

Title: The legacy of Southern American extinct megafauna on plants and biomes

Short Title: Megafauna legacy on plants and biomes

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Abstract

Large mammal herbivores are important drivers of plant evolution and vegetation patterns, but whether current plant traits and ecosystem geography reflect the historical distribution of extinct megafauna is unknown. We address this question for Southern America (Neotropical biogeographic realm) by relating plant defense trait information at the ecoregion scale to climate, soil, fire, and the historical distribution of megafauna. Here we show that megafauna history explains substantial trait variability and detected three distinct regions (called “Antiherbiomes”) characterized by convergent plant defense strategies, environmental and megafauna patterns. We also identified 10 ecoregions that experienced biome shift, from grassy- to forest- dominated, following the Pleistocene megafauna extinction. These results suggest that extinct megafauna left a significant imprint in the current plant trait and ecosystems biogeography of Southern America.

Main Text

In pre-historical times (specially more than 10,000 years ago) many more regions of our planet were populated by giant mammals (the so-called megafauna) than today.

Whereas most of these animals went extinct during the Pleistocene and Early Holocene (1), important exceptions are Africa and Asia, where a high diversity of large mammal still roam the continent. These regions provide unique opportunities to understand megafauna ecology and its effects on ecosystems (2). For instance, consumption of plant biomass and related disturbances by African megaherbivores can drive and maintain woodlands in alternative grassland states (3–5). Moreover, large herbivores impose limits to ecosystem susceptibility to fire (i.e. grazers) and increase soil fertility in the long term (5–7). Thus, large mammal herbivores create and maintain their own grassy habitat(3). Considering the key role of megafauna in maintaining these ecosystems, their extinction probably resulted in the replacement of many herbivory-maintained savannas by forests and woodlands, or by fire-maintained savannas, across the world (8–10).

Woody plant species living in herbivory-maintained ecosystems evolved morphological and physiological traits (i.e. antiherbivory defenses) to reduce damage caused by large herbivores (8). While defense traits that are disadvantageous under present condition are likely to have been lost after megafauna extinction, other traits may have persisted as anachronical features(10, 11). These anachronisms provide a valuable opportunity to understand plant-megafauna interactions and could provide insights on switches from open grassy ecosystems, with abundant megafauna, to closed canopy ecosystems. There are multiple mechanism by which plants defend themselves from large herbivores (6, 8, 12–14), and these mechanisms differ with climate and availability of soil resources (13–

19). As a result, two broad savanna regions can be recognized in Africa characterized by specific patterns of megafauna-environment-vegetation associations and convergence in antiherbivory defense traits (hereafter called “Antitherbiomes”, as analogous to biomes) (14, 15, 20): 1) Dry nutrient-rich savannas dominated by small-leaved heavily armed plants, defended with thorns, densely branched crowns, low leaf nutrients, and nitrogen-based chemical defenses; and 2) Mesic nutrient-poor savannas dominated by broad-leaved plants that mainly rely on leaf defenses (e.g. spines, acid deterrents, lignin). A third ecosystem type can be readily identified in tropical Africa, that is, forests in which low levels of megafauna herbivory and high productivity, allow fast canopy escape, and make plants largely undefended (8, 13). We hypothesized that extinct megafauna distribution in Southern America (Neotropical biogeographic realm) left an imprint on current patterns of plant functional trait geography allowing the recognition of similar Antitherbiomes. To test this hypothesis, we compiled data on wood density, spinescence (leaf and stem/branches) and leaf size for woody species, scaled up these traits to the core region (21), and correlated them with pre-historical extinct megafauna (last 130,000 years) and modern mammal (extant and recently extinct species) richness, mean body mass, and dominant diet type, as well as with climate, soil, and disturbances, such as fire and cyclones (see Methods for details). We also combined plant and megafauna with fossil information on Pleistocene/Holocene vegetation to identify biome shifts following megafauna extinction.

Drivers of South American’s extinct megafauna distribution

Ecoregions with high megafauna richness were located in central and south portions of South America, and were specially associated with current subtropical grassland-forest mosaics (e.g. Gran Chaco of northern Argentina/southern Paraguay, southern Brazilian grassland-forest mosaics; Fig. 1A). This region also concentrated the highest number of 5grazing species, followed by the Brazilian Cerrado savanna region (Fig. 1C) which also concentrated numerous megabrowsers (Fig. 1d). Richness of megafauna, megagrazer, megabrowsers and mixed-feeders were all strongly positively correlated ($r \geq 0.70$). Ecoregions characterized by large megafauna species tended to coincide with those containing fewer species (except in islands, which tended to have fewer and smaller 10species; Fig. 1b). Larger species occurred mostly in Central America and western South America (Fig. 1b).

Megafauna richness in general and megabrowser richness were mostly related to low soil cation exchange capacity and high fire frequency (Fig 2A,D, Table S1-S4). These conditions are typically found in mesic nutrient-poor grassy ecosystems in the 15Neotropics. In Africa, savannas are also associated with high megafauna species richness (2). The nutrient-poor soils and frequent fires of savannas favor low stature plants whose post-fire leaf resprouts are a valuable food resource for mammal herbivores (8, 22). Grazer species richness were favored by similar conditions, although, for these species, insularity was the most important driving factor, a pattern 20that more closely resembled body mass patterns (Fig. 2B,C). This is consistent with evidence that most megagrazer species in South America were large(1), a disadvantageous trait in islands(23). Consistent with the tendency for larger continental megafauna species occurring in megafauna species-poor areas (on islands both richness and body mass were low), these megafauna indicators were favored by opposing 25climatic and soil conditions (Fig. 2A and B; Table S1-S4). In African savannas, the

largest species are also common in soil nutrient-rich areas, whereas smaller species occur along the entire fertility gradient(2).

Megafauna history and the geography of antiherbivory defense traits

5All of the studied traits had substantial variance explained by megafauna history and most traits (except leaf size) had more variability explained by megafauna than by any other factor (Fig. 2E-H; Table S1-S4). In contrast, extant and recently extinct (i.e. extinct by modern humans) herbivores did not influence antiherbivory defenses (Table S2). In fact, for leaf traits and woody density, the relationships were more consistent
10with plants negatively affecting herbivores. As we were looking for herbivore effects instead, such relationships were discarded (see Methods). We did not find any relationship between traits and megafauna diet type dominance.

Megafauna richness was the main predictor of wood density (Fig. 2E; Table S3). Wood density is a widely studied functional trait whose variation is often attributed to climate
15and soil (8). However recent research suggested that high wood density may have been selected to minimize damage caused by mammal browsers (8). Here we not only provide the first direct evidence that variability in wood density is primarily explained by megafauna history, but also show that other disturbances, such as hurricanes, are also important, highlighting the key role of disturbances in selecting for this trait(8, 24).
20Differences in wood density between savannas and forests ecoregions were not easily visualized (Fig. 1E), in contrast to previous results(8), probably because savanna and forest occur as mosaics within many of these ecoregions.

