 4 Size of dispersed seeds by duikers based on dispersal pathway. Variation in average seed size in nests (regurgitated) versus dungs (defecated) for (A) all three duiker species combined and (B) each duiker species separately (Peter's and bay dungs combined due to difficulty of differentiation). Red dots show the means and whiskers show 95% CI from linear regression.

defecate in significantly different proportions across forest types, such that a higher percentage of nests than dungs were in étobé, the forest type with lower canopy height but the highest understory plant densities, while a higher percentage of dungs than nests were in mandja and woundo, the forest types with lower understory plant density (contingency table chi-square: $\chi^2_3 = 15.86$, $p = 0.001$; Figure 6C). We did not find a significant difference in forest type between nests and dungs for Peter's and yellow-backed duikers. Bay duikers disproportionately nested in étobé relative to the other duiker species. We can only use this data to make comparisons within each habitat type, but not across them since they were not sampled equally with our transects.

The microhabitats of regurgitated and defecated seeds also varied. We located 63% of dungs on animal trails (assumed to represent more open microhabitats) while only 44% of nests were in open microhabitats (the rest were in or next to liana thickets, fallen trees, or tree roots). We also found differences between duiker species' nesting habits. Peter's duiker nests were often in open understory, while most bay duiker nests were in thicker understory microhabitats, such as under fallen trees or in liana thickets (Figure 6D).

We tested duiker habitat selection when nesting and defecating with canopy height, LAI, and understory plant density (from ground to 5 m up) from remote sensing lidar data (10×10 m resolution). Bay duikers selected areas with lower LAI for nesting (generalized linear model: effect size = −0.74, SE = 0.38, $Z_{1453} = -1.96$, $p = 0.0506$).

Yellow-backed duikers defecated in areas with greater LAI (generalized linear model: effect size = 0.64, SE = 0.27, $Z_{1451} = 2.37$, $p = 0.018$). Elevation, canopy height, and understory plant density were not significant predictors of duiker habitat selection (Figure 7A). Lastly, when comparing nesting between duiker species, we found that locations of bay nests had a significantly lower LAI than Peter's nests (ANOVA: $F_{2,38} = 6.04$, $p = 0.005$, Figure 7B) and no significant differences for canopy height (ANOVA: $F_{2,38} = 2.35$, $p = 0.11$; Figure 7C). See Appendix S1: Table S3 for all Tukey test pairwise results.

