

ARTICLE

Defaunation changes leaf trait composition of recruit communities in tropical forests in French Guiana

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Abstract

Hunting impacts tropical vertebrate populations, causing declines of species that function as seed dispersers and predators, or that browse seedlings and saplings. Whether and how the resulting reductions in seed dispersal, seed predation, and browsing translate to changes in the tree composition is poorly understood. Here, we assess the effect of defaunation on the functional composition of communities of tree recruits in tropical rainforests in French Guiana. We selected eight sites along a gradient of defaunation, caused by differences in hunting pressure, in otherwise intact old-growth forests in French Guiana. We measured shifts in functional composition by comparing leaf and fruit traits and wood density between tree recruits (up to 5 cm diameter at breast height) and adults, and tested whether and how these compositional shifts related to defaunation. We found a positive relationship with defaunation for shifts in specific leaf area, a negative relationship for shifts of leaf toughness and wood density, and a weak relationship for shifts in fruit traits. Our results suggest that the loss of vertebrates affects ecological processes such as seed dispersal and browsing, of which browsing remains understudied. Even though these changes sometimes seem minor, together they result in major shifts in forest composition. These changes have long-term ramifications that may alter forest dynamics for generations.

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KEYWORDS

functional composition, functional trait, hunting, leaf toughness, specific leaf area, wood density

INTRODUCTION

Hunting is common throughout tropical forests, resulting in the decline of vertebrate populations in many regions (Brashares, 2004; Peres & Palacios, 2007). Hunting disproportionately affects large-bodied vertebrates, which are preferred game species, leading to compositional changes in the vertebrate community (Bugir et al., 2021; Peres & Palacios, 2007). These changes are thought to have cascading effects on forest regeneration, due to the important roles that these vertebrates play in seed dispersal, seed predation, and herbivory (Dirzo & Miranda, 1991; Galetti & Dirzo, 2013; Kurten, 2013; Kurten & Carson, 2015; Terborgh, 1988; Terborgh et al., 2008; Wright, 2003).

Interactions between vertebrates and plants are fundamentally driven by the preferences of vertebrates for leaves, seeds, and fruits with certain traits. In leaves, vertebrates generally prefer low toughness, high specific leaf area (SLA), high nutrient content, and low concentrations of secondary metabolites (Coley & Barone, 1996; Coley & Kursar, 2014; Kitajima & Poorter, 2010; Kursar & Coley, 2003; Poorter et al., 2004). Most herbivory occurs at the early stages of leaf development, before leaves toughen. Some plant species have leaves that quickly expand and toughen, supposedly to escape herbivory, but this strategy requires many nitrogen-containing enzymes and thus makes leaves more attractive to herbivores. Other plant species are thought to avoid herbivory by expanding their leaves slowly, requiring much less nitrogen (Kursar & Coley, 1991, 2003). For seeds and fruits, preferences of vertebrates are more complex and taxon-specific (Gautier-Hion et al., 1985). Vertebrates that feed on fruits and seeds often prefer specific dispersal syndromes (Gautier-Hion et al., 1985; Pijl, 1969), which are mostly defined by the color, fleshiness, and size of fruits and seeds. For example, birds typically prefer small, fleshy fruits that have a red or black color (Janson, 1983). Because of such preferences, the loss of these vertebrates will likely result in altered patterns of seed dispersal and predation, seedling herbivory and, as a consequence, tree recruitment, which may ultimately change forest composition for many functional traits.

Empirical tests of hunting-induced changes in functional trait composition have been limited mostly to seed size, in particular in relation to vertebrate body mass. Larger vertebrates are assumed to disperse larger seeds (Dirzo et al., 2007; Nunez-Iturri et al., 2008; Terborgh et al., 2008; Wright et al., 2007); hence, the loss of large-bodied dispersers has been predicted to reduce the

dispersal of large-seeded tree species, increase spatial clustering, and therefore lower the survival odds of these species due to increased density-dependent mortality (Brodie & Gibbs, 2009). Because seed mass is positively correlated to wood density and tree height (Bello et al., 2015), hunting could thus induce a shift towards lower overall forest biomass (Bello et al., 2015; Osuri et al., 2016; Peres et al., 2016). These models, however, oversimplify recruitment, which involves many ecological processes and functional traits. Moreover, recent studies suggest that defaunation-induced changes in forest community composition and structure are not driven by dispersal limitation only (Bagchi et al., 2018; Hazelwood et al., 2020). Browsing may be another mechanism through which defaunation can affect forest composition (Dirzo & Miranda, 1991), due to the preference of browsers for leaves with certain traits. However, browsing has received little attention in the context of defaunation (but see: Camargo-Sanabria et al., 2015). Thus, the relationship between vertebrate composition and functional trait composition of the plant community remains poorly understood.

