


## RESEARCH ARTICLE

# Leaf traits linked to structure and palatability drive plant–insect interactions within three forested ecosystems

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**Abstract**

**Premise:** Plant traits and insect herbivory have been highly studied within the modern record but only to a limited extent within the paleontological. Preservation influences what can be measured within the fossil record, but modern methods are also not compatible with paleobotanical methods. To remedy this knowledge gap, a comparable framework was created here using modern and paleobotanical methods, allowing for future comparisons within the fossil record.

**Methods:** Insect feeding damage on selected tree species at Harvard Forest, the Smithsonian Environmental Research Center, and La Selva were characterized using the damage type system prevalent within paleobotanical studies and compared with leaf traits. Linear models and random forest analyses tested the influence of leaf traits on total, specialized, gall, and mine frequency and diversity.

**Results:** Structural traits like leaf dry mass per area and palatability traits, including lignin and phosphorus concentrations, are important variables affecting gall and mine damage. The significance and strength of trait-herbivory relationships varied across forest types, which is likely driven by differences in local insect populations.

**Conclusions:** This work addresses the persistent gap between modern and paleoecological studies focusing on the influence of leaf traits on insect herbivory. This is important as modern climate change alters our understanding of plant–insect interactions, providing a need for contextualizing these relationships within evolutionary time. The fossil record provides information on terrestrial response to past climatic events and, thus, should be implemented when considering how to preserve biodiversity under current and future global change.

**KEYWORDS**

herbivory, leaf traits, paleoecology, plant–insect interactions

The overall fitness of broad-leaved tree species is highly influenced by photosynthetic rate (Kerchev et al., 2012), which is drastically diminished when leaf tissue is lost or damaged (Zangerl et al., 2002). Insect herbivores feeding on leaf tissue, therefore, represent a great loss of resources to plants (Züst and Agrawal, 2017; Haworth et al., 2018) and must be defended against (Agrawal and Fishbein, 2006; Züst and Agrawal, 2017). Leaf traits are one way of mitigating leaf tissue loss due to insect herbivory. Traits impact insect herbivory via structural or chemical defenses and/or by influencing the palatability or quality of the food (e.g.,

Freeman, 2008; Fürstenberg-Hägg et al., 2013). As the relationship between plants and insect herbivores has been occurring for hundreds of millions of years (Labandeira and Currano, 2013), the fossil record may provide context into how traits influence damage over evolutionary timescales and during times of pronounced evolutionary innovation, extinction, and climate change. However, many leaf traits hypothesized to affect herbivory cannot be measured within paleontological data sets, and herbivory in modern settings is rarely measured using the same metrics as in fossil studies. In this study, we fill this knowledge gap by creating

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an analogous modern comparison and testing the influence of leaf traits on different types of insect herbivory.

The term “trait” refers to any well-defined, measurable property of an organism (Dawson et al., 2021); “functional trait” (i.e., a trait related to fitness) (McGill et al., 2006; Violle et al., 2007; Dawson et al., 2021) and “morphological trait” (i.e., a physical or phenological measurement) (Violle et al., 2007) have more specific definitions. Trait-based research within modern ecology increased in popularity in 2006 (e.g., McGill et al., 2006), shifting focus away from individual species and, rather, on the influence of functional traits within an ecosystem. Specifically focusing on plant–insect interactions, plant and leaf traits have been analyzed to understand mechanistic drivers of modern patterns (e.g., Coley et al., 1996; Agrawal and Fishbein, 2006; Salazar et al., 2018). Although this transition within the mid-2000s represents a shift in modern ecology, paleontologists and paleobotanists have been using traits, specifically morphological traits, to understand ecosystems for decades (e.g., MacGinitie, 1974; Behrensmeier et al., 1992) as they are the backbone to reconstruction. Leaf traits have been used to identify and differentiate species (Ellis et al., 2009), as well as reconstruct past abiotic conditions such as temperature, precipitation, and elevation (Wolfe, 1993; Wilf, 1997; Wilf et al., 1998; Spicer et al., 2009; Blonder et al., 2014; Butrim and Royer, 2020). Although limited, the interplay between leaf traits and herbivory has also been studied within the fossil record (Müller et al., 2023), with a specific focus on leaf dry mass per area (LMA) (e.g., Wilf, 2008). Exciting new research on structural (Maccracken et al., 2019) and chemical traits (McCoy et al., 2022) builds upon paleobotanical work documenting the presence/absence of herbivory through time (e.g., Currano et al., 2021) and provides new insight into past plant species' ability to defend themselves. Although these types of traits are not groundbreaking to modern plant–insect researchers who measure many traits *in vivo*, this pushes forward the collective knowledge of positive cross-species interactions. Plant traits within the fossil record represent untapped potential for disentangling the complexities and variability in plant–insect interactions through an evolutionary viewpoint.

As sessile organisms, plants cannot migrate to evade predators or unfavorable environmental conditions; thus, leaf traits must act in place of the ability to readily relocate. Physical or morphological (i.e., margin type, LMA, trichomes), nutrition and palatability (carbon, nitrogen, lignin, cellulose, water, phosphorus), and chemical (i.e., secondary compounds) traits have been either hypothesized or documented to act as defense mechanisms (e.g., Agrawal and Fishbein, 2006). Which strategies plants invest in depends not only upon the types of insect herbivores within a plant's environment (Ali and Agrawal, 2012) but also on the overall environment itself. For example, previous work has shown that plants within nutrient–poor environments

are less defended than plants in nutrient-rich environments (e.g., Agrawal, 2007; Schemske et al., 2009), although this is debated (Moles et al., 2011). Toothed leaf margins are characteristic of deciduous species with thinner leaves, greater nitrogen concentrations, and lower LMA (Royer et al., 2012). Additionally, LMA has previously been thought to negatively influence insect herbivory in direct and indirect ways ranging from mechanical wear/stress on mouthparts to decreased nutrient quality (Clissold et al., 2009). Trichomes have many different functions, including deterring insect herbivores both physically as well as via secondary compounds that are excreted through glands (Karabourniotis et al., 2020). Margin type, LMA, and trichomes have allowed for inferences of plant defenses and palatability within the fossil record (i.e., Currano et al., 2016; Currano and Jacobs, 2021), but chemical traits related to palatability or defensive compounds have rarely been documented within the paleobotanical record (McCoy et al., 2022) because leaf fossils often lack the preservation needed to measure them. Integration of the modern literature illustrates how chemical traits influence leaf structure and overall palatability (Schädler et al., 2003). For example, tannins and phenols (secondary compounds) dissuade insect herbivory (Adams et al., 2009; Salminen and Karonen, 2011), while nitrogen and phosphorus might increase herbivory due to greater quality of food (Huberty and Denno, 2006; Tielens and Gruner, 2020). These relationships between plant defenses and insect herbivores are multidimensional with many direct and indirect pathways; as such, studies should focus on multiple traits (Agrawal and Fishbein, 2006; Farias et al., 2020) affecting insect herbivory, a strategy we deployed here.

As demonstrated above, there is a plethora of research focused on understanding the importance of plant traits and/or the influence of plant traits in modern ecosystems. Considerable fossil data has been collected to examine patterns in herbivory on bulk assemblages across time and space (e.g., Currano et al., 2021) and potential drivers of these patterns. Yet connections between these modern and fossil data sets remain elusive, limiting our understanding of why insect damage varies among species at fossil sites and the extent to which leaf traits influence spatial and temporal patterns in fossil herbivory. To fill this knowledge gap, this study implemented paleobotanical methods for characterizing insect herbivory on leaves (Labandeira et al., 2007, and subsequent revisions) within modern ecosystems and then compared herbivory frequency and diversity to leaf traits. Leaf traits readily observed within fossil data sets include margin type, LMA, and trichomes, and these were supplemented with traits that correspond to structural and chemical defenses and palatability, such as weight percent (% dry mass) carbon, nitrogen, phosphorus, water, lignin, cellulose, tannins, and phenols. Although fossil data sets currently are limited in our understanding of structural and palatability traits, that is no reason not to see the future potential of our field and include them here. Specifically, this research asks (1) how do leaf traits influence insect

herbivory measured as leaf feeding damage (2) are structural traits negatively influencing insect herbivory regardless of forest type (3) or are chemical defenses and palatability more important? To the authors' knowledge, this is the first study to investigate relationships between a diverse array of leaf traits and insect herbivore damage frequency and diversity, measured as in fossil studies, and represents a bridge between modern and paleo-ecology.