Megafauna distribution significantly explained stem spines across woody plants (Fig. 2F, Table S3). This variability was, however, explained by body mass rather than

richness. In Africa, the biomass of large browsers and social mid-size mixed-feeder species are important predictors of stem spines, although the later seems to be more important (13). This difference may be related to the fact that South America's extinct megafauna included larger herbivores and fewer grazing species(1). Like in Africa, stem spines were associated with dry areas (Fig. 2F; Table S3) (13, 14), in which the selective pressure exerted by herbivores on individual plants is likely high due to: 1) low plant density and productivity, and low plant stature; and 2) high soil fertility status (Table S5), increasing the consumption of scattered individual plants.

There was also a positive association of leaf spines with megafauna richness (Fig. 2H). However, the overall variability explained in leaf spines was small (Table S1). We suggest that this result is explained by the fact that this trait is inducible, and its variation is largely influenced by livestock, blurring the cues left by native megafauna species(19). Moreover, in many species, this trait may be replaced by other leaf defenses such as leaf chemicals (17).

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Southern American Antiherbiomes

A cluster analysis on the traits by ecoregions matrix (see Methods for details) supported the existence of three distinct antiherbiomes in Southern America (Fig. 3): (1) arid nutrient-rich ecosystems with small-leaved and thorny woody species (SLT for Small-Leaved Thorny); (2) mesic nutrient-poor ecosystems dominated by broad-leaved plants with leaf level defenses and high wood density (LDW for Leaf Defenses/Dense Wood); and, (3) megafauna sensitive forest (BLS for Broad-Leaved Sensitive). These antiherbiomes were consistent with our hypothesis based on classifications previously suggested for African ecosystems (Fig. 3).

The SLT antiherbiome mostly occurred at the northern- and southern- most parts of the Neotropical region (Fig. 3G), and was especially associated with very large megafauna species, dry, seasonal and cold climates, nutrient-rich soils (high pH and CEC), and, is currently subjected to intense fires (Figs. 3C-F, and S1). SLT includes some of the most suitable areas for megafauna in term of soil fertility (25), and many Pleistocene gigantic browser species were recorded in nearby areas (e.g. in the Humid Pampas and Patagonia Steppes), including giant sloths, glyphodonts and the elephant-like *Notiomastodont platensis* (26). The second antiherbiome (LDW) dominated in the tropical belt (Fig. 3G), and was associated with regions rich in megafauna, especially small species, high mean annual temperature and precipitation, and fire frequency and extremely cation-poor soils (Fig. 3C-F, and S1). The third antiherbiome (BLS) was associated with few but large megafauna species and tended to occur in areas of moist climate, and intermediate levels of both soil fertility (high CEC, but low pH) and mean annual temperatures (Fig. 3C-F, and S1). It was also related to forest-dominated ecoregions, most of which were located in northern Andes-drained areas, including western Amazon forests, and in Central America (Fig. 3G). These areas are presently associated with the highest richness of extant vertebrate species in South America, most of which are very small (27). Large non-ruminant herbivores, such as elephants and hippos, also occur in forests ecoregions in Africa (28), in addition to small non-megafauna mammals (2).

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Megafauna extinction and biome shifts

Antiherbiomes with highly defended plant species, rich in megafauna species and containing at least the same number megagrazer in relation to megabrowser species during the Pleistocene included mostly ecoregions currently dominated by forests (Fig.

4). These areas must have experienced recent shifts from previously open grassy (either savanna or savanna-forest mosaics) to the present forest state. This interpretation is supported by fossil evidence from 16 sites, confirming that forest ecosystems within or in the vicinity of these forest ecoregions were dominated by grasses during the Last 5Glacial Maximum and/or mid-Holocene (Fig. 4). These results are also broadly consistent with modelling evidence (9). Ecoregions that experienced shifts from savanna- to forest- dominated were concentrated in South America and included areas currently dominated by moist and dry forests (Fig 4). Climate change alone is unlikely to explain the replacement of these forest by grassy ecosystems (29). Instead, 10megaherbivores must have played a key role in maintaining Pleistocene grassy ecosystems in the Neotropics until their extinction.

Conclusion

Megafauna history explain a large fraction of the global variability in plant functional 15trait. This historical effect has been largely neglected in the ecological and biogeographical literature (8, 30). Our results emphasize the key role of history, disturbance, and species interaction to understand global patterns of plant functional trait variability. Moreover, we found that the interplay between herbivory regimes, climate, soil, and plant traits result in convergence of antiherbivory defense strategies in 20space, and the emergence of antiherbiomes with intercontinental distribution. These antiherbiomes, previously suggested for Africa, are still observable in continents that currently lack megafauna and represent one of the most striking and large-scale biological anachronisms. These trait anachronisms combined with reconstructions of megafauna distribution showed that ecosystem shifts after megafauna extinction

occurred, and many areas that are currently forest-, used to be savanna- dominated in the past, especially in central South America.

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Supplementary Materials:

Materials and Methods

15Figure S1-S2

Tables S1 – S5

References (30 - 106)

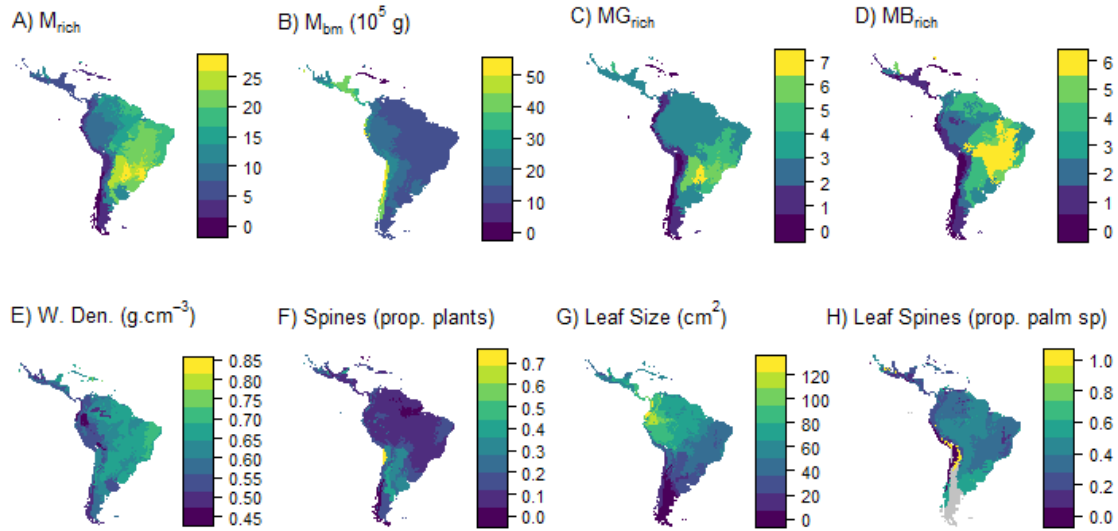


Fig. 1: Geographical variation in extinct megaherbivore species distribution (A-D) and 5antiherbivory resistance traits (E-H) among ecoregions of the Neotropical biogeographic realm. A): mean extinct megafauna species richness (M_{rich}); B): mean extinct megafauna species mean body mass (M_{bm}); C and D): mean extinct megagrazer (MG_{rich}) and megabrowser (MB_{rich}) species richness, respectively (mixed feeders excluded). Grey areas in H are ecoregions lacking data due to palm sensitivity to frost. 10prop.: proportion; sp: species.