A better understanding of the relationship between vertebrate composition and functional trait composition of the plant community could be achieved by comparing the functional composition of trees between forest sites that vary in their level of defaunation, for example as a result of hunting. Such comparison should consider traits that are linked to seed dispersal, seed predation and browsing, which each may change with defaunation. Several studies have applied this approach (Harrison et al., 2013; Kurten et al., 2015; Terborgh et al., 2008; Wright et al., 2007), but only one included traits related to browsing (Kurten et al., 2015). Furthermore, most past studies are limited to few study sites, or a small geographical area, which limits the predictive value of the results. Regional site replication would therefore be a valuable addition to such studies.

Here, we aimed to assess the effect of defaunation on the functional composition of the community of tree recruits in a tropical forest. We quantified shifts in functional composition between adults and multiple size classes of tree recruits (i.e., seedling, sapling, juvenile, and treelet) and compared these shifts across eight sites representing a gradient of defaunation in, otherwise intact, old-growth forests in French Guiana. We hypothesized that the loss of large vertebrates leads to changes in the functional composition of the tree recruit community through their roles in seed dispersal and browsing. We expected that, with increasing defaunation level,

(1) tree species with fruit traits related to dispersal by large vertebrates—large fruits and fleshy fruits—decrease in relative abundance, (2) tree species with leaf traits preferred by browsing vertebrates—leaves with low toughness, high SLA, and high nitrogen content—increase in relative abundance, (3) tree species with high wood density decrease in relative abundance.

MATERIALS AND METHODS

Study system

Field data were collected in tropical lowland forests of French Guiana, an 85,000 km² French territory in the eastern part of the Guiana shield. The area has an equatorial

climate and receives between 3000 and 4000 mm of rainfall annually, with the highest rainfall in the north-east and the lowest in the south-west (Beaufort et al., 2019). Most rain falls between December and July (Beaufort et al., 2019). We focused on the habitat type “forests of plateaus and high hills” (Code G and H in table 2 of Guitet et al., 2013), which covers ~41% of French-Guiana (Figure 1). This landscape type is defined by its geomorphological features such as its relatively high elevation and steep slopes (Guitet et al., 2013). Consequently, forests in this landscape type have relatively homogenous plant and vertebrate communities (Guitet, Pélissier, et al., 2015; Richard-Hansen et al., 2015). However, the plant- and vertebrate diversity in these forests is high; therefore, local differences in species- or functional composition within the ‘forests of plateaus and high hills’ are still to be expected. ‘Forests of plateaus and high hills’