## MATERIALS AND METHODS

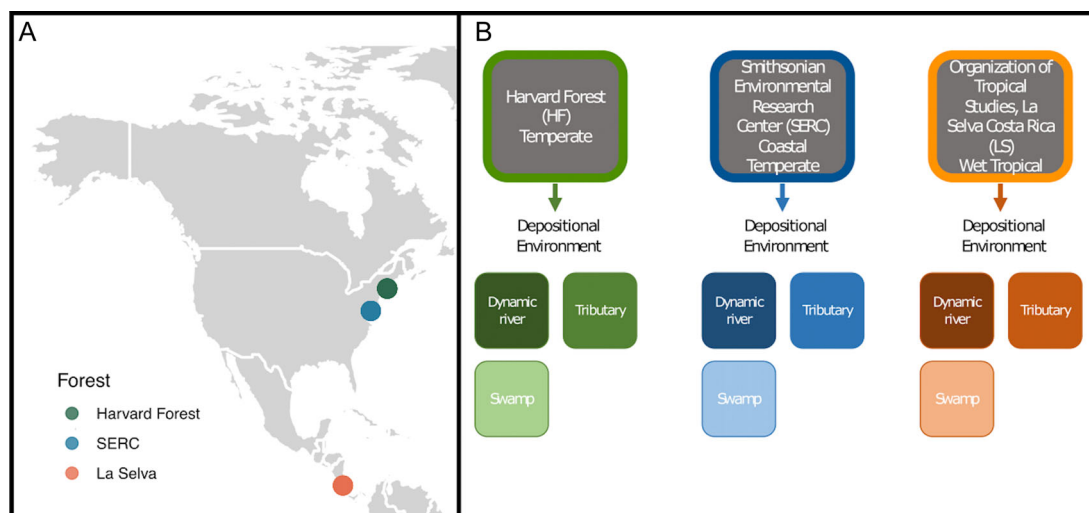
### Site descriptions

Temperate Harvard Forest, USA (HF), coastal temperate Smithsonian Environmental Research Center, USA (SERC), and wet tropical La Selva, Costa Rica (LS; Figure 1 and Appendix S1) were chosen as sampling locations due to varying forest types, climate, elevation, and dominant plant species. Differences between the two temperate forests, HF and SERC, are as follows. HF is dominated by hemlock (*Tsuga canadensis* (L.) Carrière), various birch (*Betula*) species, red maple (*Acer rubrum* L.), red oak (*Quercus rubra* L.), and American beech (*Fagus grandifolia* Ehrh.), while SERC is dominated by American beech, dogwood (*Cornus florida* L.), white (*Quercus alba* L.) and red oak, sweetgum (*Liquidambar styraciflua* L.), tulip poplar (*Liriodendron tulipifera* L.), and hickory (*Carya alba* Sarg.). Additionally, HF is approximately 1200 ft above sea level, and SERC is at sea level, with a mix of fresh and brackish water environments. Unlike the two temperate forests, LS is a low-land tropical wet forest with over 500 tree species that are variably distributed across the landscape (e.g., Newstrom et al., 1994; Clark and Clark, 2000; O. Vargas Ramírez, personal communication, December 2019). Dominant tree species within LS are *Castilla elastica* Cerv., *Ficus insipida* Willd., *Luehea seemannii* Willd., *Terminalia oblonga* (Ruiz and Pav.) Steud., and *Zygia longifolia* (Willd.) Britton and Rose (Zhang

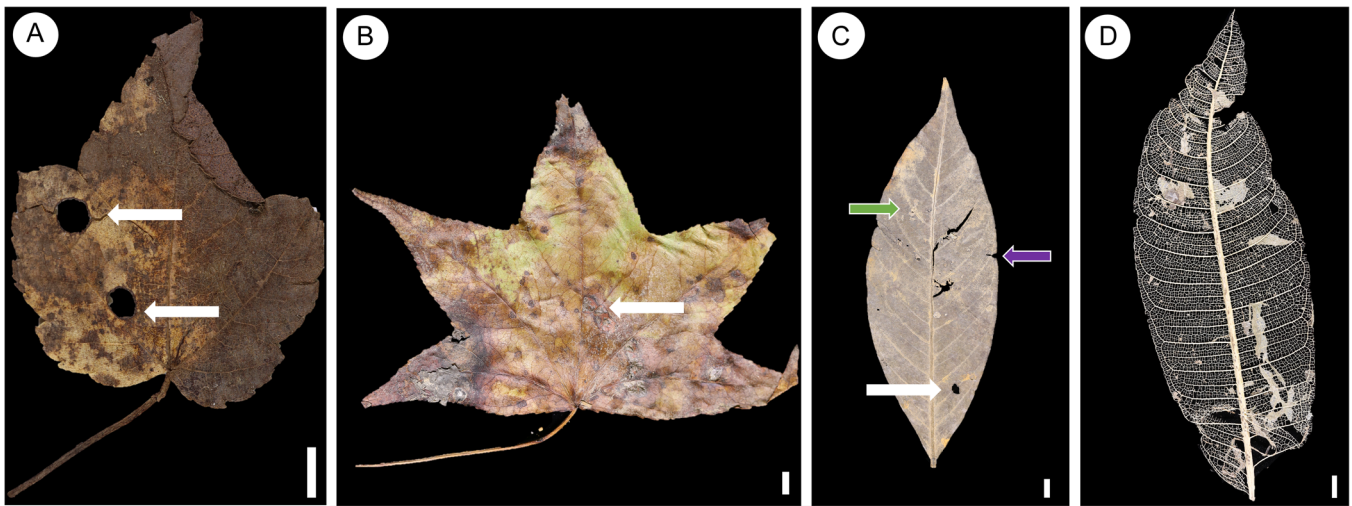
et al., 2006). The research station is surrounded by mountainous terrain and bound by two dynamic, fresh-water river systems, the Río Sarapiquí and Río Puerto Viejo.

Leaves were collected during the winter (LS) and summer (HF and SERC) of 2019. Within each forest, three depositional environments, a swamp, a small tributary, and a dynamic river, were selected to capture the variability within each forest and mimic readily fossilized environments within the paleo record. Within each depositional environment, approximately 1200 leaves were sampled from within the sediment at three sites, roughly 100 m apart, accounting for spatial variability in leaf and insect herbivore communities. Once leaves were collected, they were cleaned of sediment, pressed, and dried at approximately 70°C until dry (24–72 h depending on the ecosystem). Leaves were then transported back to the University of Wyoming for insect herbivory analysis (Figure 2).

Plant species were selected for leaf trait-herbivory analyses based on the availability of published trait data and dominance within the landscape. We also included plant species that are shared across forests. Dominant species likely represent the dominant host-plant for insect herbivores, while species that are shared across forests provide an opportunity to characterize similar herbivory interactions across different forests. Plant species dominance within each forest was identified using rank abundance curves (Figure 3). Some species that did not have high abundance, such as American beech (*F. grandifolia*) within HF, were analyzed as the species is shared across two forests (HF and SERC). Within SERC, white oak (*Q. alba*) was chosen over American hornbeam (*Carpinus caroliniana*) due to its greater overall abundance at SERC, higher quality preservation, and availability of published trait data. Similar reasoning was also used to choose American sycamore (*Platanus occidentalis*) at SERC, in addition to a high abundance of *Platanus* within the fossil record for future comparisons. Lastly, as many species within LS had similar abundances, judgment was made on the



**FIGURE 1** Map of sampling locations (A) with a conceptual schematic of sampling method across forests (B). Leaves were sampled within a dynamic river, tributary, and swamp for Harvard Forest (green), SERC (blue), and La Selva (orange). Adapted from Azevedo-Schmidt et al. (2023).



**FIGURE 2** Plate showing examples of leaves used in analyzing insect herbivory and leaf traits across all three forests. While some leaves are beautifully preserved with intact petioles (used for reconstructing leaf mass per area or LMA) on red maple (A; *Acer rubrum*) and sweetgum (B; *Liquidambar styraciflua*), they are not always this well preserved (C; *Zygia longifolia* and D; *Ficus insipida*). However, multiple damage types are preserved across forests with examples of hole damage (A; HF1901.1 #224 DT4; C; LS1902.3 #135 DT3), mine damage (B; MD1901.1 #392 DT 295), margin and surface feeding (C; LS1902.3 #135 DT12 [purple] and DT333 [green]). All scale bars are 0.5 cm.

availability of published trait data and preservation of leaves. *Cordia alliodora*, *Cordia bicolor*, *F. insipida*, *L. seemannii*, *Schnella guianensis*, *Trophis racemose*, and *Z. longifolia* were all chosen for trait analyses.

## Leaf traits

For each selected species in each forest, weight percent (wt. %) carbon, nitrogen, and carbon to nitrogen ratios (C:N) were analyzed at the University of Wyoming Stable Isotope Facility. Leaves without insect herbivory were preferred but sometimes not possible due to high levels of insect herbivory. Ten to twelve leaves were randomly selected per species and depositional environment (i.e., 10 red maple leaves from the swamp, 10 from the tributary, and 10 from the fluvial environment). This allows for comparison of the same species across multiple environments, accounting for variability within species and across depositional environments. We selected undamaged leaves to measure because herbivory may alter carbon and nitrogen composition due to down-regulation of photosynthesis (Zangerl et al., 2002). Although the leaves we chose may have been avoided by insects due to their carbon concentration, we feel confident that by selecting multiple leaves and homogenizing the sample, we minimized bias as best we could. Future research could compare damaged and undamaged leaves separately to confirm these assumptions. Petiole material and major veins were not incorporated into the samples. Tissue was ground to a very fine powder using a ball mill, weighed to 2.0–2.5 mg, and analyzed using a Costech 4010 Elemental Analyzer Thermo Delta Plus XP IRMS.