DISCUSSION

Our study is the first to assess duiker seed regurgitation directly from nests (not stomach contents) and to compare seed traits and deposition sites for seeds dispersed via regurgitation versus defecation. This approach revealed that seed traits determine the duiker dispersal pathway, which in turn, influence the likelihood of deposition in different forest types and microhabitats. Specifically, regurgitated seeds were predominantly large (10–30 mm length) drupe-type seeds with tough seed coats, preventing them from being crushed during mastication. Intact defecated seeds were typically very small (<3 mm in length), allowing them to pass farther down the digestive tract. Bay duikers disproportionately dispersed large regurgitated seeds in dense understory habitats with sparse upper canopy (étobé) compared with
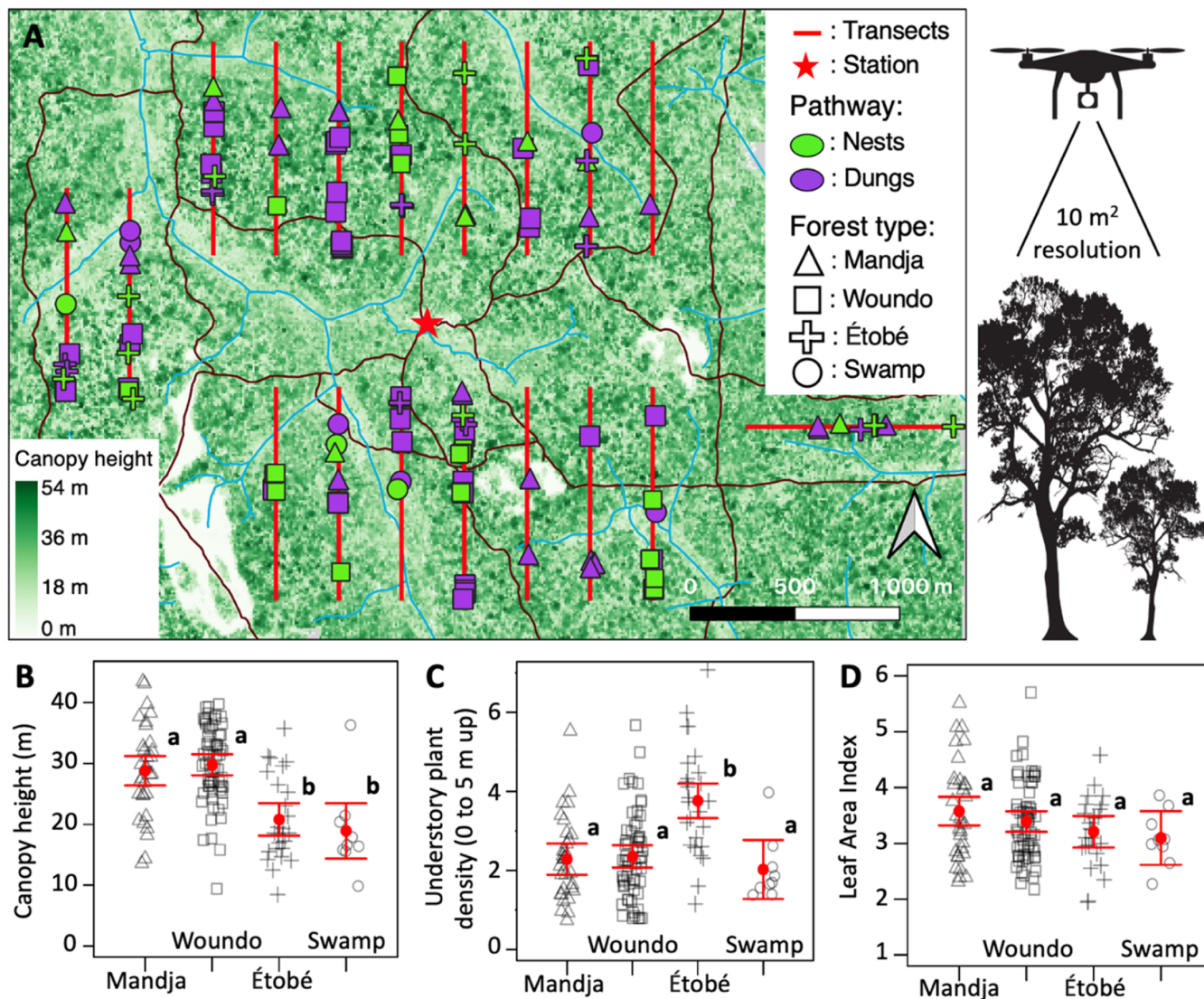
FIGURE 5 Locations of nests (green) and dungs (purple) on transects and relationships between forest types and remote sensing data. (A) The shape of symbols indicates the forest type of its location. The background layer shows canopy height. (B–D) Comparing local Baka forest classifications with remote sensing technology. (B) Canopy height, (C) understory plant density (from ground to 5 m up), and (D) leaf area index of the four forest types (points are nests and dungs in each forest type). For a detailed explanation of Baka forest type categories see *Study site*.

small defecated seeds in habitats with relatively more open understory. At the finer microhabitats scale, over 60% of duiker dungs– and the associated small seeds— were on animal trails, while nests with larger regurgitated seeds were often near structures like liana thickets, fallen trees, and tree roots. These findings show that seeds with different traits can travel via distinct dispersal pathways and end up in unique deposition sites, even after being consumed by the same animal species (and possibly individual).

The locations of nests and dungs—and thus seed deposition sites—also varied between the three duiker species. Bay duikers nested more often in denser understory habitats (étobé) and near fallen trees compared

with Peter's and yellow-backed duikers. This may be due to a difference in temporal activity since bay duikers are the only nocturnal (day-nesting) species, and more hidden nests may be advantageous in daylight. Yellow-backed duikers also dispersed more seeds in their nests than the other two species, likely due to their larger size. Similar to prior studies, these findings provide nuanced examples showing variation in dispersal by different animal species (Jordano et al., 2007; Wenny & Levey, 1998).