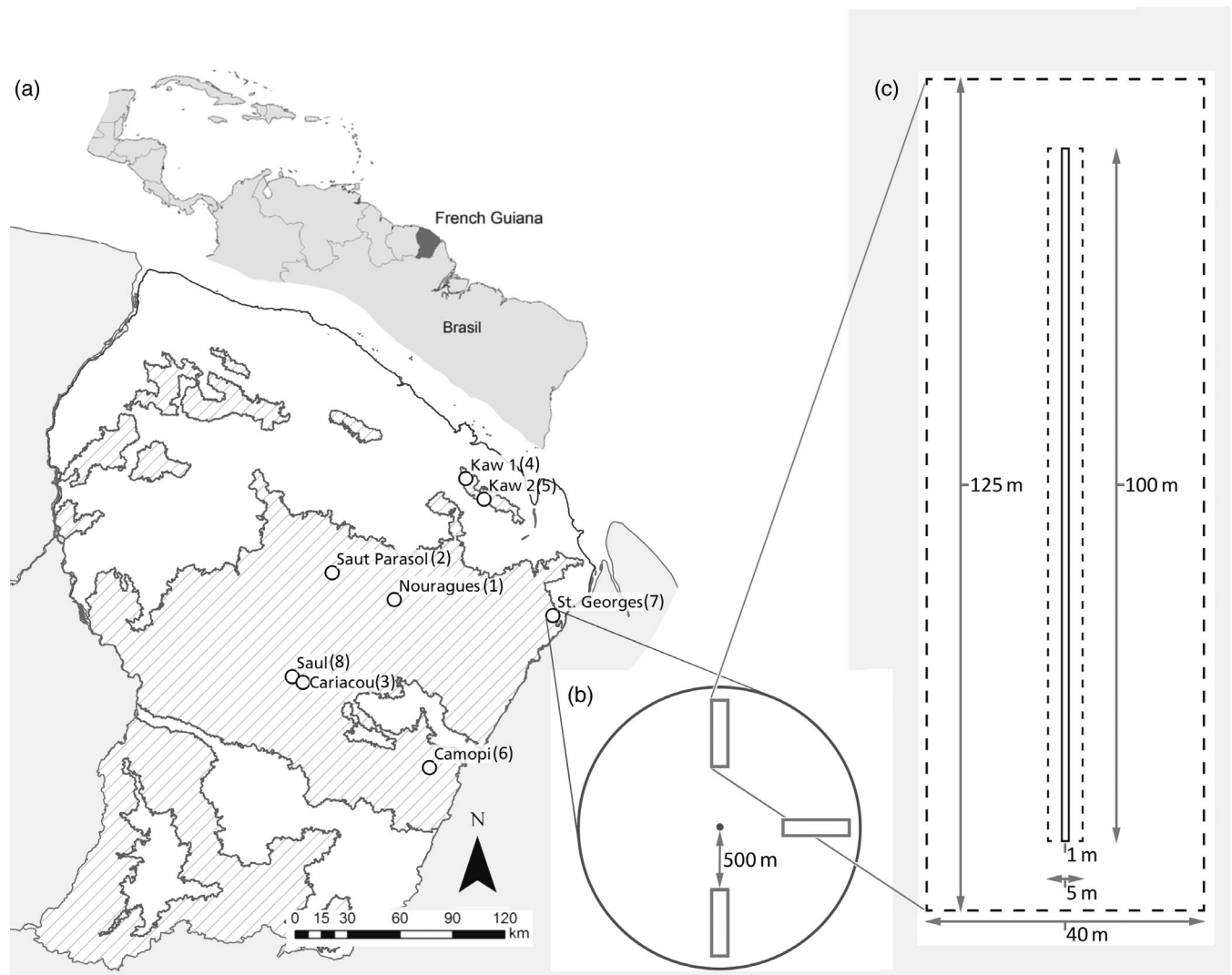


FIGURE 1 (a) Location of the eight study sites in French Guiana. The hatched area is the area with landscape type “forests of plateaus and high hills” (Guitet et al., 2013). (b) Location of plots (rectangles) relative to the site center point. Three of four cardinal directions were chosen for each site based on a set of criteria. (c) Spatial layout of a plot: Seedlings were sampled in the central plot of 1 × 100 m, saplings, juveniles, and treelets were sampled in the plot of 5 × 100 m and adults were sampled in the surrounding plot of 40 × 125 m.

are characterized by a high abundance of Fabaceae and Burseraceae trees. Species of the genera *Protium* and *Inga*, and *Dicorynia guianensis* are particularly common (Guitet, Brunaux, et al., 2015). The medium- to large vertebrate communities within 'forests of plateaus and high hills' typically include red brocket (*Mazama americana*), collared peccary (*Pecari tajacu*), black curassow (*Crax alector*), black spider monkey (*Ateles paniscus*) and tapir (*Tapirus terrestris*) (Guitet, Brunaux, et al., 2015; Richard-Hansen et al., 2015), which are also hunting sensitive species (Richard-Hansen et al., 2019).

We selected eight sites using three main criteria: (1) the site had been surveyed previously for vertebrates (Richard-Hansen et al., 2015), (2) the site had to be located in a single landscape type (Guitet, Pélissier, et al., 2015) and (3) all sites combined should represent a gradient of defaunation. Hunting was permitted at all but two of the eight sites (Table 1). Hunting pressure varied across sites, mostly as a consequence of accessibility to humans and proximity of human habitations. None of the sites had directly been recently affected by commercial logging, but selective logging has taken place in close proximity (<500 m) to the St. Georges site (7).

Level of defaunation

At each of the eight sites (Table 1), we quantified the level of defaunation with the defaunation index, D (Giacomini & Galetti, 2013) for which we used the existing vertebrate census data (Richard-Hansen et al., 2015) (Appendix S1: Section S1). D may range from 0 (no defaunation) to 1 (complete defaunation) as compared to a hypothetical undisturbed reference site. Because plants in early life stages are preferentially affected by ground-dwelling browsers, we

calculated D (Appendix S1: Section S1) for ground-dwelling browsers (D_{leaf}) (Appendix S2: Table S1), for statistical analysis concerning leaf traits. Because all censused vertebrates are known to interact with fruits, we also calculated D for all censused vertebrates (D_{all}) for statistical analysis concerning fruit and seed traits.

Vegetation sampling

Three plots were established at each site, ~500 m away from the central point that had previously been established for the vertebrate censuses, following a cardinal direction (Figure 1). The exact location of each plot was chosen following a set of criteria that were, in order of importance, the absence of gaps in the canopy over the recruits, the absence of steep inclines, and the ease of access for the fieldwork team.

In each plot, seedlings (20–100 cm height) were tagged in a strip of 1 × 100 m and were identified to lowest possible taxonomic level and measured for height. Saplings (>100 cm height, <1 cm diameter measured at breast height [DBH]), juveniles (1–2 cm DBH), and treelets (2–5 cm DBH) were tagged in a strip of 5 × 100 m, identified and measured for DBH. Adult trees were tagged, identified, and measured for DBH in a strip of 40 × 125 m. Strips were nested and centered (Figure 1).