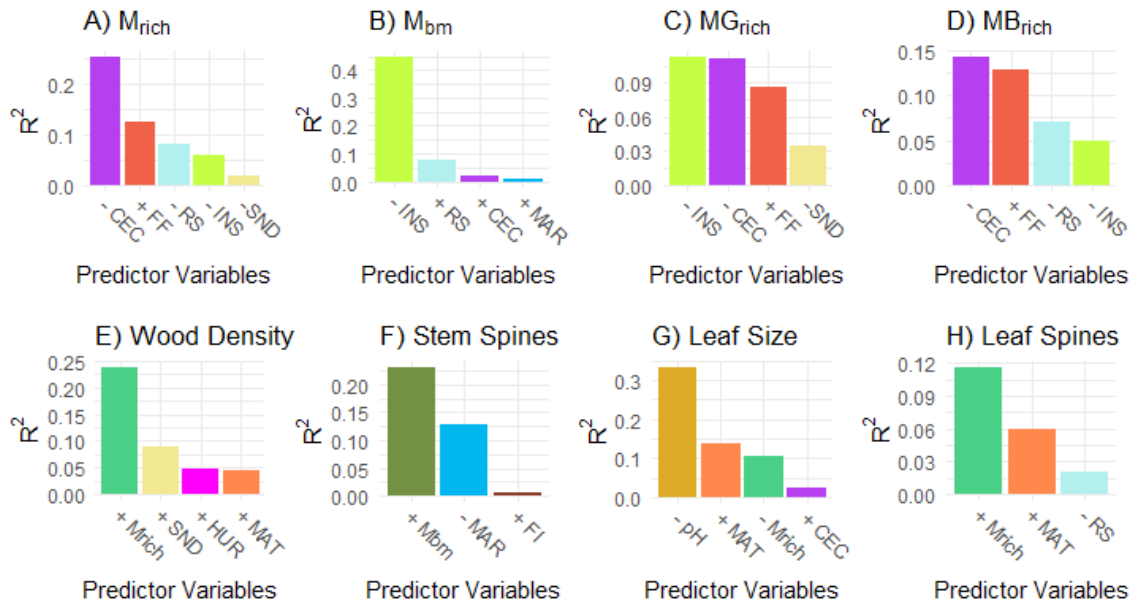


Fig. 2: Average contribution of predictor variables to regression models for megafauna (A-D) and functional traits (E-H) as response variable (see Tables S1-S5 for details).

Positive and negative signs preceding variables abbreviations indicate positive and negative associations. Results based on R^2_{adj} for A-E and H, and McFadden's pseudo- R^2 for F and G (logistic models). Islands are excluded from functional trait analyses (sample size: $N_{A-D} = 156$; $N_{E-G} = 142$; and $N_H = 131$). M_{rich} and M_{rich} : mean extinct megafauna species richness; M_{bm} and M_{bm} : mean extinct megafauna species mean body mass; MG_{rich} and MB_{rich} : mean extinct megagrazer and megabrowser species richness, respectively (mixed feeders excluded); CEC: soil cation exchange capacity; FF: (wild) fire frequency; FI: (wild) fire intensity; INS: insularity (categorical: 0/1); MAR: mean annual rainfall; MAT: mean annual temperature; pH: soil pH; RS: rainfall seasonality; SND: soil sand percentage; HUR: hurricanes.