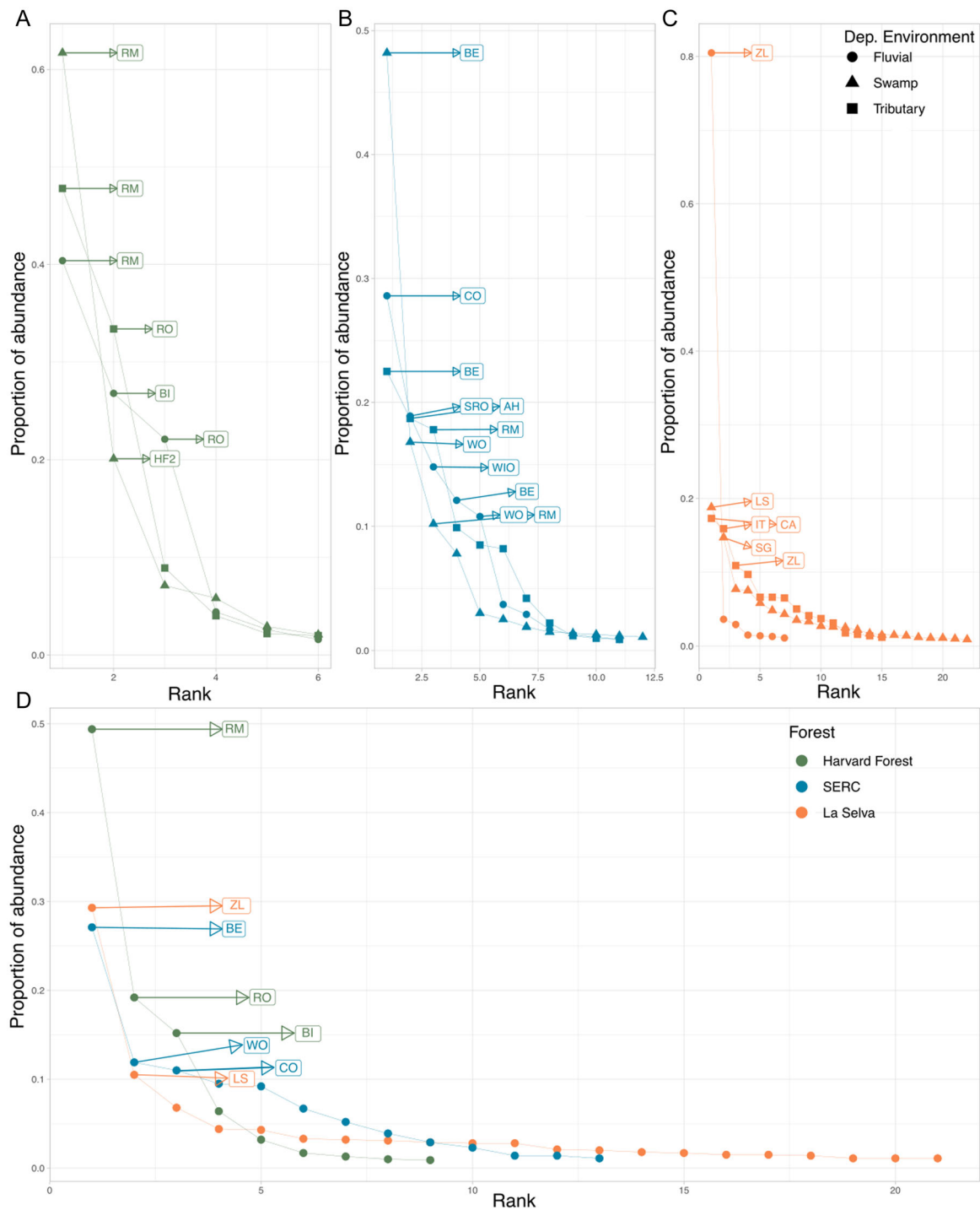
Additional leaf trait data were pulled from the literature (Appendix S2; Lee et al., 2003; Adams et al., 2009; Ardón

et al., 2009; Lind and Parker, 2010; Wind, 2013). Data were only included if they were collected within the three research sites. Some data were collected on leaf litter samples, while others came from fresh or dry leaves. Lignin (% dry mass), cellulose (% dry mass), water (% dry mass), trichomes (presence/absence & density), tannins (% dry mass), phenols (% dry mass), and phosphorus (% dry mass) values were compiled, but unfortunately, not all traits were available for all species within each forest (Table 1). The use of different sampling methods across published studies may add noise to the data set, and further research is needed to investigate how abscission, transport, and deposition/burial influence the trait values examined here.

Leaf mass per area (fresh leaf area/dry mass) could not be directly measured as leaves were never collected in their fresh state, and thus paleobotanical methods to reconstruct LMA ( $\text{g}/\text{m}^2$ ) were used (Royer et al., 2007). Approximately 50 leaves per depositional environment (Appendix S3),  $\geq 75\%$  intact, were randomly selected and measured for each dominant species (Table 1). If 50 well-preserved leaves with an attached petiole were not present within a depositional environment, we used the largest sampling number available (Table 1). Each leaf was carefully photographed, and measurements were made using ImageJ. LMA was reconstructed for each leaf where the percent area damaged was measured (see the “Insect herbivory” section).

## Insect herbivory

Insect herbivory, measured as damage types and grouped into functional feeding groups (DTs, FFGs; Labandeira et al., 2007), was recorded for each individual leaf collected ( $n = 7825$ ) in order to assess the influence of leaf traits on



**FIGURE 3** Rank abundance curves for Harvard Forest (A), SERC (B), and La Selva (C). Forests are grouped together (D) to compare the abundance of species across forests. Dominant tree species for Harvard Forest, red maple (*Acer rubrum*; RM), red oak (*Quercus rubra*; RO), and birch sp. (*Betula* sp.; BI), SERC, American beech (*Fagus grandifolia*; BE), chestnut oak (*Quercus montana*; CO), southern red oak (*Quercus falcata*; SRO), American Hornbeam (*Carpinus caroliniana*; AH), white oak (*Quercus alba*; WO), red maple (RM) and willow oak (*Quercus phellos*; WIO), and La Selva, *Zygia longifolia* (ZL), *Luehea seemannii* (LS), *Cordia alliodora* (CA), *Inga thibaudiana* (IT), and *Schnella guinensis* (SG) are labeled. Shape of points in A–C denotes the depositional environment.

insect herbivory across all three forests. DTs are morphologically distinct patterns of insect feeding preserved on leaf lamina or tissue, with many DTs making up a FFG. FFGs correspond to groups of insects that make similar types of feeding damage and are standard within fossil data sets. For

example, DT2 is a circular perforation 1–5 mm in diameter, while DT3 is a polylobate perforation 1–5 mm in diameter; each DT is distinct in morphology, but both DTs are within the hole feeding FFG (Labandeira et al., 2007). In total, there are seven folivorous FFGs: hole feeding, margin feeding,

TABLE 1 Summary table of plant traits used in this study which were directly measured or previously published.

Direct measure		Literature													
Forest	Species	n	Nitrogen (wt. %)	Carbon (wt. %)	C:N	Margin type (teeth = 1)	Trichomes (presence = 1)	LMA (g/m <sup>2</sup> ; mean)	Phosphorus (wt.%)	Lignin (wt. %)	Cellulose (wt. %)	Tannins (wt. %)	Phenols (wt. %)	Trichome density (cm <sup>-2</sup> )	Water (wt. %)
Harvard Forest	<i>Acer rubrum</i>	150	1.10	40.90	38.17	1	1	76.11 (1.53)	0.24	22.60	26.49	12.78	7.64	-	-
	<i>Quercus rubra</i>	134	1.45	40.20	28.20	0	0	60.24 (0.33)	0.16	31.05	31.94	7.20	9.31	-	-
	<i>Betula</i> sp.	150	1.57	36.73	24.37	1	1	69.64 (0.70)	0.39	29.43	34.99	3.07	6.30	-	-
	<i>Fagus grandifolia</i>	93	1.10	31.37	29.00	1	1	52.27 (0.82)	-	-	-	-	-	-	-
SERC	<i>Quercus alba</i>	93	1.19	38.19	33.04	0	1	68.79 (1.09)	0.19	-	-	-	-	669.10	40.04
	<i>Quercus montana</i> or <i>michauxii</i>	70	1.15	43.20	38.05	0	1	62.01 (1.14)	0.00	-	-	-	-	270.00	59.48
	<i>Platanus occidentalis</i>	36	1.50	33.45	22.05	1	1	81.83 (3.98)	0.21	-	-	-	-	0.00	65.20
	<i>Fagus grandifolia</i>	115	1.05	29.15	25.57	1	1	58.56 (1.23)	0.13	-	-	-	-	118.80	50.77
	<i>Quercus falcata</i>	93	1.08	35.75	33.95	0	1	65.36 (0.83)	0.24	-	-	-	-	15.94	57.34
	<i>Acer rubrum</i>	15	1.18	28.53	25.35	1	1	59.96 (3.52)	0.15	-	-	-	-	0.70	59.42
La Selva	<i>Zygia longifolia</i>	83	2.18	45.10	20.98	0	1	93.94 (1.98)	0.06	28.30	24.60	8.10	10.00	-	-
	<i>Luehea seemannii</i>	97	1.43	34.28	24.07	1	1	105.01 (3.34)	0.11	16.30	19.90	13.60	7.40	-	-
	<i>Trophis racemose</i>	28	2.30	45.35	19.65	1	0	80.16 (2.33)	-	-	-	-	-	-	-
	<i>Ficus insipida</i>	45	1.70	37.65	22.13	1	0	90.00 (3.14)	0.08	8.30	19.10	0.50	11.80	-	-
	<i>Cordia bicolor</i>	49	1.60	37.90	24.10	1	0	102.72 (2.84)	-	-	-	-	-	-	-
	<i>Schnella guianensis</i>	40	1.50	46.05	31.20	1	0	79.65 (1.56)	-	-	-	-	-	-	-
	<i>Cordia alliodora</i>	56	1.50	36.73	36.73	0	1	79.93 (1.93)	-	-	-	-	-	-	-