Plant traits

We measured leaf toughness and leaf thickness of tree recruits, and we collected leaf samples to determine SLA and leaf nitrogen content (Appendix S1: Section S1).

TABLE 1 Characteristics of the study sites.

Site name	Site no.	Latitude	Longitude	Hunting permitted	Vertebrate census year	Vegetation census year	D_{leaf}	D_{all}
Nouragues	1	4.0380569	-52.6729167	No	2007	2018	0.37	0.40
Saut Parasol	2	4.1774043	-52.9955918	No	2009	2018	0.20	0.30
Cariacou	3	3.6088132	-53.1472023	Yes	2008	2017	0.49	0.53
Kaw 1	4	4.6653857	-52.3010218	Yes	2012	2017	0.53	0.55
Kaw 2	5	4.5588718	-52.2065257	Yes	2011	2017	0.50	0.53
Camopi	6	3.1824930	-52.5130750	Yes	2002	2018	0.32	0.45
St. Georges	7	3.9551317	-51.8487714	Yes	2018	2019	0.58	0.62
Saül	8	3.6382591	-53.2029712	Yes	2002	2017	0.61	0.60

Note: D_{leaf} represents the level of defaunation for all censused ground dwelling browsers and D_{all} represents the level of defaunation for all censused browsers.

For each identified species, we collected fruit traits from the literature through a systematic review (Vaessen et al., in preparation). Wood density data we collected from the global wood density database (Zanne et al., 2009; Appendix S1: Section S1). We pooled plot data by site and then calculated the community weighted mean for continuous traits (CWM), or community weighted proportion for categorical traits (CWP), of each of the leaf and fruit traits for each size class (i.e., seedlings or saplings, size classes described under “vegetation sampling”). A common issue in studies on defaunation is that size classes are often directly compared between sites, but the inherent natural variation in tree functional composition between sites, would likely overshadow any short-term (i.e., <50 years) impacts of defaunation. To overcome this, we looked at relative changes in trait composition by calculating the ratio between the CWM or CWP of each of the size classes of tree recruits and the adult size class (see Hazelwood et al., 2020; Terborgh et al., 2008; Wright et al., 2007).

Analysis

The analysis focused on the relationships between the defaunation index and shifts in functional traits between adult trees and recruits. To test the predictions that defaunation explained differences in abundance of species with specific traits, we performed two types of analysis. First, a linear least squares regression was used to test the relationship between the level of defaunation and CWMs or CWPs of the different functional traits. Second, we created linear mixed effects models to test if significant relationships from the first analysis could be attributed to trait related shifts in species abundances. Statistical analyses were done in R version 3.6.1 (R Development Core Team, 2019).

For our first analysis, we pooled data by site. We calculated the CWM per site and per size class, for leaf toughness, leaf thickness, SLA, leaf nitrogen content, mean fruit length, and wood density, and calculated the CWP for the categorical variables of fruit fleshiness and color. As we did not have enough data for mean seed length, it was excluded from analyses.

Natural variation in trait composition makes the comparison of absolute shifts (i.e., directly comparing recruit communities) between sites unsuitable. For example, if the adult community between two sites would differ largely in leaf toughness, it is likely that, under similar the circumstances, the recruit communities at the same two site would show similar differences in leaf toughness. We therefore controlled for the natural variation in the undisturbed adult communities by calculating the ratio between each of the size classes of tree recruits and the adult size class for

the CWM or CWP of each functional trait (recruit:adult ratio). The ratios were tested for relationships with D , using Pearson correlation and least squares regression.

For our second analysis, to test if potential differences in CWM or CWP were due to trait-related changes in relative abundance of species as opposed to natural variation in CWM or CWP, we fitted linear mixed effects models, using the lme4 package (Bates et al., 2015). We calculated the relative abundance of each species by site and the overall species means for each functional trait, and then fitted and compared the following models:

$$\log(\text{ratio}_{i,s}) = \text{trait}_i \times D_s + (1|\text{species}).$$

$$\log(\text{ratio}_{i,s}) = \text{trait}_i + D_s + (1|\text{species}).$$

Here $\log(\text{ratio}_{i,s})$ represents the logarithm of the ratio of the abundance of the recruits to the abundance of adults for species i and for site s , trait_i represents a trait value for species i for each of the leaf and fruit traits, D_s represents either D_{all} or D_{leaf} (depending on the trait) for site s , and $(1|\text{species})$ is a random effect where each species is allowed to have its own intercept. If changes to the CWM are related to changes in the relative abundance of tree species because of defaunation, then we expect the interaction term ($\text{trait}_i \times D_s$) to result in a better model fit than the model without the interaction term.