The forest type and microhabitat where seeds are deposited are crucial for determining their germination success and survival into adulthood, with significant consequences for plant species distribution, forest regeneration,
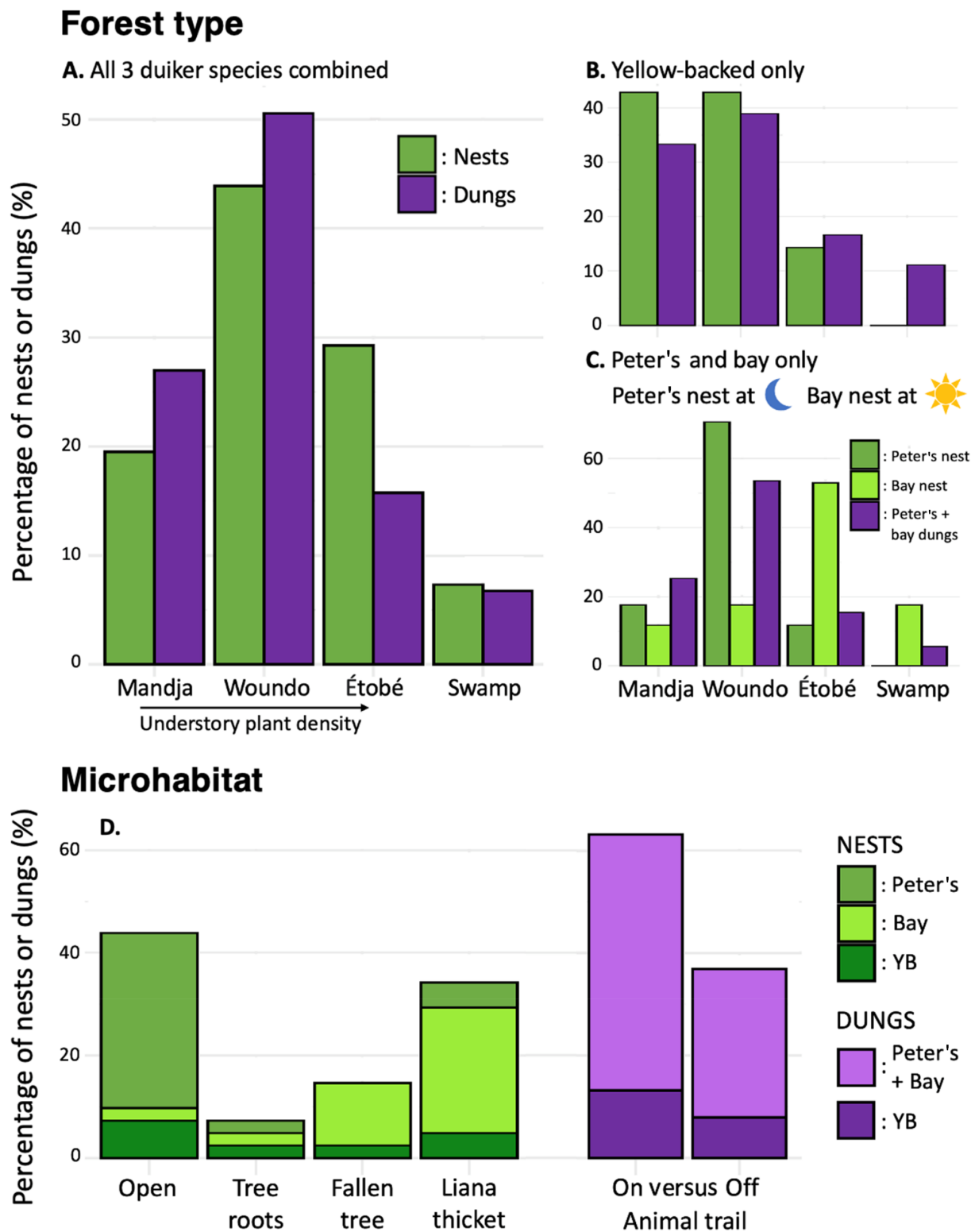
FIGURE 6 Association of duiker nests and dungs with forest type and microhabitats. Forest types follow local Baka classifications (see *Study site* for a detailed explanation). (A) Proportion of nests and dungs found in each forest type during transects. (B) Same but only for yellow-backed duikers. (C) Only for Peter's and bay nests (green and light green) and their dungs (purple) which are combined due to difficulties telling them apart. (D) The percentages of nest microhabitats (greens) and of dungs on or off animal trails (purples), with shading to show how each duiker species contributed. YB, yellow-backed duiker.