Note: Values given are the average of samples within a species and depositional environment. Standard error for reconstructed LMA values is shown in parentheses next to LMA values. Abbreviation: LMA, leaf dry mass per area.

skeletonization, surface feeding, piercing and sucking, mining, and galling (Labandeira et al., 2007). Leaf chewing damage that occurs while the leaf is living and attached to the tree has a distinct reaction rim or thickened tissue, allowing it to be differentiated from detritivore damage that occurs post-abscission. DTs can also be classified as generalist versus specialist damage. Generalist damage is damage that occurs on many different plant-hosts and is often made by many insect groups, while specialist damage occurs on one or few, similar plant species (Labandeira et al., 2007), often only made by a smaller subset of insect species. However, assigning DTs to specific insect genera (Azevedo-Schmidt et al., 2019; Donovan et al., 2014; Maccracken et al., 2021) and species has been rare (Adroit et al., 2020; Dong et al., 2018; Winkler et al., 2010). Examining DTs and FFGs on leaves provides a proxy for insect diversity and landscape-level assessment of insect communities (Carvalho et al., 2014).

For this study, we focused on total, specialized, gall, and mine damage (measured as frequency and diversity) for the selected plant species within each forest type. These four categories of feeding were targeted as they

capture all feeding behaviors. Total damage is the measure of every instance of damage on every leaf without distinguishing between FFGs or generalists versus specialists. While both galls and mines are included within specialized damage, they generally make up less than 50% of specialized damage diversity. Focusing on galling and mining, as opposed to all specialist feeders, decreases the taxonomic breadth of insects being considered. Lastly, gall and mine damage are regularly reported in the paleobotanical literature (e.g., Wilf and Labandeira, 1999; Labandeira, 2021; Labandeira and Wappler, 2023). Once DTs were recorded for each individual leaf, frequencies of herbivory and the percent of leaves with a given type of damage were analyzed for the four damage categories listed above. Frequencies were calculated for each selected plant species by averaging across depositional environments within each forest (Table 2; Appendix S3). Diversities and the number of damage types observed were reported for total, specialized, gall, and mine damage for the selected tree species by standardizing to 30 leaves to account for uneven sample sizes (Table 2). Additionally, the percent area damaged, the area of tissue consumed via

**TABLE 2** Summary table of herbivory frequencies and diversities for the selected species in each forest.

Forest	Species	n	Frequencies (% of leaves)				Diversities (no. of DTs on 30 leaves)			
			Total	Specialized	Gall	Mine	Total	Specialized	Gall	Mine
Harvard Forest										
	<i>Acer rubrum</i>	2033	89.14	46.90	29.63	6.45	16.21	6.34	1.52	1.01
	<i>Quercus rubra</i>	792	99.04	84.05	2.37	13.71	17.78	7.10	0.65	0.92
	<i>Betula</i> sp.	627	81.05	10.22	0.90	0.36	10.90	2.62	0.27	0.11
	<i>Fagus grandifolia</i>	130	73.72	15.13	2.19	0.44	8.21	2.19	0.31	0.16
SERC										
	<i>Quercus alba</i>	430	95.85	82.23	4.77	17.13	15.85	7.20	1.05	1.32
	<i>Quercus montana</i> or <i>michauxii</i>	364	90.93	77.75	1.92	18.13	17.22	7.91	0.51	1.88
	<i>Platanus occidentalis</i>	101	59.18	10.20	0	0	7.65	1.88	0	0
	<i>Fagus grandifolia</i>	983	80.50	37.08	0.85	0	13.19	4.55	0.23	0
	<i>Quercus falcata</i>	344	88.98	62.92	4.75	2.49	15.26	6.77	0.86	0.41
	<i>Acer rubrum</i>	334	72.10	28.69	7.02	2.62	12.99	4.85	1.02	0.59
La Selva										
	<i>Zygia longifolia</i>	781	82.90	62.82	30.17	9.28	15.95	6.10	2.70	1.60
	<i>Luehea seemannii</i>	338	88.22	70.12	43.27	14.97	16.08	6.35	2.69	1.48
	<i>Trophis racemose</i>	59	96.61	77.97	18.64	10.17	18.80	6.21	0.99	0.99
	<i>Ficus insipida</i>	89	52.68	42.85	12.44	10.47	11.14	4.23	1.71	0.98
	<i>Cordia bicolor</i>	79	93.67	77.22	30.38	3.80	14.89	4.31	1.77	0.77
	<i>Schnella guianensis</i>	201	89.55	68.66	23.88	13.43	11.99	4.13	1.30	0.99
	<i>Cordia alliodora</i>	140	67.16	50.37	31.59	1.12	5.32	2.08	0.62	0.32

Note: n is the total number of leaves studied for each species at each forest, frequencies are reported as the percent of leaves with the given type of herbivory damage, and diversities are the number of damage types observed on 30 leaves.

insect herbivory divided by the area of the leaf, was quantified for each leaf that was  $\geq 75\%$  intact with an attached petiole. Leaves were carefully photographed, and leaf area and petiole width were measured using ImageJ (Schneider et al., 2012), following the Royer et al. (2007) protocol.

## Statistical analyses

Statistical analyses were performed to quantify the influence of plant traits on the frequency and diversity of total, specialized, gall, and mine damage in addition to the percent leaf area damaged.

Percent data was logit transformed prior to scaling and centered around the mean, while diversity data was only scaled. Transforming the data allows for all traits and herbivory to be on similar ranges and thus comparable. Linear models (Stats R package; lm function; R Core Team, 2013) were utilized for investigating the influence of LMA on total, specialized, gall, and mine damage frequencies and diversities. Generalized linear models (Stats R package; glm function; R Core Team, 2013) were used when forest type did not need to be accounted for, while random mixed effects models (lme4 R package; lmer function; Bates et al., 2015) were utilized when forest type was accounted for. Previous work (Azevedo-Schmidt et al., 2023) found that forest type was important when analyzing insect herbivory using the DT system for some FFGs but not all. This framework was implemented here, providing information on when forest type should and should not be accounted for. Plant species was not accounted for because many traits are shared across species (e.g., Siefert et al., 2015; Bruelheide et al., 2018) and not part of the questions posed here. All traits listed above were used in the model selection process, but only traits that improved the models via AIC scores (i.e., measure of goodness of fit) were used in the final analyses. R version 3.6.1 was used for linear model analyses.

Random forest analyses have been adopted within modern ecology to quantify complex ecosystems, specifically interactions between species and environmental variables (Cutler et al., 2007; Simon et al., 2023). Unlike linear models, random forests do not provide directionality between independent and dependent variables, but they do detect nonlinear relationships between variables that influence ecosystems. This is important for model selection but also, for understanding what are important drivers of ecosystem complexity. Random forests were used here to visualize important drivers of plant–insect interactions that may have been undetected using linear models. Random forests were created for each examined FFG and percent area damaged using 2000 iterations to maximize stability (randomForest R package; randomForest function [Liaw and Wiener, 2002]). Only forests that explained  $\geq 5\%$  of the variance were included. The majority of the forests explained approx. 40% of the herbivory types, but we felt it was important to include a few low percentages due to the complexity of plant–insect

interactions. Relative importance of a trait within the ecosystem, either all forests binned together or separated out, was measured as a percent increase in mean standard error (%IncMSE). This metric is analogous to accuracy because they are both calculated by reshuffling the out-of-bag samples, providing information about how much a variable increases the accuracy of the random forest. If the %IncMSE or relative importance values are below zero, that informs us that random noise from reshuffling is more important than the variable itself. Using this framework, plant traits were assessed for importance across all herbivory metrics. R version 4.2.2. was used for all random forest analyses.