We used the package lmerTest and Satterthwaite’s approximation to estimate denominator degrees of freedom and thereby p -values from these models (Kuznetsova et al., 2017). To compare the two models, we used Akaike’s Information Criterion (AIC). If models differed by more than two, we considered models to be significantly different. In cases where the AIC’s did not differ by more than two, we compared the AIC to the Bayesian Information Criteria (BIC) and selected the model with the lowest BIC. We present both the AIC and the BIC for all models in Appendix S2: Tables S3 and S4.

We used a mixed effects ANOVA to identify whether some species had a larger influence than others on the relationships between D and the CWM or CWP of the tested traits. We performed the ANOVA with Tukey posthoc analysis using the “emmeans” package (Lenth, 2021), to check for differences in recruit:adult abundance ratios between species. After identifying species that displayed large differences, we excluded these species from the data and reran the first analysis. If the selected species had a disproportionately large influence on the hunting-induced changes in CWMs and CWPs of the different traits, we expect that the removal of these species from the data will change

the significant results from the first analysis to become non-significant.

In addition we performed two PCAs on all sites for all size classes, using the package Vegan V2.5-6 (Oksanen et al., 2019). The first PCA was intended to distinguish major patterns in functional trait composition. Covariates included were the CWM of leaf toughness, leaf thickness, SLA, leaf nitrogen content, mean fruit length, and wood density, and the CWP of fruit fleshiness (using only one of two categories). The second PCA was intended to identify patterns in fruit color composition. Covariates included in the PCA were CWPs of fruit colors.

RESULTS

The surveys yielded a range of 892–1562 seedlings (average density 11.88 m^{-2}), 230–658 saplings (1.33 m^{-2}), 134–325 juveniles (0.46 m^{-2}), 110–279 treelets (0.38 m^{-2}), and 156–334 adults (0.06 m^{-2}) per site. For leaf traits, we obtained a value for at least 64% of the individuals, depending on the trait and the size class (Appendix S2: Table S2). For fruit and seed traits, these proportions were generally lower (Appendix S2: Table S2), as these traits could not be imputed for unidentified individuals and we did not have trait data for all species. The proportions were particularly low for the seedling communities, as the proportion identified was lower for seedlings than for any of the other size classes. Therefore the seedling size class was excluded from the least squares regression analysis between D (defaunation index) and the CWM and the CWP of fruit traits, and was entirely excluded from the analysis with linear mixed effects models, which required species means.

Trait composition differed clearly between the size classes within each of the eight sites, which was reflected in the PCA (Appendix S2: Figure S1A). Three more or less distinct clusters were observed, in which saplings, juveniles, and treelets formed one cluster, and adults and seedlings each formed a cluster (Appendix S2: Figure S1A). However, there was a large spread of the sites, particularly for the seedling size class, and Euclidean distances between sites were often larger than between seedlings and other size classes. The PCA based on fruit colors showed no distinct patterns (Appendix S2: Figure S2).

The CWMs of leaf toughness of sapling, juvenile, and treelet communities significantly decreased, relative to the adult community, as D_{leaf} (defaunation index of ground-dwelling browsers) increased (Figure 2c, Table 2a). SLA was significantly positively related to D_{leaf} for all classes of recruits (Figure 2d, Table 2b). Leaf nitrogen content and leaf thickness did not show any significant

relationships with D_{leaf} , while fruit length, fruit fleshiness did not show significant relationships with D_{all} (defaunation index of all censused vertebrates). Wood density of seedling and sapling communities significantly decreased relative to the adult community with increasing defaunation, whereas relationships were not significant for juvenile and treelet community (Figure 2h, Table 2b).

The linear mixed effects models showed significant or marginally significant relationships of the interaction between D and wood density on the species ratio of abundance of recruits and adults, and between D and SLA (Appendix S2: Tables S3 and S4). Fruit fleshiness, fruit color, wood density, leaf nitrogen content, and leaf toughness all showed model improvements when including an interaction of these traits with D compared to the model without an interaction (i.e., AIC or BIC improved by at least 2) (Appendix S2: Table S3 and S4). For fruit length and leaf thickness, the model neither improved nor had a significant interaction with D . Our follow-up analysis showed no species to be true outliers in terms of recruit:adult abundance ratios (Appendix S2: Figure S3), but visual inspection revealed that multiple species seemed to have notably higher or lower recruit:adult ratios and trait values, and therefore potentially had a disproportionate effect on the CWMs or CWPs. Based on these criteria, we removed *Quararibea duckei*, *Protium apiculatum*, and *Dicorynia guianensis*, after which none of the previously significant relationships between D and the CWMs and CWPs of the functional traits remained significant, indicating that changes in these species drive the observed relationships between hunting and community trait values. We did not observe these changes in significance when only removing two of the three species. Furthermore, there are perhaps more species that may have a similar influence on the relationships between D and the CWMs and CWPs of the functional traits, but for which we did not test the effect of removing them from the first analysis.