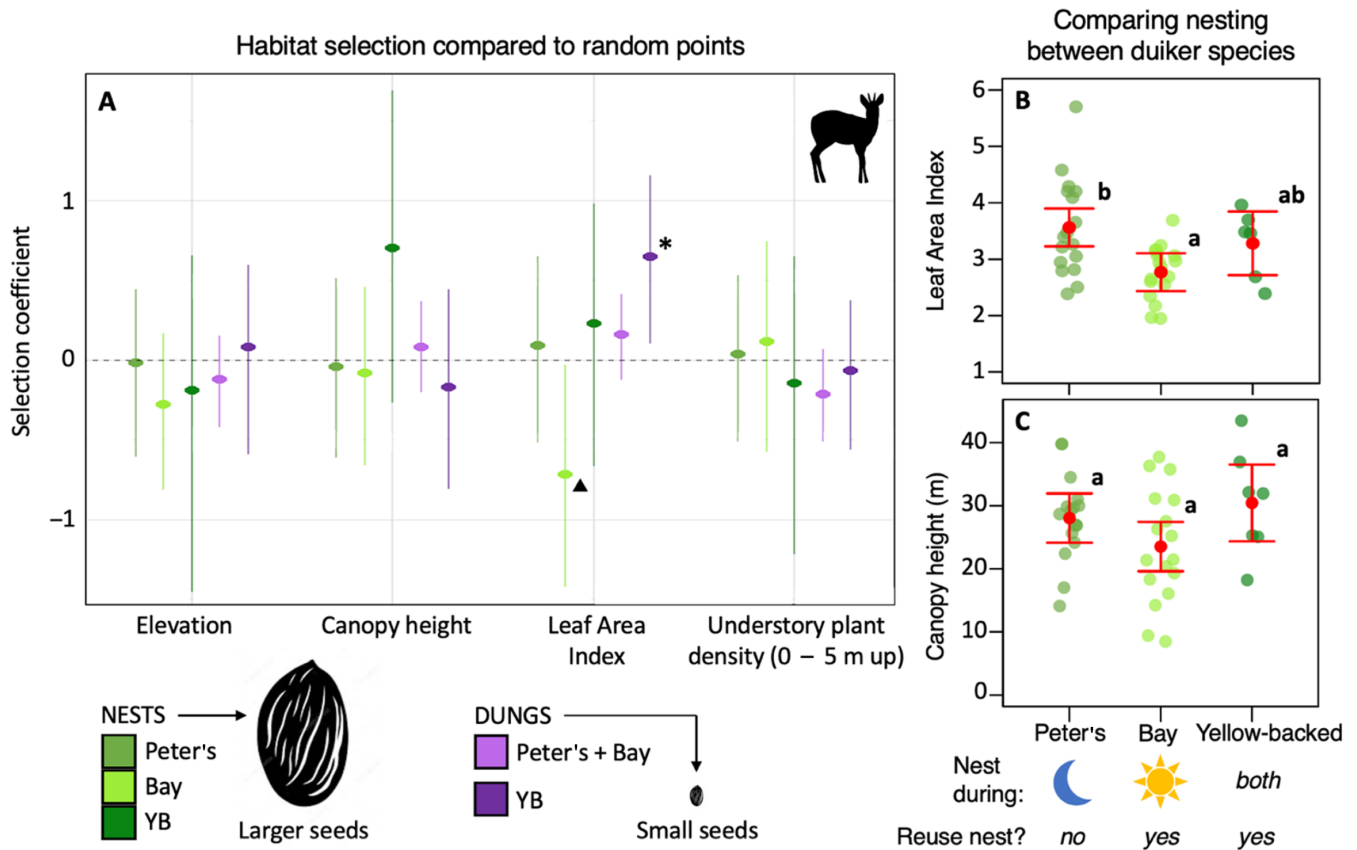
FIGURE 7 Duiker nesting and defecating associations and site selection. (A) Nesting and defecating site selection for each duiker species. Points represent estimates of selection coefficients for the covariates of interest and lines show 95% CI. The dotted line at zero represents no selection, and positive and negative values correspond to the sign of selection. Asterisks denote significance at $p < 0.05$ and the triangle denotes marginal significance at $p = 0.05$. Variation in (B) leaf area index and (C) canopy height between locations of nests of the three different duiker species. Letters denote statistical difference among species pairs of $p < 0.05$. The nesting habits of each species are described.