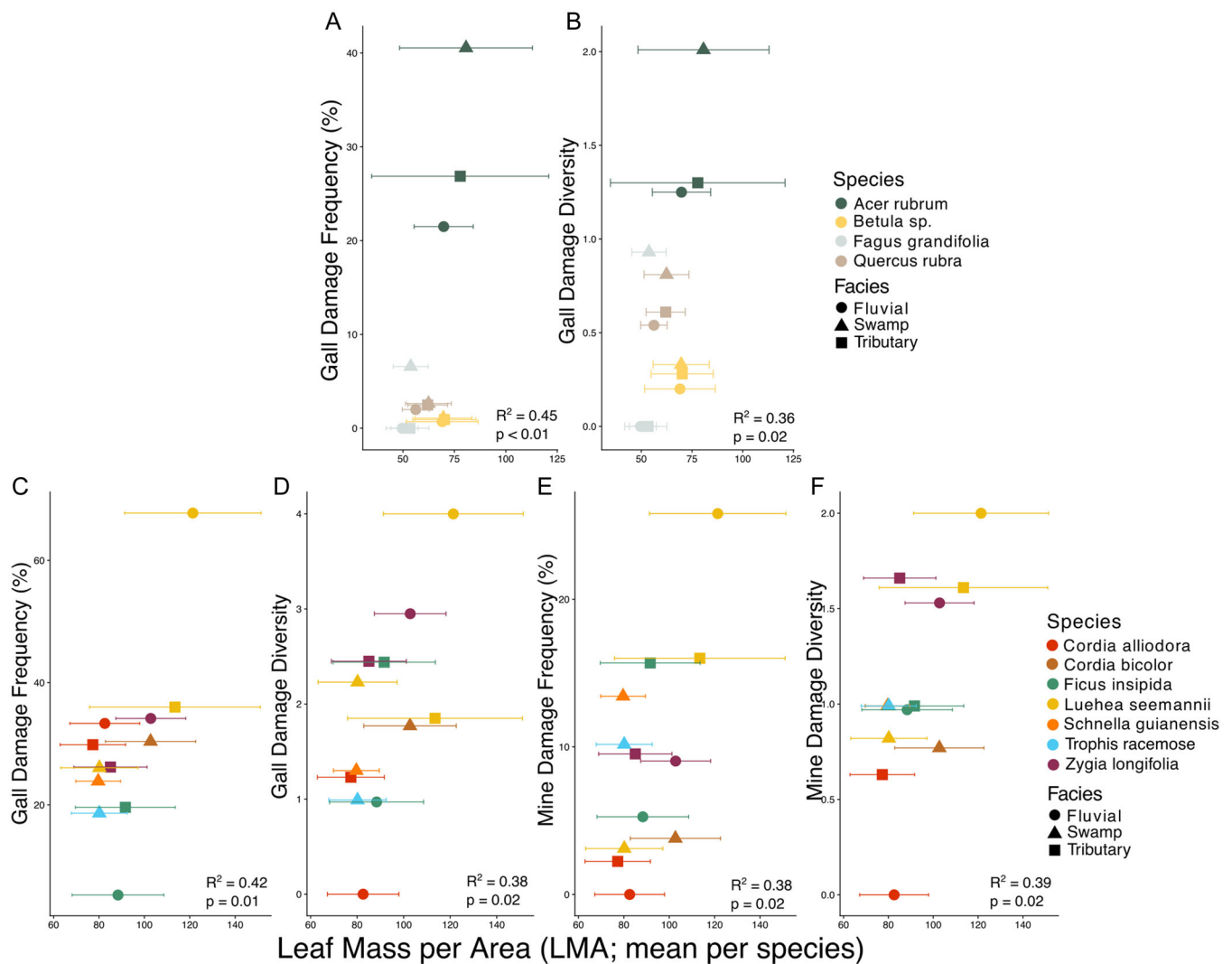
## RESULTS

### Linear models

Because LMA ( $\text{g}/\text{m}^2$ ) reconstructions are widely used in paleobotanical studies, we analyzed how this structural trait influences insect herbivory across forest types. LMA ranged from approx. 60–120, with the lowest values at HF and the highest values at LS (Table 1). Significant relationships between LMA and herbivory exist only within HF and LS (Figure 4) and not SERC. At HF, gall frequency and diversity are the only FFGs with significant relationships ( $R^2 = 0.45$ ,  $p \leq 0.01$ ;  $R^2 = 0.36$ ,  $p = 0.02$ ), and both show a positive interaction with increasing LMA (Figure 4A, B). This relationship is driven by red maple (*A. rubrum*), which has the highest LMA and is greatest within the swamp environment, followed by the tributary and fluvial environments. Other plant species share this pattern of higher values within the swamp compared with the tributary and/or fluvial environments (Figure 4A, B). As with HF, LS gall frequency and diversity were positively correlated with LMA ( $R^2 = 0.42$ ,  $p = 0.01$ ;  $R^2 = 0.38$ ,  $p = 0.02$ ), along with mine frequency and diversity ( $R^2 = 0.38$ ,  $p = 0.02$ ;  $R^2 = 0.39$ ,  $p = 0.02$ ); Figure 4C–F). *L. seemannii* within the fluvial environment drives these relationships, with the tributary environment also influencing these relationships. The pattern of higher LMA values and damage within the HF swamp environment does not hold within LS (Figure 4C–F). For *L. seemannii*, the fluvial environment has the highest LMA, followed by the tributary environment, and then the swamp. Unlike the patterns present within HF, the depositional environment within LS has less of an influence on the relationship between LMA and herbivory types. Full model outputs can be found in Appendix S4.

Generalized linear models (glm and lmer) show the influence of predictor variables (here, plant traits) on the frequency and diversity of total, specialized, gall, and mine damage across all three forests. HF has the most significant, both positive and negative, relationships between plant traits and insect herbivory metrics (Table 3). There are 14 significant interactions between plant traits and insect herbivory within HF, 11 within SERC, four within LS, and three when all forests are





**FIGURE 4** Leaf dry mass per area (LMA; mean per species) against herbivory types. Colored points represent different species, and the shape of points corresponds to the depositional environment. Figure only shows significant relationships between LMA and herbivory. Harvard forest (A and B) gall damage frequency and diversity were significantly influenced by LMA, while La Selva showed positive relationships for gall and mine frequency and diversity (C–F). Error bars represent one standard deviation.  $R^2$  and  $p$  values are shown in the bottom right corner of each graph. Full summary outputs for all models can be found in Appendix S4.

binned together. Within SERC, total damage frequency and diversity have significant negative correlations with LMA and margin type (i.e., low LMA leaves and untoothed leaves have more frequent and diverse damage); total damage frequency additionally has a negative correlation with weight percent nitrogen and a positive correlation with weight percent carbon (Table 3). Total damage frequency within HF, on the other hand, has a negative correlation with the presence/absence of trichomes. HF displays a strong, positive relationship between LMA and total damage diversity and a negative relationship between wt. % phosphorus and total damage diversity (Table 3). Specialized damage frequency has the most significant relationships within HF, where LMA positively correlates with frequency and diversity. Weight percent phosphorus negatively correlates with specialized damage diversity

within HF as well as when all forests are binned together, and when all forests are binned together, wt. % phosphorus is also a negative predictor of specialized frequency. Lastly, within SERC, specialized damage frequency negatively correlates with margin type.

Gall damage frequency and diversity have strong relationships with multiple trait variables across all forest bins (Table 3). LMA positively correlates with gall frequency and diversity for all forest bins (i.e., all, HF, and LS) (Table 3). This is expected as it is a result shared by previous analyses (Figure 4). Within SERC, gall frequency is positively predicted by C:N and wt. % nitrogen, and the interaction between C:N and LMA while wt. % carbon is a negative predictor. A negative correlation with phosphorus is also observed at HF for both gall diversity and frequency (Table 3). Echoing gall frequency and diversity, mine

TABLE 3 Summary output of generalized linear models for frequency and diversity of total, specialized, gall, and mine damage.