DISCUSSION

We studied the functional composition of tree recruit communities at eight sites in French Guiana that ranged widely in their level of defaunation, to test the hypothesis that the loss of large vertebrates leads to changes in functional composition of the tree recruit community due to trait-related changes in species abundance. The CWMs of leaf toughness, SLA, and wood density (expressed as a ratio between size classes of tree recruits and the adult size class) showed significant relationships with defaunation. Shifts in CWMs were, however, only partially explained by trait-related shifts in species abundance. Rather, the shifts

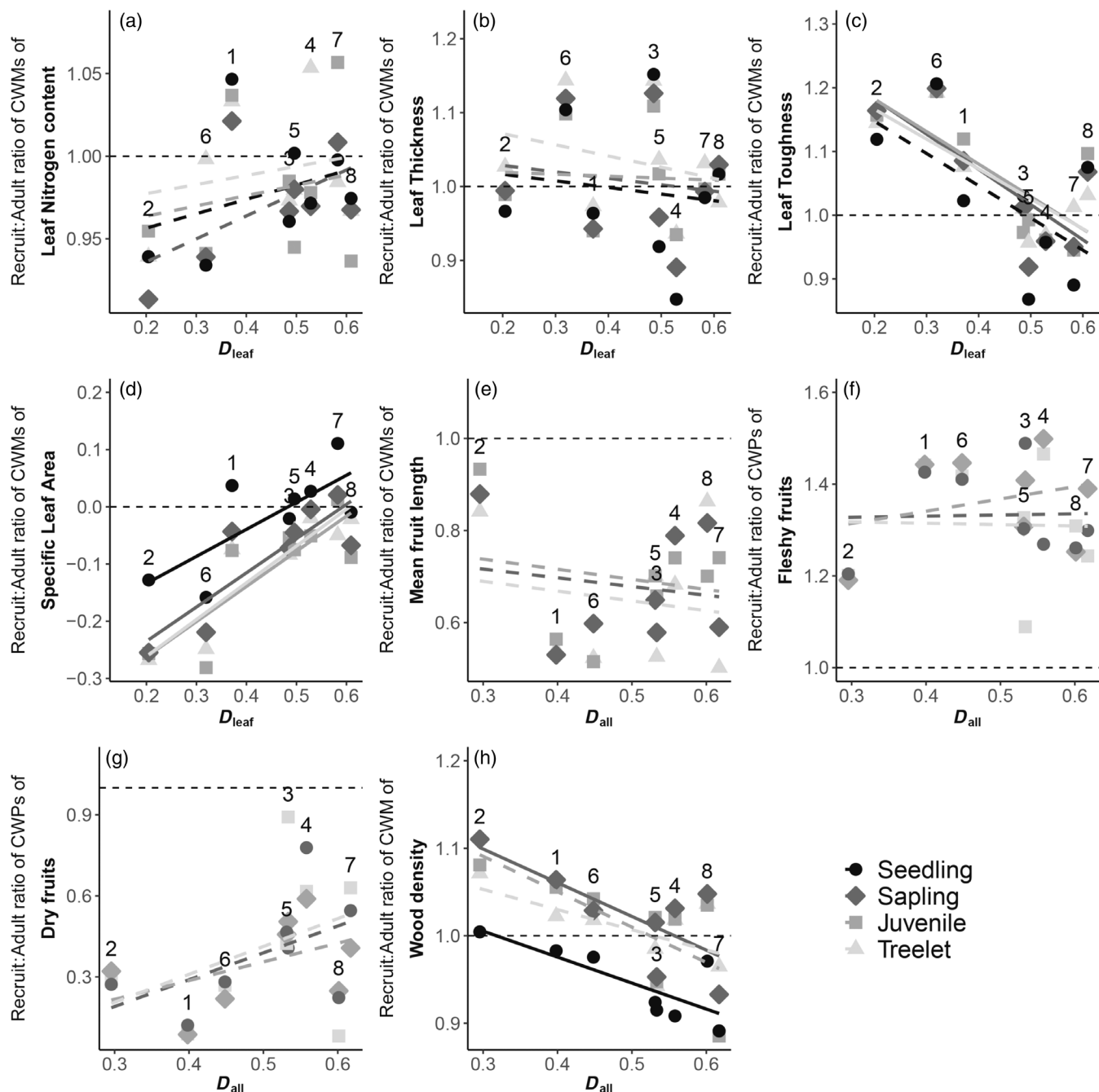


FIGURE 2 Relationship between the recruit:adult ratios of the community weighted means (CWM) or community weighted proportions (CWP) of eight functional traits and defaunation index based on all censused vertebrate species (D_{all}) or only ground dwelling browsers (D_{leaf}). Values greater than one indicate that the CWM or CWP is higher in the recruit community, compared to the adult community. Numbers correspond with site numbers. (a) Leaf nitrogen content, (b) leaf thickness, (c) leaf toughness, (d) specific leaf area, (e) mean fruit length, (f) fleshy fruits, (g) dry fruits, (h) wood density. Seedlings, saplings, juveniles, and treelet communities are represented, respectively, by circles, diamonds, squares, and triangles. Lines indicate least squares regression, with solid lines indicating significant relationships and dashed line indicating non-significant relationships. Color coding represents, from dark to light, seedling, sapling, juvenile, and treelet classes. Pearson correlations are shown in Table 2.

seemed associated with a combination of factors, including weak trait-related shifts in species abundance and the disproportionate influence of three tree species that were relatively abundant and had trait values towards the further ends of the spectra for multiple traits.

The CWMs of SLA and leaf toughness showed significant relationships with defaunation. These results suggest that recruits with palatable leaves (e.g., high SLA and low leaf toughness) are more abundant with high defaunation, in agreement with our prediction that tree species with