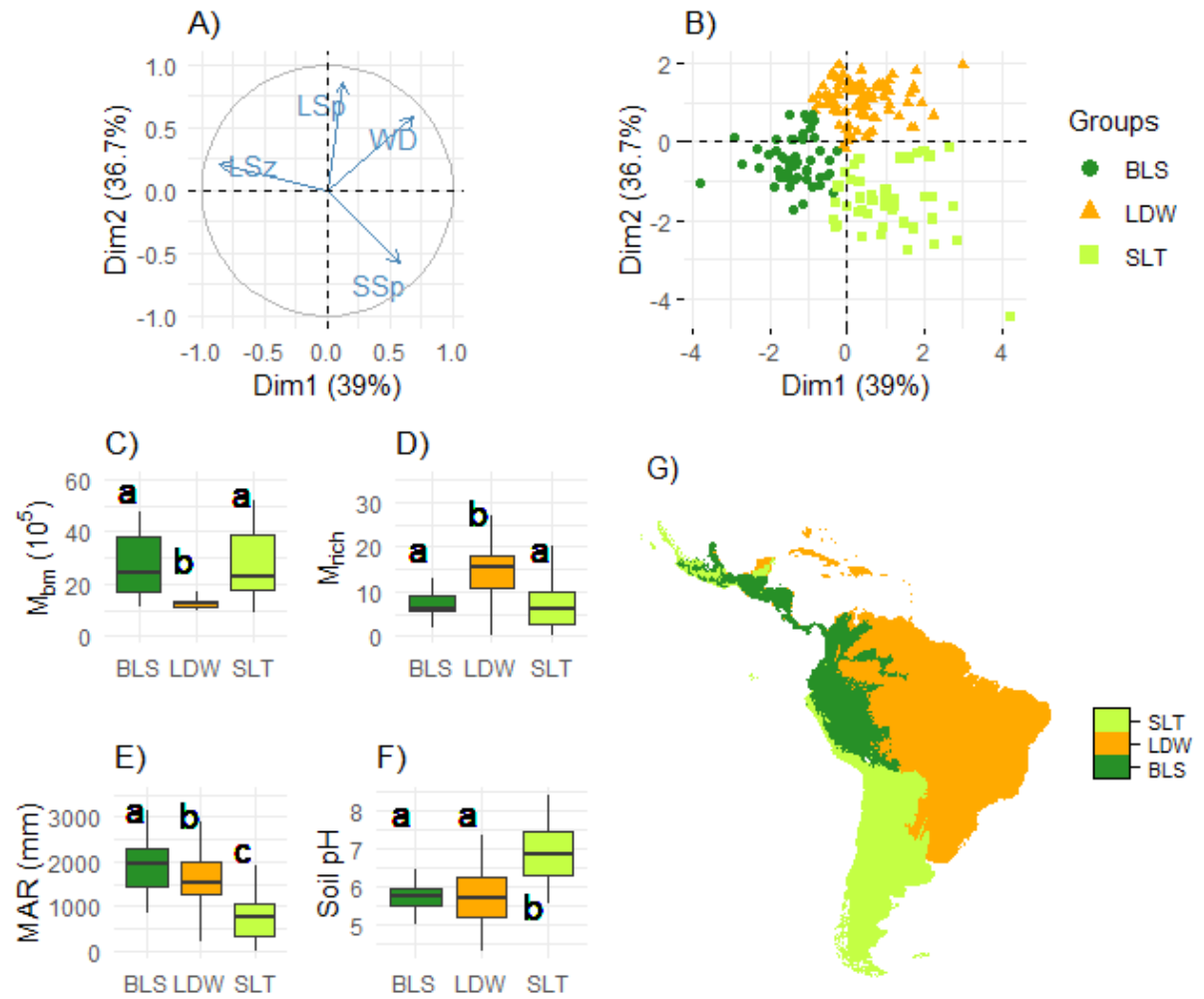


Fig. 3: Classification (A-B), characterization (C-F) and mapping (G) of Neotropical Antiherbiomes (large regions characterized by similar plant antiherbivory resistance traits). (A) Trait correlations to the principal component axes of plant defenses; (B) Cluster analysis on these axes; (C-D) megafauna, (E) mean annual rainfall (MAR) and (F) soil pH differences among the three Antiherbiomes (see Fig. S1 for other significant differences; P-values corrected using the Benjamini & Hochberg method). LSz: Leaf Size; SSz: Stem spines; WD: Wood Density; LSp: Leaf Spines; BLS: Broad-Leaved 10Sensitive; SLT: Small-Leaved Thorny; LDW: Leaf Defenses/Dense Wood; M_{rich} : extinct megafauna species richness; M_{bm} : extinct megafauna species mean body mass.

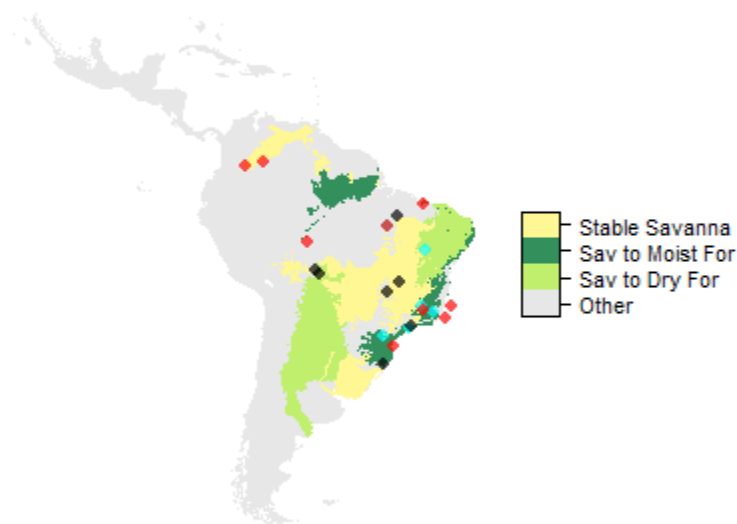


Fig. 4: Regions (colored area) formerly characterized by grassy ecosystems (Pleistocene) and fossil sites recording past vegetation patterns (diamonds; references in 5methods). Pleistocene grassy ecoregions are those with extinct megafauna richness equal or higher than 14 species (75% quantile), with number of grazing equal or higher than number of browsing extinct megafauna species, and containing highly defended plant assemblages (SLT or LDW antiherbiomes). They include ecoregions that are currently dominated by savannas (Sav; yellow area), dry forests (Dry For; light green 10area) and moist forests (Moist For; dark green area). Megafauna-poorer Llanos savanna (13 megafauna species) is also shown. Fossil sites supported grassy vegetation during the Last Glacial Maximum (red), Mid-Holocene (cyan) or in both periods (black diamonds). Only five out of the 21 fossil sites are from currently non-forest ecosystems (the partially coinciding three north- and west- most red points, totalizing four, and the 15leftmost black point in the central savanna patch - i.e. in Brazilian Cerrado region).

Supplementary Material

Materials and methods

Species level trait data

We compiled species level data on wood density (WD), spinescence, and leaf size for 5tropical and extra-tropical South and Central American woody species (the Neotropical biogeographic realm). WD was obtained for 2577 species from (31). The presence or absence of stem (and branch) spines (mostly thorns, but also prickles) were obtained from (32) for Neotropical savanna and forest species (1004 species) and complemented with other literature sources (33, 34, 43–52, 35, 53–62, 36, 63–72, 37, 73–75, 38–42). 10Thus, our final stem spine dataset included 2520 woody species. Leaf size data was obtained for 2660 woody species from (76). For some species we had more than one trait value, so we computed the species trait value as the mean for quantitative traits (WD, leaf size), and as the maximum for binary traits (spinescence; 0 for absence and 1 for presence).

15Because of the lack of a large dataset of leaf level defenses for woody species (e.g. chemical leaf defenses, leaf spines), we used data on leaf spinescence of palm species as a proxy (694 species) from the global Palm Traits Database 1.0 (34). In African savannas, leaf spines tend to be associated with resource conservative plants and is positively correlated with other leaf level defenses, such as acid detergent fiber and 20lignin (18).

From Species to Ecoregions

We obtained geographical distribution data (coordinates) from GBIF for all species in each species-trait dataset (Data available from GBIF; WD: <https://doi.org/10.15468/dl.3vua3x>; Stem spines: <https://doi.org/10.15468/dl.sj8hj5>; Leaf spines: <https://doi.org/10.15468/dl.vv8gw4>; Leaf size: <https://doi.org/10.15468/dl.k98nxc>). From these coordinates, we determined the ecoregion and biogeographical realm(77) and cropped out occurrences falling outside of the Neotropical realm. Based on the occurrence data, we built a species abundance (columns) by ecoregion (rows) matrix for each trait.

Using the respective matrices, we obtained ecoregion scale abundance weighted mean for WD, Stem Spines, and Leaf Size by: 1) Multiplying species abundance by the trait value of the corresponding woody species; 2) Summing up the row values; and 3) dividing the resulting row sum by the total species abundance per ecoregion (row sum prior to trait multiplication). This process resulted in weighted means for WD and stem spinescence for 173 ecoregions, and Leaf Size for 174, out of 179 Neotropical ecoregions. For leaf spinescence we used a similar approach but because of the fewer species, the abundance estimate from GBIF was unreliable. Thus, we transformed the ecoregion species abundance to presence/absence before multiplying the trait values (0/1 for absence/presence). We obtained leaf spinescence data for 159 out of the 179 Neotropical ecoregions.