Predictors	Total damage (%)			Specialized damage (%)			Gall damage (%)			Mine damage (%)		
	Estimates	CI	p	Estimates	CI	p	Estimates	CI	p	Estimates	CI	p
C:N							1.13	0.68 to 1.59	<0.01			
LMA	-0.50	-0.96 to -0.04	0.03	0.89	0.18 to 1.61	0.01	0.83	0.55 to 1.11	<0.01	0.57	0.17 to 0.98	<0.01
wt. % N	-1.95	-3.58 to -0.32	0.02				0.59	0.13 to 1.05	0.01			
wt. % C	1.39	0.06 to 2.73	0.04				1.28	0.64 to 1.92	<0.01			
wt. % P				-0.75	-0.72 to -0.09	0.02	1.22	0.75 to 1.68	<0.01			
Margin type	-0.60	-1.00 to -0.20	<0.01	-0.96	-1.54 to -0.38	<0.01	-0.95	-1.34 to -0.57	<0.01			
C:N × LMA							-0.37	-0.68 to -0.06	0.02	-0.99	-1.95 to -0.03	0.04
Trichomes	-0.68	-1.21 to -0.14	0.01	-1.02	-1.33 to -0.71	<0.01	0.37	0.08 to 0.67	0.01			
Predictors	Total damage diversity			Specialized damage diversity			Gall damage diversity			Mine damage diversity		
	Estimates	CI	p	Estimates	CI	p	Estimates	CI	p	Estimates	CI	p
LMA	-0.58	-0.93 to -0.22	<0.01	2.39	1.02 to 3.77	<0.01	0.59	0.24 to 0.94	<0.01	0.70	0.23 to 1.17	<0.01
wt. % P	2.18	0.75 to 3.62	<0.01				0.89	0.10 to 1.68	0.03	0.93	-0.03 to 1.89	0.06
Margin type	-0.90	-1.66 to -0.13	0.02	-1.10	-1.83 to -0.37	<0.01	0.96	0.60 to 1.31	<0.01			
	-0.32	-0.62 to 0.04	0.04				-0.40	-0.62 to -0.19	<0.01	-0.64	-1.15 to -0.13	0.02

Note: Colors correspond to forest type, with all forests binned together (purple), Harvard Forest (green), SERC (blue), and La Selva (orange). Table has been simplified to only show significant relationships between predictors, plant traits, and herbivory. Full tables can be found in Appendices S5–S8.

Abbreviations: CI, confidence interval; LMA, leaf dry mass per area.

frequency and diversity were positively predicted by LMA at LS. The relationship between LMA and mine diversity is only marginally significant within HF (Table 3). Additionally, wt. % phosphorus negatively predicts mine frequency and diversity within HF (Table 3).

## Random forests

Random forests provide insight into the complex relationship between plant traits and herbivory (Figure 5) not previously detected using analyses that focus on linear relationships. Given previous results, it is expected that LMA, C:N, and wt. % phosphorus influences the frequency and diversity of total, specialized, gall, and/or mine damage. Random forests demonstrate that chemical compounds also influence insect herbivory, which was not possible in linear model analyses due to low sampling resolution and issues with singularity. Additionally, only significant relationships are shown, while non-significant relationships are omitted (Figure 5).

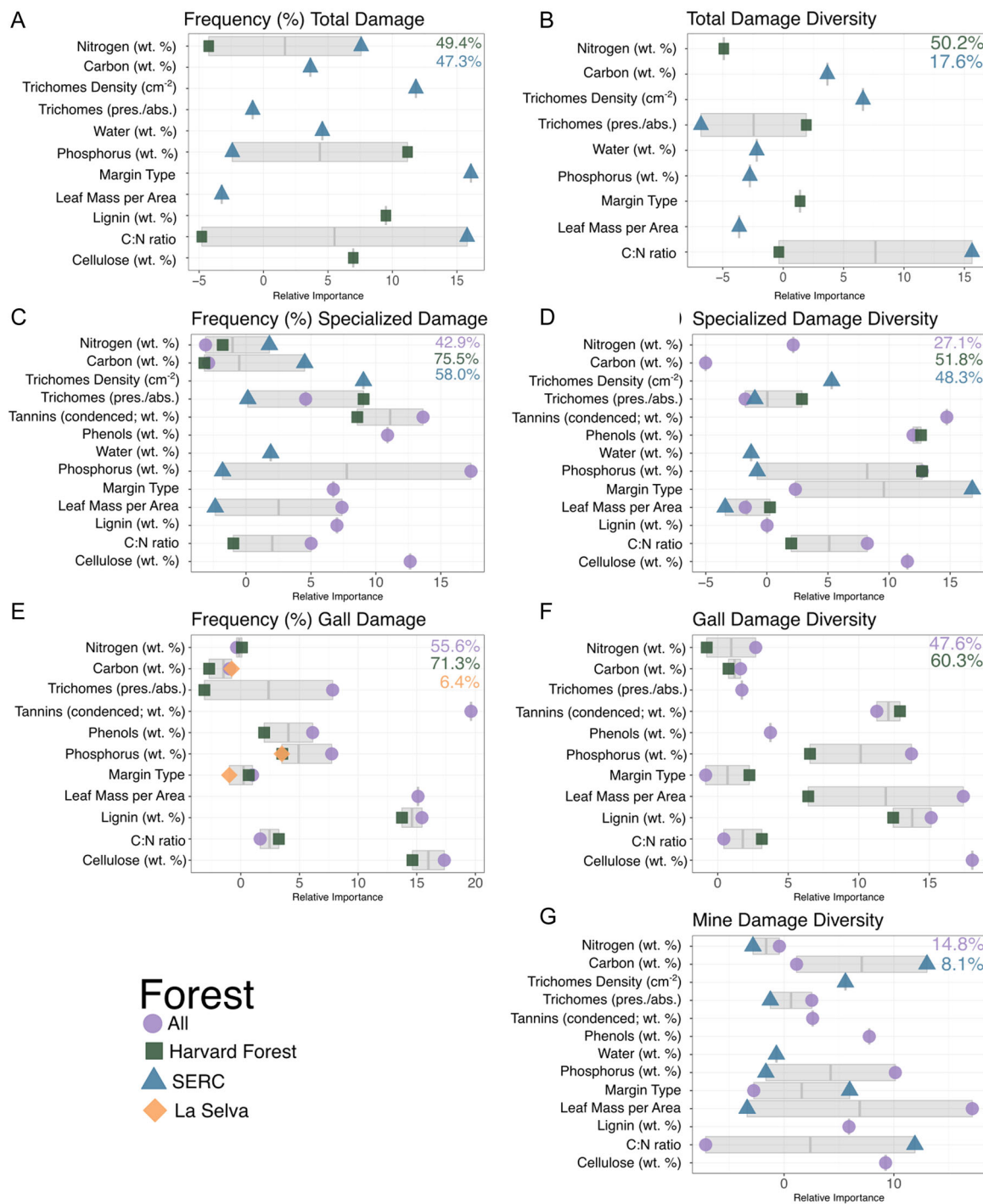
Within HF, wt. % phosphorus, wt. % lignin, and wt. % cellulose were the most important for predicting total damage frequency, while nitrogen (wt. %), as well as the C:N ratio, were not important (Figure 5A; var. explained = 49.4%). This pattern of nitrogen (wt. %) and C:N ratio was shared with total damage diversity (Figure 5B; var. explained = 50.2%), while trichomes (presence/absence) and margin type were minimally important (Figure 5B; var. explained = 50.2%). In contrast, wt. % nitrogen and carbon, trichome density ( $\text{cm}^{-2}$ ), wt. % water, margin type, and C:N ratio were important for predicting total damage frequency within SERC (Figure 5A; var. explained = 47.3%), while carbon, trichome density ( $\text{cm}^{-2}$ ), and C:N were strong predictors of total damage diversity within SERC (Figure 5B; var. explained = 17.6%). Interestingly, trichomes (presence/absence), wt. % phosphorus, and LMA ( $\text{g}/\text{m}^2$ ) were not important for predicting total damage frequency within SERC, a pattern shared with total damage diversity (Figure 5A, B).

More variables were important for predicting specialized damage frequency and diversity, especially when forest type was binned. Interestingly, wt. % nitrogen was not important for predicting specialized damage frequency. Trichomes (presence/absence), wt. % tannins, phenols, phosphorus, as well as margin type, LMA ( $\text{g}/\text{m}^2$ ), and wt. % lignin, C:N, and wt. % cellulose influenced specialized damage frequency (Figure 5C; var. explained = 42.9%). Within HF, trichomes (presence/absence) and wt. % tannins were important (var. explained = 75.5%). These particular variables within HF explain approx. 75% of the variance in the data, suggesting that they are strong drivers of specialized damage frequency. Variables related to leaf palatability such as wt. % nitrogen and carbon, and the ratio of C:N were not important predictors of specialized damage frequency within HF (Figure 5C). Variables within SERC had less relative importance, with wt. % nitrogen and carbon, trichome density ( $\text{cm}^{-2}$ ), and wt. % water predicting specialized damage frequency, while trichomes (presence/absence), phosphorus (wt. %), and LMA ( $\text{g}/\text{m}^2$ ) were not

strong predictors (Figure 5C; var. explained = 58.0%). Some of these patterns hold when looking at specialized diversity and forest type; however, new patterns emerge as well. Wt. % nitrogen, tannins, and phenols, along with margin type, C:N ratio, and wt. % cellulose were all important for predicting specialized diversity when forests were binned together, while wt. % carbon, trichomes (presence/absence), LMA ( $\text{g}/\text{m}^2$ ), and wt. % lignin were not (Figure 5D; var. explained = 27.1%). Within HF, trichomes (presence/absence), wt. % phenols and phosphorus, as well as C:N ratio were all important predictors of specialized damage diversity, with only LMA ( $\text{g}/\text{m}^2$ ) minimally unimportant (Figure 5D; var. explained = 51.8%). Lastly, only trichome density ( $\text{cm}^{-2}$ ) and margin type are important predictors for SERC while trichomes (presence/absence), wt. % water and phosphorus, and LMA ( $\text{g}/\text{m}^2$ ) are not (Figure 5D; var. explained = 48.3%). Again, no significant relationships were observed at LS.