and forest structure (Carson et al., 2008; Russo et al., 2023). Both duiker regurgitation and defecation can disperse viable seeds (see Appendix S1; Houngbégnon et al., 2023), and our findings suggest that directed seed dispersal by duikers contributes to succession and forest structure turnover. In tropical forests, seed and seedling survival is often limited by the low amounts of light reaching the forest floor (Carson et al., 2008). We found that bay duikers selected nesting sites at locations with lower LAI and, thus, likely more light. We also found 15% of duiker nests near fallen trees (29% for bay duikers only). These suggest that a portion of regurgitated large seeds are dispersed at advantageous locations for germination and succession. Many plants dispersed by duikers are pioneer colonizers (Houngbégnon et al., 2023) and over half of the regurgitated seed species we identified were medium-to-high wood density (Appendix S1: Figure S2). In Afrotropical rainforests, large-seeded species are often taller and store more carbon (Osuri et al., 2016); thus, duiker regurgitation of large seeds in liana- and palm-dominated parts of the forest (étobé) and near

fallen trees likely play a key role in forest succession, structure, and carbon storage.

Our integration of traditional indigenous knowledge (Baka forest type classifications) with cutting-edge technologies (drone lidar) showed both consistency and complementarity. While lidar-derived metrics detected the expected differences in forest structure between forest types, only traditional forest type classifications detected trends in seed deposition sites with understory density. This is likely due to a combination of factors such as: noise from GPS and lidar errors, variation in structure within forest types, and too much complexity for high-resolution continuous variables (vs. categorical). Unlike high-tech approaches, indigenous locals can provide complementary on-the-ground data (Gagnon & Berteaux, 2009). Local knowledge used in conjunction with science can also inform us on the status of local species, foster partnership, improve the transmission of results to locals, and increase the likelihood of success for restoration projects (Braga-Pereira et al., 2024; Pierotti & Wildcat, 2000; Uprety et al., 2012). In this

case, collaborating with indigenous colleagues allowed us to find results that we would have missed had we only used technology.

What happens to rainforests when seed dispersers are gone? Many tropical forests in Africa suffer severe defaunation, and duikers are a primary source of bushmeat there (Amin et al., 2022; Benítez-López et al., 2019). Notably, some duiker species seem to be tolerant to some human disturbances, while others are declining (Amin et al., 2022). We found evidence of redundancy in seed dispersal services across the three duiker species, such as for seed size, but also of complementarity since duiker species differed in where they dispersed these seeds. Duiker can disperse a wide variety of plant species, including large seeds through regurgitation, which could help alleviate some seed dispersal loss from already diminished populations of large seed dispersers, like elephants and primates (Campos-Arceiz & Blake, 2011; Poulsen et al., 2001). Losing duiker species would likely negatively impact plant community richness and forest regeneration. By regurgitating and defecating, duikers proved to be important seed dispersers for African tropical forests.

Seed regurgitation is not a process limited to duikers, and ruminants worldwide likely shape plant distribution in similar fashion, yet ungulate seed dispersal via regurgitation remains poorly studied. Ruminating ungulates are present on every continent except Antarctica, with almost 200 living species, ~75 million wild individuals, and 3.6 billion domestic animals (Hackmann & Spain, 2010). As they are often large-bodied and abundant, ruminants can have an important effect on plant community dynamics (Delibes et al., 2019). Deer (Cervidae; which are common, widespread, and often tolerant of human disturbance), goats, and antelopes have been observed spitting seeds while chewing the cud (Castañeda et al., 2018; Janzen, 1985; Muñoz-Gallego et al., 2019; Pegg, 2014; Prasad et al., 2006; Şahin et al., 2021). However, the prevalence and ecological importance of ruminant seed dispersal via regurgitation, and its influence on plant communities, remain poorly understood and often overlooked in seed dispersal research (Delibes et al., 2019). Our study shows that ruminant seed regurgitation can be an important mechanism shaping seed deposition, and thus vegetation composition and structure, and will hopefully stimulate similar work in other biomes.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data and code (Dehaudt, 2024) are available in figshare at https://doi.org/10.6084/m9.figshare.26264246.

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REFERENCES

Albert, A., A. G. Auffret, E. Cosyns, S. A. O. Cousins, B. D'hondt, C. Eichberg, A. E. Eycott, et al. 2015. "Seed Dispersal by Ungulates as an Ecological Filter: A Trait-Based Meta-Analysis." *Oikos* 124(9): 1109–20. https://doi.org/10.1111/oik.02512.

Albert, A., A. Mårell, M. Picard, and C. Baltzinger. 2015. "Using Basic Plant Traits to Predict Ungulate Seed Dispersal Potential." *Ecography* 38(5): 440–49. https://doi.org/10.1111/ecog.00709.

Amin, R., T. Bruce, O. Fankem, T. Wacher, G. Oum Ndjock, A. S. Kobla, D. Olson, and A. Fowler. 2023. *The Conservation Value of Dja Faunal Reserve for Tropical Forest Mammal Communities* 1–102. Zoological Society of London.