20

Historical Megafauna and Herbivore Mammal Distributions

We obtained data on historical distribution of megafauna species from the MegaPast2Future/PHYLACINE_1.2 dataset (78), a dataset containing distribution maps (96.5 km of spatial resolution) and functional traits for mammal species of the last

130,000 years. Specifically, we extracted the potential present distribution of extinct mammal (coded “EP” for IUNC status) whose body mass was higher than 50 kg (megafauna), and for which at least 90 % of their diet consisted on plants (i.e. strict herbivores). The potential distribution of extinct species in this database was based on the present distribution of extant species that are known, from the fossil record, to have coexisted with the extinct species. In this approach, the extinct species was considered to have been present in a given grid cell if at least 50% of the extant species found in the fossil (and subfossil) sites of the extinct species presently occur in the cell (79). This approach assumes that, since extant and extinct species coexisted in the same locations, they must have had similar ecological requirements. It also assumes that megafauna extinction had anthropogenic causes, instead of causes related to climate change (79), which is largely accepted in the literature (80, 81).

For each Ecoregion, we calculated two megafauna-related metrics: extinct megafauna species richness (M_{rich}) and their mean body mass (M_{bm}). First, we cropped the distribution maps of the selected megaherbivores to the Neotropical realm and assigned the corresponding dominant ecoregion to each of the grid cells of the megafauna map. Then, for each ecoregion, we built a grid cell (rows) by megafauna species (columns) matrix containing presence or absence of each megafauna species. For M_{rich} , we summed the matrix rows to obtain pixel level richness and calculated the ecoregion level mean of each ecoregion. For M_{bm} , we multiplied the matrix columns by the body mass of the respective megafauna species before computing the grid cell mean, and then we calculated the ecoregion level mean. We also obtained diet information from the literature for most megafauna species that occurred in the Neotropical region in this dataset (1, 82–98). Based on this information, we calculated the richness of megabrowser (MB_{rich}), megagrazer (MG_{rich}), and mega mixed-feeder (MM_{rich}) species

We also compiled data on the distribution of extant and recently extinct (i.e. during modern times) to test the alternative hypothesis that trait variation is actually explained by recent herbivory history. This data was obtained from the same dataset and represent the hypothesized original distribution of these species prior to anthropogenic reduction of their ranges. For this, the dataset was produced using the same approach described above for extinct megafauna species. Here, we also included strict herbivores (at least 90% of the diet constituted of plants), but did not use a size threshold. We subsequently calculated the same metrics as for the extinct megafauna species (except for mixed-feeders as our source for diets labelled species according to dominant feeding pattern). Dietary patterns of extant and recently extinct mammals were taken from (99).

Climate, Soil, Fire, Insularity and Hurricanes

For each Ecoregion, we obtained data on climate (mean annual precipitation and temperature, and rainfall seasonality) and soil (sand content, pH, and cation exchange capacity). Climate data was obtained from WorldClim 2.1 (10 minute spatial resolution) and was based on climate data from 1970 and 2000 (100). Soil data were obtained from SoilGrids dataset (5 km of spatial resolution) (101), and consisted of average values for two depths, 0.05 and 2 m. We calculate Ecoregion level means for all soil and climate variables after intersecting the grid maps (climate and soil) with the ecoregion map.

We obtained the number (a proxy for frequency) and intensity of wildfires per ecoregion area using the MODIS active fire location product (MCD14ML) (102). We only considered fires with detection confidence of 95% or higher occurring from November 2000 to December 2019 (both included). To ensure that only wildfires were considered, we associated each fire pixel to a land cover type (300 m of spatial

resolution) from (103) for a buffer area of 1000 m surrounding the fire pixel centroid.

We excluded all fires occurring in areas in which more than 10% of the surrounding land cover pixels corresponded to agricultural, urban and water classes. We calculated the number of wildfires per ecoregion area by dividing the fire count of each Ecoregion 5 by the ecoregion area, multiplied by the proportion of vegetated land cover pixels (same classes used to exclude fires on anthropogenic areas and water bodies above). Fire intensity was calculated as the average fire radiative power of all wildfires in the ecoregion. Ecoregions lacking large preserved vegetated areas (criteria above) were excluded from subsequent analyses.

10 Using the ecoregion map, we also classified ecoregions into insular (1), when most of the ecoregion area was located in islands, vs. continental (0), otherwise. This was performed because island biogeography theory predicts island to be species poor due to limited dispersal from continental areas. Insularity was also expected to reduce megafauna body mass due to specific density dependent population dynamics (the 15 island rule) (23). We also compiled data on hurricane activity, as woody density confers resistance against this disturbance(24). We used data from 1990 to 2019 from the HURDAT2 dataset(104), containing six-hourly information on the location of all known tropical and subtropical cyclones (0.1° latitude/longitude). We used the sum of hurricane occurrences per ecoregions divided by ecoregion area as an indicator of the 20 hurricane activity.

Statistical Analysis

To better understand patterns in the megafauna indicators, we fit (multiple) regression models with fire, climate, soil variables and insularity as predictors. Before the analyses,

we tested the correlation among all the variables that would enter as predictors in a given model (Table S5), in order to avoid the inclusion of highly correlated variables (i.e. $r \geq 0.60$) and, thus, collinearity. Since only mean annual precipitation and pH were strongly positively correlated ($r = -0.78$), for all analyses, model selection was performed separately for these two variables (i.e. two different model selection procedures, one containing each of the two variables in the initial set of predictors). We selected the best among the two models as that with the lowest AIC (differences higher than two points in all cases). To double check for multicollinearity in predictor variables we also used the Variation Inflation Factor (VIF; calculated as one minus the R^2 of the model when regressing a predictive variable against all the others). All models present VIF of 0.30 or higher indicating absence of multicollinearity.

Model simplification was carried by interactively stepwise (i.e. with both forward and backward variable deletion) searching for the model with the lowest AIC (using R's "step" function) and subsequently retaining only significant variables ($p \leq 0.05$). We calculated the average contribution of each predictor variable in the selected model as the mean difference in R^2 between models including and removing the target variable for all possible subset models containing the variable and the selected predictors. This was performed with the R package "dominanceanalysis".