TABLE 2 Pearson correlations and corresponding *p*-values between (A) D_{leaf} and recruit:adult ratios of the community weighted means (CWMs) of four leaf traits, (B) D_{all} and recruit:adult ratios of the CWM (mean fruit length, wood density) or community weighted proportions (CWP) (dry fruits, fleshy fruits) of three fruit traits and wood density, significant correlations ($p \leq 0.05$) are in bold.

Tested ratio	<i>r</i>	<i>p</i> -value	Figure
A			
Leaf nitrogen content			
Treelet:Adult	0.40	0.33	2a
Juvenile:Adult	0.29	0.48	2a
Sapling:Adult	0.59	0.13	2a
Seedling:Adult	0.35	0.39	2a
Leaf thickness			
Treelet:Adult	-0.25	0.56	2b
Juvenile:Adult	-0.04	0.93	2b
Sapling:Adult	-0.17	0.68	2b
Seedling:Adult	-0.14	0.74	2b
Leaf toughness			
Treelet:Adult	-0.82	0.01	2c
Juvenile:Adult	-0.73	0.04	2c
Sapling:Adult	-0.79	0.02	2c
Seedling:Adult	-0.62	0.10	2c
Specific leaf area			
Treelet:Adult	0.91	0.00	2d
Juvenile:Adult	0.80	0.02	2d
Sapling:Adult	0.86	0.01	2d
Seedling:Adult	0.74	0.04	2d
B			
Mean fruit length			
Treelet:Adult	-0.16	0.71	2e
Juvenile:Adult	-0.10	0.82	2e
Sapling:Adult	-0.12	0.78	2e
Fleshy fruits			
Treelet:Adult	-0.02	0.96	2f
Juvenile:Adult	0.29	0.48	2f
Sapling:Adult	0.04	0.92	2f
Dry fruits			
Treelet:Adult	0.27	0.52	2g
Juvenile:Adult	0.45	0.26	2g
Sapling:Adult	0.53	0.18	2g
Wood density			
Treelet:Adult	-0.60	0.11	2h
Juvenile:Adult	-0.68	0.06	2h

(Continues)

TABLE 2 (Continued)

Tested ratio	<i>r</i>	<i>p</i> -value	Figure
Sapling:Adult	-0.73	0.04	2h
Seedling:Adult	-0.78	0.02	2h

Note: The header "figure" refers to the corresponding figures.

traits preferred by browsers benefit from defaunation. Contrary to our prediction, leaf nitrogen content was independent of the level of defaunation, even though nitrogen content is often associated with leaf palatability in recruits (Coley, 1983; Coley & Barone, 1996). However, the association observed in past studies concerns leaves that have not yet fully matured, which lack the leaf toughening that often provides protection against herbivory. Other studies have found that leaf toughness is a better predictor for herbivory than nitrogen (Coley, 1983; Poorter et al., 2004). In this study, we measured leaf nitrogen content on leaves that had seemingly fully developed, at which point nitrogen content may play a limited factor in leaf palatability. Our results in leaf traits differ from Kurten et al. (2015), who found no significant differences in leaf mass per area and leaf toughness between a hunted and control site. However, they directly compared size classes of recruits, without controlling for the functional composition of the adult community.