Amin, R., T. Wacher, O. Fankem, O. N. Gilbert, M. S. Ndimbe, and A. Fowler. 2022. "Status and Ecology of Forest Ungulates in the Dja Faunal Reserve, Cameroon." *Mammalia* 86(5): 444–454. https://doi.org/10.1515/mammalia-2021-0175.

Avila Martin, E., G. Ros Brull, S. M. Funk, L. Luiselli, R. Okale, and J. E. Fa. 2020. "Wild Meat Hunting and Use by Sedentarised Baka Pygmies in Southeastern Cameroon." *PeerJ* 8: e9906. https://doi.org/10.7717/peerj.9906.

Baltzinger, C., S. Karimi, and U. Shukla. 2019. "Plants on the Move: Hitch-Hiking with Ungulates Distributes Diaspores across Landscapes." *Frontiers in Ecology and Evolution* 7: 38. https://doi.org/10.3389/fevo.2019.00038.

Benítez-López, A., L. Santini, A. M. Schipper, M. Busana, and M. A. J. Huijbregts. 2019. "Intact but Empty Forests? Patterns of Hunting-Induced Mammal Defaunation in the Tropics."

PLoS Biology 17(5): e3000247. https://doi.org/10.1371/journal.pbio.3000247.

Braga-Pereira, F., P. Mayor, T. Q. Morcatty, P. E. Pérez-Peña, M. T. Bowler, M. A. R. De Mattos Vieira, R. R. D. N. Alves, et al. 2024. "Predicting Animal Abundance through Local Ecological Knowledge: An Internal Validation Using Consensus Analysis." *People and Nature* 6: 535–547. https://doi.org/10.1002/pan3.10587.

Campos-Arceiz, A., and S. Blake. 2011. "Megagardeners of the Forest – The Role of Elephants in Seed Dispersal." *Acta Oecologica* 37(6): 542–553. https://doi.org/10.1016/j.actao.2011.01.014.

Carson, W. P., S. A. Schnitzer, and Smithsonian Tropical Research Institute, eds. 2008. *Tropical Forest Community Ecology.* Wiley-Blackwell Publishing, Hoboken, New Jersey.

Castañeda, I., J. M. Fedriani, and M. Delibes. 2018. "Potential of Red Deer (*Cervus elaphus*) to Disperse Viable Seeds by Spitting them from the Cud." *Mammalian Biology* 90: 89–91. https://doi.org/10.1016/j.mambio.2017.10.004.

Davies, A. B., F. Oram, M. Ancrenaz, and G. P. Asner. 2019. "Combining Behavioural and LiDAR Data to Reveal Relationships between Canopy Structure and Orangutan Nest Site Selection in Disturbed Forests." *Biological Conservation* 232: 97–107. https://doi.org/10.1016/j.biocon.2019.01.032.

Dehaudt, B. 2024. "Data and R Code for 'Divergent Seed Dispersal Outcomes: Interactions between Seed, Disperser, and Forest Traits'." figshare. Dataset. https://doi.org/10.6084/m9.figshare.26264246.v1.

Delibes, M., I. Castañeda, and J. M. Fedriani. 2019. "Spitting Seeds from the Cud: A Review of an Endozoochory Exclusive to Ruminants." *Frontiers in Ecology and Evolution* 7: 265. https://doi.org/10.3389/fevo.2019.00265.

Deshpande, K., and N. Kelkar. 2015. "How Do Fruit Bat Seed Shadows Benefit Agroforestry? Insights from Local Perceptions in Kerala, India." *Biotropica* 47(6): 654–59. https://doi.org/10.1111/btp.12275.

Feer, F. 1989a. "Occupation de l'espace par deux bovidés sympatriques de la forêt dense africaine (*Cephalophus callipygus* et *C. dorsalis*): Influence du rythme d'activité." *Revue d'Écologie (La Terre et La Vie)* 44(3): 225–248.

Feer, F. 1989b. "Comparaison des régimes alimentaires de *Cephalophus callipygus* et *C. dorsalis*, Bovidés sympatriques de la fôret sempervirente africaine." *Mammalia* 53(4): 563–604. https://doi.org/10.1515/mamm.1989.53.4.563.

Feer, F. 1995. "Seed Dispersal in African Forest Ruminants." *Journal of Tropical Ecology* 11(4): 683–89. https://doi.org/10.1017/S0266467400009238.

Gagnon, C. A., and D. Berteaux. 2009. "Integrating Traditional Ecological Knowledge and Ecological Science: A Question of Scale." *Ecology and Society* 14(2): 19.