To test whether the studied plant functional traits were related to megafauna indicators, we fit linear models to WD and leaf size, and generalized linear models (GLM; binomial family) for spinescence, using ecoregion as the unit. For the spinescence, we used the matrix containing spiny and non-spiny species abundance (for stem spines) or number of species with or without spines (for leaf spines; see above) as response variables. The predictor variables included the extinct megafauna indicators, extant and recently extinct herbivore indicators, as well as climate, soil, and fire predictors (and

hurricanes, for wood density). Because total, as well as megagrazer, megabrowser and mega mixed feeder species richness were all strongly positively correlated (Table S5), we only used megafauna richness, body mass, and the difference between grazer and browser species richness as indicators. We used the same variables for extant and recently extinct species (i.e. herbivore richness, body mass and richness difference between grazers and browsers). We did not identified strong correlations between extinct megafauna and extant/recently extinct herbivore indicators (Table S5), therefore, all these indicators were entered in the same initial models at once. We also calculated the average predictor contribution in these models. For this, we used the MacFadden Pseudo- R^2 in the GLM models as implemented in the “pscl” and “dominanceAnalysis” packages for R, as this statistic is the most comparable with R^2 from linear multiple regression (Maximum Likelihood and Cragg and Uhler’s Pseudo- R^2 were also calculated for the full logistic models). Islands were not included in these models, as islands plants were expected to respond differently due to insularity effect on animal species richness.

For all the general linear regression analyses, assumptions of normality and homoscedasticity in model residuals were checked using the Kolmogorov-Smirnov and Breusch-Pagan tests, respectively. In some cases, heteroscedasticity was detected and, thus, the significance of the coefficients was tested using heteroskedasticity-consistent covariance matrix estimation. Overdispersion in the generalized linear model was also detected and dealt with using overdispersed binomial logit models, as implemented in the “dispmod” package for R, in which weights are interactively calculated and used to maintain the residual deviance lower than degrees of freedom.

To test the prediction that Neotropical ecoregions could be broadly classified into the three hypothesized antiherbiomes, we used hierarchical clustering on principal

component axes of ecoregion by trait matrix (four traits, standardized to zero mean and unit variance prior to this analysis). We selected the number of clusters associated with the highest loss of inertia (within group variability) when progressively increasing the number of clusters using the R package “FactoMiner”. For stem spines, we used the proportion of spinescent plants/species (rather than the number of “yes” and “no” used in previous analyses), whereas, for leaf spines, we used the predicted probability from the selected GLM model as palms were missing from 20 ecoregions. To address the impact of using predicted leaf spine data, we also performed the classification without leaf spines. The two results were very similar, so we maintained leaf spines (Fig. S2).

This procedure defined large regions characterized by specific patterns of megafauna-environment-vegetation interactions, promoting plant assemblages that converge in antiherbivory defense traits (that is, what we call an antiherbiomes). We then tested for megafauna and environmental differences between the resulting antiherbiomes to see if climate and soil matched the patterns reported for Africa, and to understand how the megafauna was distributed in these antiherbiomes. For these comparisons, we used Kruskal-Wallis and post-hoc pairwise Dunn tests, using the Benjamini & Hochberg (1995) correction of P-values for multiple comparisons in both cases.

Finally, we identified forest ecoregions that were likely to have experienced biome shifts after megafauna extinction. We considered these ecoregions to be those that: 1) are currently forest; 2) pertained to antiherbiomes characterized by highly defended species; 3) were megafauna-rich during the Pleistocene (equal or higher number of species than the 75% quantile of M_{rich} , that is, 14 species); and 4) had an equal or higher number of grazing compared to browsing species. We compared the distribution of these areas with fossil evidence from the Last Glacial Maximum and mid-Holocene (29, 25/06). For this, we also used information on the present dominant vegetation type to

segregate savanna-forest shifts from data coming from savanna patches within forest or long-term savannas.

All statistical analyses and data handling were carried out in the R environment, using the previously mentioned packages, as well as the packages gridExtra, grid, lattice, 5latticeExtra, raster, rgbif, lmtest, sandwich, olsrr and rgdal.

3. Supplementary Figures

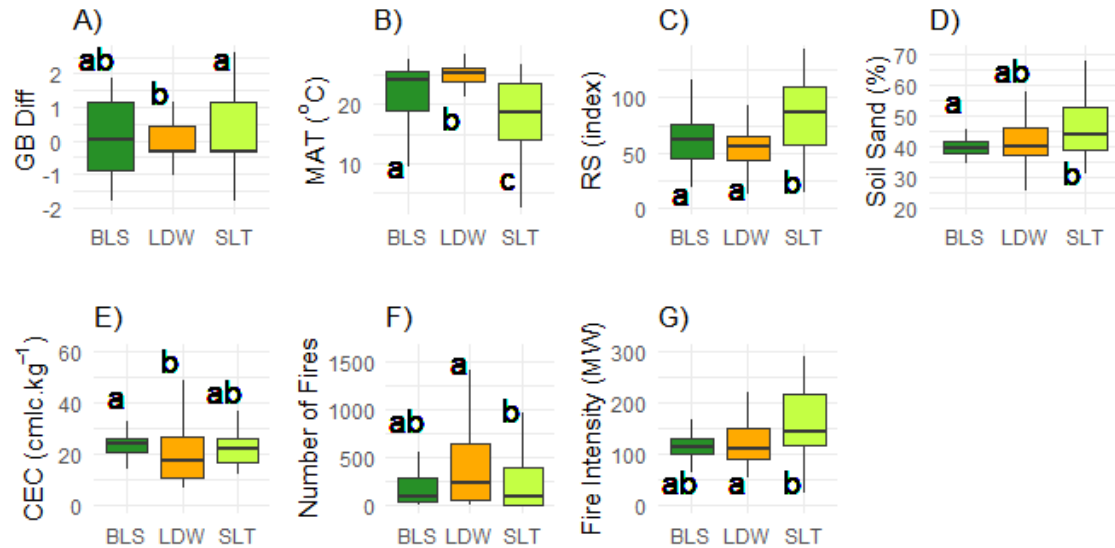


Fig. S1: Significant differences in predictor variables among herbivomes that were not shown in Fig 3. P-values corrected using the Benjamini & Hochberg method.

Differences are based on Dunn tests. For difference between number of megagrazer and megabrowser (GB_diff), statistical results indicated lower values for SLT. BLS: Broad Leaf Sensitive; SLT: Small Leaved Thorny; LDW: Leaf Defense Dense Wood; MAT: mean annual precipitation; RS: rainfall seasonality; CEC: cation exchange capacity.