We found no support for our prediction that species with fruit traits related to dispersal by large vertebrates would decrease in abundance with increased defaunation. Neither the CWMs and CWPs showed significant relationships with defaunation level, nor did the species abundance ratio. Despite these results, we consider it highly unlikely that hunting-induced changes in seed dispersal do not affect recruit functional composition, as Wright et al. (2007) found higher mean seed mass associated to seedling communities at hunted sites, and Kurten et al. (2015) found a significant higher mean seed mass of seedlings in vertebrate exclosures. A plausible explanation is that we were not able to test seed size or mass, two important fruit traits, due to the relatively low availability of data for the surveyed species. Yet, the tested fruit traits are also known as important determinants of vertebrate dispersal syndromes (Janson, 1983), and we therefore expected these traits to be affected by defaunation. The results of our linear mixed effects models suggested that there is a relationship between fruit color or fruit fleshiness and defaunation, showed by the great improvement in model fit with the inclusion of the interaction term, but that this relationship was weak, explaining the non-significance of the interaction. This weak effect may be due to the fact that vertebrates rely on these cues in combination with other traits, which form a full dispersal syndrome rather than just the fruit fleshiness

or color alone (Gautier-Hion et al., 1985; Janson, 1983; Pijl, 1969). These trait syndromes are not fully captured by analyses of single traits like ours. Therefore, multivariate analyses may be more suitable to investigate defaunation impacts on forest composition, as they probably better capture the complexity of vertebrate-fruit interactions.

Wood density showed a significant negative relationship with defaunation at the seedling and sapling stage, in concurrence with the findings of Kurten et al. (2015), and there were significant wood density-related shifts in species abundance in line with earlier predictions (Harrison, 2011; Jansen et al., 2010). It is the only trait that showed clear significant relationships in all analyses. This is striking, because wood density can only be indirectly affected through its correlations with leaf traits (Kitajima & Poorter, 2010) and fruit traits (Bello et al., 2015). This result thus illustrates that a combination of—sometimes weak—trait related shifts in species abundance together can result in large changes in forest composition.

We observed changes in the CWM of wood density only in the seedling and sapling size class. This pattern may occur because defaunation induced changes through browsing and seed dispersal are most apparent in the earliest life stages (Kurten et al., 2015; Wright et al., 2007), but can be diluted in later life stages through density dependent mortality, environmental filtering, or a higher proportion of individuals that sprouted pre-hunting. As a result of defaunation-induced lower mean wood density, it is likely that forests will store less biomass in the long-term, as has been modeled by previous studies (Bello et al., 2015; Osuri et al., 2016; Peres et al., 2015). Contrary to these studies, however, we link reduced forest biomass not only to changes in seed dispersal, but also to changes in browsing.

Our results showed that shifts in functional composition are largely driven by a few species. In particular, three species, *Quararibea duckei*, *Protium apiculatum*, and *Dicorynia guianensis*, were able to disproportionately influence these relationships. These species were relatively abundant and had more extreme trait values for several traits. Of these three species, *Quararibea duckei* and *Protium apiculatum* influenced relationships due to their relative increase in abundance with higher defaunation, while *Dicorynia Guianensis*, a popular timber species, decreased in relative abundance with higher defaunation. Because we did not test all species, it may be that some other tree species have a similar influence on the relationship between defaunation and the functional traits. These results suggest that species whose traits are negatively influenced by defaunation may experience local populations reductions. Species with one or few negatively influenced traits, are, in the short-term,

perhaps less affected by defaunation at the populations level.

Defaunation has been linked to changes in the composition of recruit communities (Dirzo & Miranda, 1991; Effiom et al., 2013; Terborgh et al., 2008; Wright et al., 2007), and is often assumed to be the result of changes in seed dispersal (Bello et al., 2015; Osuri et al., 2016). Indeed, there is evidence that defaunation results in reduced dispersal, and higher clustering of recruits, but the impacts of defaunation on the clustering of recruits seem to be small in the short term (i.e., <50 years) (Bagchi et al., 2018; Harrison et al., 2013), and it is questionable whether reduced seed dispersal is the only mechanism causing changes in composition of recruit communities (Bagchi et al., 2018; Hazelwood et al., 2020). Our results support the hypothesis that hunting induces changes in forest composition via multiple mechanisms, of which browsing has been underappreciated. Dirzo and Miranda (1991) linked the lower species diversity in a defaunated forest compared to an undisturbed forest to the striking difference in herbivore damage between the two sites. However, since then only few studies have focused on the impact of a reduction in browsing for recruit community composition (Camargo-Sanabria et al., 2015; Kurten et al., 2015).

Our results suggest that minor changes in ecological processes such as browsing and seed dispersal may combine to lead to major changes in forest composition. Hunting thus not only affects the faunal composition of these ecosystems but may have cascading effects on the nature of tree recruitment and thus the composition and dynamics of future forests that may be visible for centuries after.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data (Vaessen et al., 2022) are available in DataverseNL at <https://doi.org/10.34894/QMYHNNH>. Part of the fruit trait data were downloaded from <https://bioportal.naturalis.nl/> by searching for species name (see Appendix S1: Section S1).

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