Hackmann, T. J., and J. N. Spain. 2010. "Invited Review: Ruminant Ecology and Evolution: Perspectives Useful to Ruminant Livestock Research and Production." *Journal of Dairy Science* 93(4): 1320–34. https://doi.org/10.3168/jds.2009-2071.

Hirsch, B. T., R. Kays, V. E. Pereira, and P. A. Jansen. 2012. "Directed Seed Dispersal towards Areas with Low Conspecific Tree Density by a Scatter-Hoarding Rodent." *Ecology Letters* 15(12): 1423–29. https://doi.org/10.1111/ele.12000.

Houngbégnon, F. G. A., J.-F. Gillet, J. Michaux, Y. Brostaux, D. Zébazé, S. Lhoest, C. Vermeulen, B. Sonké, and J.-L. Doucet. 2023. "Seed Dispersal by Duikers in Selectively Logged Rainforests: Overlooked Dispersal of an Important Animal Community." *Forest Ecology and Management* 529: 120650. https://doi.org/10.1016/j.foreco.2022.120650.

Janzen, D. H. 1985. "*Spondias mombin* Is Culturally Deprived in Megafauna-Free Forest." *Journal of Tropical Ecology* 1(2): 131–155. https://doi.org/10.1017/S0266467400000195.

Jordano, P., C. García, J. A. Godoy, and J. L. García-Castaño. 2007. "Differential Contribution of Frugivores to Complex Seed Dispersal Patterns." *Proceedings of the National Academy of Sciences* 104(9): 3278–82. https://doi.org/10.1073/pnas.0606793104.

Kitamura, S., T. Yumoto, P. Poonswad, N. Noma, P. Chuailua, K. Plongmai, T. Maruhashi, and C. Suckasam. 2004. "Pattern and Impact of Hornbill Seed Dispersal at Nest Trees in a Moist Evergreen Forest in Thailand." *Journal of Tropical Ecology* 20(5): 545–553. https://doi.org/10.1017/S0266467404001518.

Manfred, A. E., and G. Oum Ndjock. 2020. "Dja Faunal Reserve A Natural Treasure with Diversesocial Representations." *World Heritage Review* 2020(96): 30–35. https://doi.org/10.18356/27887138-2020-96-4.

Martínez, I., D. García, and J. R. Obeso. 2008. "Differential Seed Dispersal Patterns Generated by a Common Assemblage of Vertebrate Frugivores in Three Fleshy-Fruited Trees." *Écoscience* 15(2): 189–199. https://doi.org/10.2980/15-2-3096.

MINFOF Atlas Forestier du Cameroun. 2017. "*Lieux habites.*" Dataset. https://cmr.forest-atlas.org

Muñoz-Gallego, R., J. M. Fedriani, and A. Traveset. 2019. "Non-Native Mammals Are the Main Seed Dispersers of the Ancient Mediterranean Palm *Chamaerops humilis* L. in the Balearic Islands: Rescuers of a Lost Seed Dispersal Service?" *Frontiers in Ecology and Evolution* 7: 161. https://doi.org/10.3389/fevo.2019.00161.

Newing, H. 2001. "Bushmeat Hunting and Management: Implications of Duiker Ecology and Interspecific Competition." *Biodiversity and Conservation* 10(1): 99–118. https://doi.org/10.1023/A:1016671524034.

Osuri, A. M., J. Ratnam, V. Varma, P. Alvarez-Loayza, J. Hurtado Astaiza, M. Bradford, C. Fletcher, et al. 2016. "Contrasting Effects of Defaunation on Aboveground Carbon Storage across the Global Tropics." *Nature Communications* 7(1): 11351. https://doi.org/10.1038/ncomms11351.

Parolin, P., F. Wittmann, and L. V. Ferreira. 2013. "Fruit and Seed Dispersal in Amazonian Floodplain Trees—A Review." *Ecotropica* 19(1/2): 15–32.

Pearson, K. M., and T. C. Theimer. 2004. "Seed-Caching Responses to Substrate and Rock Cover by Two Peromyscus Species: Implications for Pinyon Pine Establishment." *Oecologia* 141(1): 76–83. https://doi.org/10.1007/s00442-004-1638-8.

Pegg, N. 2014. "Antelope Ingestion Enhances Germination of the Marula (*Sclerocarya birrea*), An Important African Savannah Tree." *African Journal of Ecology* 52(4): 499–505. https://doi.org/10.1111/aje.12152.

Pierotti, R., and D. Wildcat. 2000. "Traditional Ecological Knowledge: The Third Alternative (Commentary)." *Ecological Applications* 10(5): 1333–40. https://doi.org/10.1890/1051-0761(2000)010[1333:TEKTTA]2.0.CO;2.