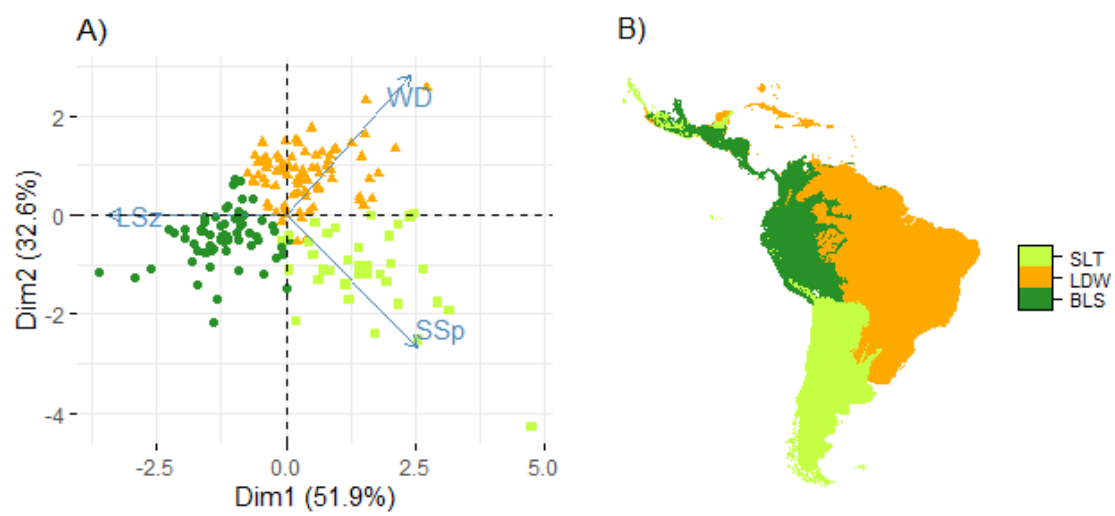


Fig. S2: Antiherbivore classification when leaf spines was excluded.

2. Supplementary Tables

Table S1: General and generalized linear model results regressing megafauna indicators and plant functional traits against biotic (megafauna), and abiotic 5(climate, soil, fire and hurricane) predictors.

Assemblages	Response variables	N	R^2_{adj}	R^2_{McFadd}	R^2_{ML}	R^2_{CU}	ΔAIC
Megafauna	M_{rich} (number)	156	0.53				-112.34
	$\text{Log}(M_{bm} \text{ (index)} + 1)$	156	0.55				-122.12
	MB_{rich} (number)	156	0.38				-69.58
	MG_{rich} (number)	156	0.33				-57.81
	MM_{rich} (number)	156	0.57				-126.4
Woody plants	Wood Density (g.cm^3)	142	0.40				-69.52
	Stem Spines (yes/no)	142		0.38	0.50	0.59	-92.93
	Leaf Size (cm^2)	142	0.59				-122.85
	Leaf Spines (yes/no)	131		0.15	0.40	0.41	-59.87

Selected models and coefficients are shown in Table S2. Model diagnostics are shown in Table S3. Models were selected after a stepwise backward procedure (until all retained variables were significant). All models are general linear regression except for the response variables stem and leaf spines, which are generalized linear model instead 10with a binomial family distribution. For these, MacFadden's (R^2_{McFadd}), maximum likelihood (R^2_{ML}), and Cragg and Uhler's (R^2_{CU}) pseudo- R^2 are shown instead of R^2_{adj} . M_{rich} : mean extinct megafauna species richness; M_{bm} : mean extinct megafauna species mean body mass; MG_{rich} , MB_{rich} and MM_{rich} (C and D): mean extinct megagazer, megabrowser and megamixed-feeders species richness, respectively.

Table S2: Selected models and coefficients for the models in Table S1.

Assemblages	Response variables	Coefficients
Megafauna	M_{rich} (number)	33.799-3.770*INSUL-0.002*MAR-0.101*RS- 0.416*CEC-0.165*SND+0.004*FF
	Log (M_{bm} (index) + 1)	0.130- 2.072*INSUL+0.000*MAP+0.009*RS+0.023*CEC
	MB_{rich} (number)	4.696-0.994*INSUL-0.021*RS- 0.069*CEC+0.001*FF
	MG_{rich} (number)	6.627-1.75*INSUL-0.062*CEC- 0.064*SND+0.001*FF
	MM_{rich} (number)	10.738-1.474*INSUL-0.001*MAR-0.042*RS - 0.178*CEC+0.002*FF
Plants	Wood Density (g.cm ³)	0.364+0.010* M_{rich} - +0.002*MAT+0.004*HUR+0.003*SND
	Stem Spines (yes/no)	-1.63+0.000* M_{bm} +0.002*FI-0.001*MAR
	Leaf Size (cm ²)	144.498-1.621* M_{rich} +1.365*MAT+0.538*CEC- 1.825*pH
	Leaf Spines (yes/no)	-2.278+0.05* M_{rich} +0.055*MAT-0.007*RS

M_{rich} : mean extinct megafauna species richness; M_{bm} : mean extinct megafauna species mean body mass; MG_{rich} , MB_{rich} and MM_{rich} : mean extinct megagrazer, megabrowser and megamixed-feeders species richness, respectively. MAT: mean annual temperature; 5MAP: mean annual (rainfall) precipitation; RS: rainfall seasonality; SND: soil sand content; pH: soil pH; CEC: soil cation exchange capacity; FI: (wild)fire intensity; FF: (wild)fire frequency; HUR: hurricane activity (count per area).

Table S3: Model Diagnostics for the models presented in Table S1 and S2

Response	Kolmogorov-Smirnov		Breusch-Pagan		HCE	Dispersal	
	Statistic	P	Statistic	P	Robust predictors?	Resid. Dev.	DF
M _{rich}	0.103	0.073	17.607	0.007	yes	-	-
M _{bm}	0.096	0.112	78.614	<0.001	yes	-	-
MG _{rich}	0.055	0.743	0.724	0.948	-	-	-
MB _{rich}	0.077	0.315	5.468	0.243	-	-	-
MM _{rich}	0.078	0.296	30.063	<0.001	yes	-	-
WD	0.089	0.211	1.926	0.750	-	-	-
LSz	0.086	0.241	31.009	<0.001	yes	-	-
SSp	-	-	-	-	-	131.49	138
LSp	-	-	-	-	-	122.39	127

Kolmogorov-Smirnov and Breusch-Pagan tests were used to test model residuals

against the null hypothesis of normality and homoscedasticity, respectively. When the later assumption was violated (i.e. M_{rich}, M_{bm}, MM_{rich}, and Lsz), we used

5heteroskedasticity-consistent estimation (HCE) of covariance matrices to check

predictor significance (confirmed in all cases). For the logistic models (generalized

linear regression with binomial error), residual deviance (Resid. Dev.) and degrees of

freedoms (DF) are shown for overdispersal diagnostics. M_{rich}: mean extinct megafauna

species richness; M_{bm}: mean extinct megafauna species mean body mass; MG_{rich}, MB_{rich}

10and MM_{rich}: mean extinct megagrazer, megabrowser and megamixed-feeders species

richness, respectively. WD: wood density; Lsz: leaf size; SSp: stem spines; LSp: leaf

spines.