Gall damage frequency was influenced by trichomes (presences/absence), wt. % tannins, phenols, and phosphorus, margin type (minimally), LMA ( $\text{g}/\text{m}^2$ ), wt. % lignin, C:N, and wt. % cellulose when forests were binned together (Figure 5E; var. explained = 55.6%). Wt. % carbon was the only variable with a relative importance below zero when all forests were binned. HF and LS also showed relationships between leaf traits and gall damage frequency. Within HF wt. % phenols, phosphorus, and lignin, C:N, and wt. % cellulose were important for gall frequency, while wt. % nitrogen and margin type were minimally important (Figure 5E; var. explained = 71.3%). Carbon was not important and while nitrogen was marginally important, we see similar patterns of low/no importance with total damage frequency (Figure 5A) and diversity (Figure 5B), as well as specialized damage frequency (Figure 5C). LS has much lower variance explained by traits, but phosphorus was an important predictor, while wt. % carbon and margin type were not (Figure 5E; var. explained = 6.4%). Gall diversity was affected by wt. % nitrogen and carbon, trichome (presence/absence), wt. % tannins, phenols, and phosphorus, LMA ( $\text{g}/\text{m}^2$ ), wt. % lignin, C:N, and wt. % cellulose, while margin type was minimally unimportant when all forests are binned together (Figure 5E; var. explained = 47.6%). Important drivers of gall diversity within HF are wt. % carbon, tannins, and phosphorus, margin type, LMA, wt. % lignin, and C:N (Figure 5F; var. explained = 60.3%). Again, wt. % nitrogen and carbon have minimal importance (Figure 5F) as seen with gall frequency (Figure 5E).

Mine frequency showed no significant relationships when all forests were binned together or when forests were separate however, mine diversity did show some patterns. Wt. % carbon, trichomes (presences/absence), wt. % tannins, phenols, and phosphorus, LMA, wt. % lignin and cellulose were predictors of mine diversity when all forests were binned together, while wt. % nitrogen, margin type and C:N were not important predictors (Figure 5G; var. explained = 14.8%). Within SERC, wt. % carbon, trichome density ( $\text{cm}^{-2}$ ), margin type, and C:N predicted mine diversity, while wt. % nitrogen, trichomes (presence/



**FIGURE 5** Random forest importance plotted for frequency and diversity of total, specialized, gall, and mine damage (A–G). Forests were binned together (all = purple) to see the influence of traits on herbivory regardless of forest type or separated by forest type (Harvard Forest = green, SERC = blue, La Selva = orange). Percent variance explained is shown in the top right corner of each panel, with colors corresponding to forest type. Relative importance (% IncMSE) expresses how the model accuracy improves with the variable. Negative values show that random noise is more important than the trait itself. Gray boxes show the variability between forests in relative importance for a single plant trait; min, mean, and max values are shown. All leaf trait percentage values are weight percent (wt. %).

absence), wt. % water and phosphorus, and LMA (g/m<sup>2</sup>) did not (Figure 5G; var. explained = 8.1%).

Percent (%) area damaged showed no significant relationships in any linear model (Appendices S5–S8) or random forest analysis (Appendices S9–S12).

## DISCUSSION

Insect herbivory measured within Harvard Forest (HF), the Smithsonian Environmental Research Center (SERC), and La Selva (LS) was compared with leaf traits to investigate

their influence on insect herbivore damage recorded on leaf tissue. As plant–insect interactions are being disrupted by human influence (Zvereva and Kozlov, 2006; Dyer et al., 2018; Azevedo-Schmidt et al., 2022) and forest health is influenced by insect herbivory (e.g., Nability et al., 2009), it is increasingly important to understand these relationships across a wider breadth of evolutionary time via the geologic record. To facilitate collaboration between modern and deep-time ecology, paleobotanical methods were replicated in modern ecosystems. Additionally, the adoption of paleobotanical methods within modern ecosystems benefits modern ecologists as these methods provide an inexpensive and time-efficient way to capture forest-scale community interactions and can be linked to insect diversity (Carvalho et al., 2014). The results presented here suggest that leaf traits, specifically structural and palatability traits, are important drivers of plant–insect interactions, and the methods used here capture the variability across forests (Table 3 and Figure 5). Linear models show how structural traits such as LMA affect total, specialized, gall, and mine damage frequency and diversity (Table 3), while random forest analyses detect the non-linear relationships between palatability traits (% tannins, phenols, lignin, and cellulose; Figure 5E–G) and herbivory. Potential mechanisms influencing the patterns reported here are described below.

As leaf traits are shared across species, they allow for comparisons across plant clades and environments. Variability across plant groups and within species (Niklas et al., 2007) has been linked to environmental variables (Bruelheide et al., 2018), as well as insect herbivore interactions (Caldwell et al., 2016; Züst and Agrawal, 2017). The relationship between traits and herbivory is complicated, as a group of traits is often needed, and various insect groups respond differently (Carvajal Acosta et al., 2023). The plant “defense syndrome” (Agrawal and Fishbein, 2006; Agrawal, 2007) characterizes different groups of traits responsible for defense, such as structural (trichomes, leaf toughness, etc.), chemical (secondary chemicals such as latex) and nutrition or palatability (C:N ratio, water content, etc.). Plants utilize different defenses to protect from generalist herbivores, feeding on many host-plants, and specialists, feeding on one or a few closely related host-plant species. Protection from generalist herbivores requires a wide variety of traits because they have adapted to many different plants, whereas specialists have evolved to the specific defenses of one or a few host-plants (e.g., Agrawal and Fishbein, 2006). However, herbivores themselves adapt in response to plant defensive traits, causing the inevitable “arms race” between interacting species. Previous work, (Volf et al., 2018) that focused on closely related New Guinean figs (*Ficus* sp.) and specialist moths (*Asota* sp.) demonstrated this relationship. They found that differences in traits among closely related figs affected specialist herbivores negatively but extreme specialists positively. Given the intricate balance and multitude of feedbacks between host-plants and insect herbivores, it is perhaps unsurprising that total and specialized frequency and

diversity were influenced by multiple traits (Table 3, Figure 5).

The influence of structural traits on insect herbivory is seen in the data presented here. Within SERC, LMA and margin type negatively influence total damage frequency and diversity, and margin type is negatively associated with specialized damage frequency (Table 3). There are no significant relationships at SERC between galling and mining damage and LMA or margin type, suggesting that leaf chewing and piercing insects are more strongly impacted by LMA than gallers or miners. In contrast, LMA positively correlates with total damage diversity, specialized damage frequency, and specialized damage diversity within HF, and significant positive relationships between LMA and gall damage frequency and diversity are observed when all forests are binned together, as well as within HF and LS (Table 3 and Figure 4). These results resemble similar studies that found that mechanistic traits such as thickness and specific leaf area were strong indicators of gall and mine inducing insects (Baird et al., 2010). Within HF, this positive relationship between galling damage and LMA seems to be driven by red maple (Figure 4A, B), which is interesting as red maple is phylogenetically different than the other selected species within this analysis. Red maple is within the Sapindales order, while birch, American beech, and red oak belong to Fagales. Phylogenetic similarity, rather than LMA, may, therefore, be affecting gall inducing insect communities; however, LMA does explain 45% and 36% of the gall frequency and diversity variance within HF (Figure 4A, B), indicating that the trait itself is an important driver. Hypothesized mechanisms for why LMA is a strong driver of galling herbivory are as follows, and future research is needed to test these. Galling insects may choose thicker leaves because the weight of a gall or multiple galls could cause the leaf to droop, increasing susceptibility to mechanical damage or the likelihood of abscission. Similarly, the presence and weight of galls could negatively impact a leaf's ability to capture sunlight, prompting a species to increase thickness and/or density due to continued galling insect pressure over time.

Galls and mines are created by shared orders of insects such as moths (Lepidoptera), flies (Diptera), and wasps, specifically sawflies (Hymenoptera). These orders of insects are similar in how they deposit their eggs into leaf tissue, inducing a gall (Price et al., 1987) or mine (Sinclair and Hughes, 2010), and their unique life cycles. Unlike other herbivorous insects, gall and mine inducing herbivores have two distinct life stages with varying interactions with the host-plant. The adult phase is mobile and independent of the leaf tissue as a food source (e.g., Hickman et al., 1995; Azzouz et al., 2004; Krenn, 2010) but dependent on it for depositing eggs. Conversely, the larval stage is completely dependent on the leaf tissue as it is confined, protected from predators, and feeding within (Cornelissen et al., 2016). Thus, life stage is an important factor influencing the effectiveness of leaf traits because a trait may deter one life

stage (Peeters, 2002; Caldwell et al., 2016) while benefiting another (Cornelissen, 2011). Although all insects are susceptible to traits influencing palatability and nutrient content, both galling and mining insect larvae are confined within a leaf, making them unique from other FFGs, such as leaf chewing or piercing and sucking insects. Because of these similarities, life stages should be considered when investigating the influence of plant traits on plant–insect interactions.