Poulsen, J. R., C. J. Clark, and T. B. Smith. 2001. "Seed Dispersal by a Diurnal Primate Community in the Dja Reserve, Cameroon." *Journal of Tropical Ecology* 17(6): 787–808. https://doi.org/10.1017/S0266467401001602.

Prasad, S., J. Krishnaswamy, R. Chellam, and S. P. Goyal. 2006. "Ruminant-Mediated Seed Dispersal of an Economically Valuable Tree in Indian Dry Forests." *Biotropica* 38(5): 679–682. https://doi.org/10.1111/j.1744-7429.2006.00182.x.

R Core Team. 2023. *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing. https://www.R-project.org/.

Rehm, E., E. Fricke, J. Bender, J. Savidge, and H. Rogers. 2019. "Animal Movement Drives Variation in Seed Dispersal Distance in a Plant–Animal Network." *Proceedings of the Royal Society B: Biological Sciences* 286(1894): 20182007. https://doi.org/10.1098/rspb.2018.2007.

Russo, N. J., A. B. Davies, R. V. Blakey, E. M. Ordway, and T. B. Smith. 2023. "Feedback Loops between 3D Vegetation Structure and Ecological Functions of Animals." *Ecology Letters* 26: 1597–1613. https://doi.org/10.1111/ele.14272.

Şahin, B., S. Kar, and N. Şafak Odabaşı. 2021. "Spitting Type Seed Dispersal by Domestic Goat in the Zoochorial Process of Blackthorn Plum." *Annali Di Botanica* 11: 43–54. https://doi.org/10.13133/2239-3129/16736.

Schupp, E. W., P. Jordano, and J. M. Gómez. 2010. "Seed Dispersal Effectiveness Revisited: A Conceptual Review." *New Phytologist* 188(2): 333–353. https://doi.org/10.1111/j.1469-8137.2010.03402.x.

Signer, J., J. Fieberg, and T. Avgar. 2019. "Animal Movement Tools (amt): R Package for Managing Tracking Data and Conducting Habitat Selection Analyses." *Ecology and Evolution* 9(2): 880–890. https://doi.org/10.1002/ece3.4823.

Uprety, Y., H. Asselin, Y. Bergeron, F. Doyon, and J.-F. Boucher. 2012. "Contribution of Traditional Knowledge to Ecological Restoration: Practices and Applications." *Écoscience* 19(3): 225–237. https://doi.org/10.2980/19-3-3530.

Van Leeuwen, C. H. A., N. Villar, I. Mendoza Sagrera, A. J. Green, E. S. Bakker, M. B. Soons, M. Galetti, P. A. Jansen, B. A. Nolet, and L. Santamaría. 2022. "A Seed Dispersal Effectiveness Framework across the Mutualism–Antagonism Continuum." *Oikos* 2022: e09254. https://doi.org/10.1111/oik.09254.

Van Vliet, N., R. Nasi, L. Emmons, F. Feer, P. Mbazza, and M. Bourgarel. 2007. "Evidence for the Local Depletion of Bay Duiker *Cephalophus dorsalis*, within the Ipassa Man and Biosphere Reserve, North-East Gabon." *African Journal of Ecology* 45(3): 440–43. https://doi.org/10.1111/j.1365-2028.2007.00783.x.

Van Vliet, N., S. Zundel, C. Miquel, P. Taberlet, and R. Nasi. 2008. "Distinguishing Dung from Blue, Red and Yellow-Backed Duikers through Noninvasive Genetic Techniques." *African Journal of Ecology* 46(3): 411–17. https://doi.org/10.1111/j.1365-2028.2007.00879.x.

Wenny, D. G., and D. J. Levey. 1998. "Directed Seed Dispersal by Bellbirds in a Tropical Cloud Forest." *Proceedings of the National Academy of Sciences* 95(11): 6204–7. https://doi.org/10.1073/pnas.95.11.6204.

Willson, M. F., and A. Traveset. 2000. "The Ecology of Seed Dispersal." In *Seeds: The Ecology of Regeneration in Plant Communities*, 2nd ed., edited by M. Fenner, 85–110. CABI. https://doi.org/10.1079/9780851994321.0085.

Zwolak, R. 2018. "How Intraspecific Variation in Seed-Dispersing Animals Matters for Plants: Intraspecific Variation and Seed Dispersal." *Biological Reviews* 93(2): 897–913. https://doi.org/10.1111/brv.12377.

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