Table S4: Average contribution (%) of the selected variables in the selected regression models (shown in details in Table S1 and S2)

	M _{rich}	M _{bm}	M _{gbr}	MAT	MAR	RS	CEC	pH	SND	FF	FI	HUR	INS
M _{rich}	NE	NE	NE	0%	1%	8%	26%	0%	2%	13%	0%	NE	6%
M _{bm}	NE	NE	NE	0%	1%	8%	3%	0%	0%	0%	0%	NE	45%
MB _{rich}	NE	NE	NE	0%	0%	7%	14%	0%	0%	13%	0%	NE	5%
MG _{rich}	NE	NE	NE	0%	0%	0%	11%	0%	3%	9%	0%	NE	11%
MM _{rich}	NE	NE	NE	0%	1%	8%	30%	0%	0%	12%	0%	NE	7%
WD	24%	0%	1%	4%	0%	0%	0%	0%	9%	0%	0%	5%	NE
SSp	0%	23%	0%	0%	13%	0%	0%	0%	0%	0%	1%	NE	NE
LSp	12%	0%	0%	6%	0%	2%	0%	0%	0%	0%	0%	NE	NE
LSz	10%	0%	0%	14%	0%	0%	3%	33%	0%	0%	0%	NE	NE

The values of the three most important predictors of each response variable are

highlighted in bold. The average contribution is the mean R^2 (or McFadden's Pseudo- $5R^2$, for stem and leaf spine) difference between all subset models containing the variable and the same models without the variable, multiplied by 100. M_{rich}: mean extinct megafauna species richness; M_{bm}: mean extinct megafauna species mean body mass; MG_{rich}, MB_{rich} and MM_{rich}: mean extinct megagrazer, megabrowser and megamixed-feeders species richness, respectively; M_{gbr}: difference between number of megagrazer and megabrowser species. MAR: mean annual rainfall; MAT: mean annual temperature; RS: rainfall seasonality; SND: soil sand content; CEC: soil cation exchange capacity; pH: soil pH; FI: fire intensity; FF: fire frequency; HU: hurricanes; INS: insularity; NE: Not Evaluated.

Table S5: Pairwise correlation table showing correlations between all pairs of variables used as predictor variables in the regression models (Table S2-S4)

Row	Column	r
M_rich	M_bm	-0.44
M_rich	MGB_diff	-0.14
M_bm	MGB_diff	0.39
M_rich	MH_rich	0.27
M_bm	MH_rich	0.06
MGB_diff	MH_rich	-0.15
M_rich	MH_bm	0.16
M_bm	MH_bm	0.37
MGB_diff	MH_bm	0.09
MH_rich	MH_bm	0.11
M_rich	MHGB_diff	0.40
M_bm	MHGB_diff	0.02
MGB_diff	MHGB_diff	0.29
MH_rich	MHGB_diff	0.14
MH_bm	MHGB_diff	0.08
M_rich	pH	-0.37
M_bm	pH	0.27
MGB_diff	pH	0.14
MH_rich	pH	-0.55

MH_bm	pH	0.12
MHGB_diff	pH	0.14
M_rich	INSUL	-0.32
M_bm	INSUL	-0.35
MGB_diff	INSUL	-0.09
MH_rich	INSUL	-0.50
MH_bm	INSUL	-0.56
MHGB_diff	INSUL	-0.28
pH	INSUL	0.16
M_rich	FF	0.40
M_bm	FF	-0.08
MGB_diff	FF	-0.12
MH_rich	FF	-0.09
MH_bm	FF	0.14
MHGB_diff	FF	0.16
pH	FF	-0.04
INSUL	FF	-0.11
M_rich	MAR	0.04
M_bm	MAR	-0.12
MGB_diff	MAR	-0.03
MH_rich	MAR	0.38
MH_bm	MAR	-0.16

MHGB_diff	MAR	-0.33
pH	MAR	-0.78
INSUL	MAR	0.05
FF	MAR	-0.11
M_rich	FI	0.21
M_bm	FI	-0.11
MGB_diff	FI	-0.10
MH_rich	FI	-0.12
MH_bm	FI	0.19
MHGB_diff	FI	0.25
pH	FI	0.09
INSUL	FI	-0.15
FF	FI	0.22
MAR	FI	-0.23
M_rich	MAT	0.14
M_bm	MAT	-0.13
MGB_diff	MAT	0.03
MH_rich	MAT	0.05
MH_bm	MAT	-0.33
MHGB_diff	MAT	-0.29
pH	MAT	-0.37
INSUL	MAT	0.17

FF	MAT	0.22
MAR	MAT	0.48
FI	MAT	-0.36
M_rich	RS	-0.22
M_bm	RS	0.43
MGB_diff	RS	0.21
MH_rich	RS	-0.17
MH_bm	RS	0.01
MHGB_diff	RS	0.26
pH	RS	0.43
INSUL	RS	-0.13
FF	RS	0.16
MAR	RS	-0.49
FI	RS	-0.07
MAT	RS	-0.03
M_rich	CEC	-0.58
M_bm	CEC	0.19
MGB_diff	CEC	0.12
MH_rich	CEC	-0.24
MH_bm	CEC	-0.06
MHGB_diff	CEC	-0.15
pH	CEC	0.42

INSUL	CEC	0.39
FF	CEC	-0.25
MAR	CEC	-0.07
FI	CEC	-0.13
MAT	CEC	-0.19
RS	CEC	-0.03
M_rich	SND	0.18
M_bm	SND	-0.04
MGB_diff	SND	-0.22
MH_rich	SND	-0.17
MH_bm	SND	0.33
MHGB_diff	SND	0.09
pH	SND	0.16
INSUL	SND	-0.26
FF	SND	0.24
MAR	SND	-0.31
FI	SND	0.38
MAT	SND	-0.39
RS	SND	0.06
CEC	SND	-0.39
M_rich	HUR	-0.34
M_bm	HUR	0.20

MGB_diff	HUR	0.29
MH_rich	HUR	-0.40
MH_bm	HUR	-0.12
MHGB_diff	HUR	0.06
pH	HUR	0.41
INSUL	HUR	0.29
FF	HUR	-0.04
MAR	HUR	-0.22
FI	HUR	-0.22
MAT	HUR	0.08
RS	HUR	0.34
CEC	HUR	0.29
SND	HUR	-0.21

M_rich: mean extinct megafauna species richness; M_bm: mean extinct megafauna species body mass; MGB_diff: difference between number of megagrazer and megabrowser species; MH_rich: mean herbivore mammal species richness; MH_bm: mean herbivore mammal species body mass; MHGB_diff: difference between number of mammal herbivore grazer and browser species; MAR: mean annual rainfall; MAT: mean annual temperature; RS: rainfall seasonality; SND: soil sand content; CEC: soil cation exchange capacity; pH: soil pH; FI: fire intensity; FF: fire frequency; HU: hurricanes; INS: insularity;