The similarities between gall and mine damage are supported by recent research that has shown how these two groups of insects physiologically alter the host-plant (Giron et al., 2016). Leaf carbon, nitrogen, and phosphorus content and C:N ratio are important for insect larval development (Giron et al., 2016), driving positive correlations between these traits and gall and mine damage (Figures 3 and 5E–G). Previous work has shown that galls alter nutrient flow within leaves, high-jacking the host-plant to concentrate nutrients within the gall and/or larval chamber and thereby providing more nutrients for the developing insect (e.g., Giron et al., 2016). These mechanistic studies provide a framework with which to interpret our data. The results presented here show no linear relationships between secondary defensive compounds (tannins, phenols) and insect herbivory, but when non-linear relationships are accounted for via random forests, patterns emerge (Figure 5). Because both gall and mine inducing insects have the ability to downregulate secondary compounds such as tannins and phenols near the feeding sites (e.g., Giron et al., 2016), we hypothesize that this ability could explain why secondary compounds measured here are less important for predicting gall or mine frequency and diversity than structural traits.

There are many significant relationships between leaf traits and herbivory across the forested ecosystems examined here; however, the strength of these relationships, as evident by the estimates or slopes, varies (Table 3 and Figure 5). The strongest observed relationships between leaf traits and insect herbivory are within cool-temperate HF (estimates = 2.18 for LMA—total damage diversity and 2.39 for LMA—specialized damage diversity) (Table 3). The HF landscape is unlike the other forests in that it has experienced periodic outbreaks of spongy moths (*Lymantria dispar*; Henry et al., 1947; Liebhold et al., 2021), with the most recent outbreak event occurring from 2016 to 2018 (Barker Plotkin et al., 2021), and so we consider leaf trait—herbivory relationships in this context. *Lymantria dispar* caterpillars cause a range of mandibular chewing damage types, such as hole (DT2-5) and margin (DT12-15) feeding, thereby impacting total and specialized damage diversity. Trichomes have been previously shown to negatively influence mandibular chewing insect herbivores like *L. dispar* (Hadley et al., 2005), and we observe a negative correlation between the presence/absence of trichomes and total and specialized damage frequency (Table 3). Other structural traits such as LMA positively influence total and specialized damage at HF, indicating that thicker leaves with

higher weight percent carbon are either more desirable or must be consumed in greater quantities to meet nutritional requirements (Coll and Hughes, 2008). However, wt. % carbon appears to have an indirect relationship as the variable itself has low relative importance (Figure 5). Wt. % phosphorus, which is required for nucleic acid synthesis and protein production and can drastically influence larval fitness (Huberty and Denno, 2006), is very important within HF (Figure 5). Carbon and nitrogen are important building blocks of leaf compounds such as lignin and cellulose (e.g., Bryant et al., 1983; Dyer et al., 2018), which were shown to influence herbivory in the random forests (Figure 5). Lastly, spongy moth outbreaks have likely been influencing how insect herbivores interact with each other (Azevedo-Schmidt et al., 2023) and the acquisition of resources (i.e., leaf material). Gall frequency and diversity may be higher on red maple (*A. rubrum*; Figure 4A, B) because (1) the outbreak of *L. dispar* has isolated galling insects to red maples as they are not its “preferred” plant host, and/or (2) the outbreak released gall-inducing insects from competitive pressure. Alternatively, galling insects may prefer red maple due to other leaf traits not examined here.

The interplay between host-plants and insect herbivory is complex, with relationships that have evolved over many millions of years (Labandeira and Curran, 2013). Fossil data sets shed light on both stable and changing patterns of plant–insect interactions as they respond to local or global environmental change on long time scales (e.g., Curran et al., 2021). In the delicate arms race between host-plants and the insects that feed on them, there is a give and take, balancing crucial and often costly resources (e.g., Karabourniotis et al., 2020). The strategies deployed by plants to mitigate insect herbivory target generalist and specialist insects, and thus, the “defense syndrome” or diversity of traits needed to control herbivory is dynamic (Agrawal and Fishbein, 2006; Farias et al., 2020). Examining insect herbivory at the forest level allows large-scale patterns to emerge, including functional relationships isolated from taxonomic species identifications. It is imperative to apply trait-based research to fossil data sets, as taxonomic species identifications are not always feasible, especially in older fossil assemblages. Characterizing host-plants and their insect herbivores roots our understanding of evolutionary relationships; however, as species are extirpated from their native habitat or go extinct, traits may be more important. With the ever-present influence of humans on modern landscapes, conservation and/or protection of trait diversity may be more important in supporting a robust environment and safeguarding against further biodiversity loss (Tscharrntke and Brandl, 2004). If we can preserve species that promote a diverse community of insect herbivores within a forest, we could properly manage and/or mitigate further insect decline (van der Sluijs, 2020; Wagner, 2020; Wagner, Fox, et al., 2021; Wagner, Grames, et al., 2021). Integrating modern research with fossil data sets can shed light on local, regional, and global extinctions and hopefully create a bridge that allows us to better

understand the importance of leaf traits and insect herbivory within a changing world.

## CONCLUSIONS

Plant and insect herbivore interactions are influenced by a multitude of variables ranging from abiotic (climate) to biotic, including leaf traits. The influence of leaf traits on herbivory within the modern record has been well studied (Kattge and Str, 2020) and represents a promising avenue for research in paleoecology (Wilf, 2008; Müller et al., 2023). However, the use of different methods in modern ecology and paleoecology presents a barrier. Here, we show how to compare across spatiotemporally distinct records by utilizing paleobotanical techniques in modern ecosystems. Our results demonstrate that traits that can be measured or reconstructed in the fossil record (LMA, margin type, presence/absence of trichomes) are important drivers of insect herbivory (Table 3). Although not all traits measured here, such as secondary compounds, can be determined from fossils at present, we have hope that new techniques could someday make this a reality. By expanding plant–insect interaction research to include leaf traits, we may be able to answer questions regarding the efficacy of leaf traits and defense through evolutionary time.

## AUTHOR CONTRIBUTIONS

L.A.S. and E.D.C. contributed to the design and implementation of the research, to the analysis of the results, and to the writing of the manuscript.

## OPEN RESEARCH BADGES



This article has earned Open Data and Open Materials badges. Data and materials are available at [https://github.com/lazevedoschmidt/DT\\_leaf.traits](https://github.com/lazevedoschmidt/DT_leaf.traits).

## DATA AVAILABILITY STATEMENT

The datasets and code generated and/or analysed during the current study are available on our Github repository, [https://github.com/lazevedoschmidt/DT\\_leaf.traits](https://github.com/lazevedoschmidt/DT_leaf.traits).

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## CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**Appendix S1.** Summary table of gps points where leaves were collected across all forest types.

**Appendix S2.** Literature citations for previously published trait data used in the analyses.

**Appendix S3.** Summary table of leaf sample numbers used to quantify herbivory frequencies for each species across depositional environment and forest. Values in parentheses show sample number for leaf mass per area (LMA; mean) calculations.

**Appendix S4.** Linear model outputs for investigating the relationship between leaf mass per area (LMA) and total, specialized, gall, and mine damage frequency and diversity. Models have been color coded to reflect the various forests as consistent with the manuscript.

**Appendix S5.** Full summary table of generalized linear model output when all forests are binned together. Frequency and diversity of total, specialization, gall, mine damage, and percent area damaged are shown again predictor variables. When random mixed effects models were used, random effects values are provided, otherwise generalized linear models were used. Traits were only included in the model if they improved model fit without causing singularity.

**Appendix S6.** Full summary table of generalized linear model output for Harvard Forest. Frequency and diversity of total, specialization, gall, mine damage, and percent area damaged are shown again predictor variables. When random mixed effects models were used, random effects values are provided, otherwise generalized linear models were used. Traits were only included in

the model if they improved model fit without causing singularity.

**Appendix S7.** Full summary table of generalized linear model output for SERC. Frequency and diversity of total, specialization, gall, mine damage, and percent area damaged are shown again predictor variables. When random mixed effects models were used, random effects values are provided, otherwise generalized linear models were used. Traits were only included in the model if they improved model fit without causing singularity.

**Appendix S8.** Full summary table of generalized linear model output for La Selva. Frequency and diversity of total, specialization, gall, mine damage, and percent area damaged are shown again predictor variables. When random mixed effects models were used, random effects values are provided, otherwise generalized linear models were used. Traits were only included in the model if they improved model fit without causing singularity.

**Appendix S9.** Random forest output for herbivory and percent area damaged when forest isn't accounted for.

**Appendix S10.** Random forest output for herbivory and percent area damaged within Harvard Forest.

**Appendix S11.** Random forest output for herbivory and percent area damaged within SERC.

**Appendix S12.** Random forest output for herbivory and percent area damaged within La Selva.

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