



Effects of Dam-Induced Landscape Fragmentation on Amazonian Ant–Plant Mutualistic Networks

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Abstract: *Mutualistic networks are critical to biological diversity maintenance; however, their structures and functionality may be threatened by a swiftly changing world. In the Amazon, the increasing number of dams poses a large threat to biological diversity because they greatly alter and fragment the surrounding landscape. Tight coevolutionary interactions typical of tropical forests, such as the ant–myrmecophyte mutualism, where the myrmecophyte plants provide domatia nesting space to their symbiotic ants, may be jeopardized by the landscape changes caused by dams. We analyzed 31 ant–myrmecophyte mutualistic networks in undisturbed and disturbed sites surrounding Balbina, the largest Central Amazonian dam. We tested how ant–myrmecophyte networks differ among dam-induced islands, lake edges, and undisturbed forests in terms of species richness, composition, structure, and robustness (number of species remaining in the network after partner extinctions). We also tested how landscape configuration in terms of area, isolation, shape, and neighborhood alters the structure of the ant–myrmecophyte networks on islands. Ant–myrmecophytic networks were highly compartmentalized in undisturbed forests, and the compartments had few strongly connected mutualistic partners. In contrast, networks at lake edges and on islands were not compartmentalized and were negatively affected by island area and isolation in terms of species richness, density, and composition. Habitat loss and fragmentation led to coextinction cascades that contributed to the elimination of entire ant–plant compartments. Furthermore, many myrmecophytic plants in disturbed sites lost their mutualistic ant partners or were colonized by opportunistic, nonspecialized ants. Robustness of ant–myrmecophyte networks on islands was lower than robustness near lake edges and in undisturbed forest and was particularly susceptible to the extinction of plants. Beyond the immediate habitat loss caused by the building of large dams in Amazonia, persistent edge effects and habitat fragmentation associated with dams had large negative effects on animal–plant mutualistic networks.*

Keywords: coextinction, compartmentalization, edge effect, habitat change, habitat loss, myrmecophyte, nestedness, species interaction

Efectos de la Fragmentación del Paisaje Inducida por Presas sobre Redes Mutualistas Hormiga-Planta Amazónicas

Resumen: *Las redes mutualistas son críticas para el mantenimiento de la biodiversidad; sin embargo, sus estructuras y funcionalidad pueden estar amenazadas por un mundo que cambia rápidamente. En el Amazonas, el número creciente de presas representa una gran amenaza para la biodiversidad porque alteran y fragmentan el paisaje circundante drásticamente. Interacciones coevolutivas estrechas típicas de los bosques tropicales, como el mutualismo hormigas–mirmecofitas en el que las plantas mirmecofitas proporcionan espacio para que aniden sus hormigas simbióticas, pueden estar en peligro por los cambios en el paisaje provocados por las presas. Analizamos 31 redes mutualistas hormigas–mirmecofitas en sitios perturbados y no perturbados alrededor de Balbina, la mayor presa en el Amazonas central. Probamos como difieren las redes hormiga–mirmecofitas entre islas inducidas por la presa, bordes del lago y bosques no perturbados en términos de la riqueza, composición, estructura y robustez (número de especies que permanecen en la red*

después de la extinción de otras integrantes de la red). También probamos como altera la configuración del paisaje, en términos de área, aislamiento, forma y vecindad (proporción de tierra y agua alrededor del fragmento), la estructura de las redes de hormigas-mirmecofitas en las islas. Las redes de hormigas-mirmecofitas estuvieron muy compartimentadas en los bosques no perturbados, y los compartimientos tenían pocos socios mutualistas conectados fuertemente. En contraste, las redes en los bordes del lago y en las islas no estuvieron compartimentadas y fueron afectadas negativamente por la área de la isla y el aislamiento en términos de la riqueza, densidad y composición de especies. La pérdida y fragmentación del hábitat produjo cascadas de coextinción que contribuyeron a la eliminación de compartimientos enteros de hormigas-plantas. Más aun, muchas plantas mirmecofitas en sitios perturbados perdieron sus hormigas mutualistas o fueron colonizadas por hormigas oportunistas, no especializadas. La robustez de las redes hormigas-mirmecofitas en islas fue menor a la robustez cerca de los bordes del lago y en bosque no perturbado y fue particularmente susceptible a la extinción de plantas. Más allá de la pérdida inmediata de hábitat provocada por la construcción de presas en la Amazonía, la persistencia de efectos de borde y fragmentación del hábitat asociadas con presas tuvieron efectos negativos importantes sobre las redes mutualistas animales-plantas.

Palabras Clave: anidamiento, cambio de hábitat, coextinción, compartimentación, efecto de borde, interacción de especies, mirmecofita, pérdida de hábitat

Introduction

Conservation of mutualistic interactions is essential for the maintenance of biological diversity (Dunn et al. 2009; Tylianakis et al. 2010). In tropical forests, mutualisms are widespread and a single plant can interact with a diversity of pollinators, seed dispersers, phosphorus-fixing mycorrhizae, nitrogen-fixing bacteria, and symbiotic ants. These interactions shape mutualistic networks that differ in size, structure, and stability (Bascompte et al. 2003; Bascompte & Jordano 2007). Recently, network analyses have been used to examine how species interactions respond to anthropogenic disturbance (e.g., Tylianakis et al. 2007; Sabatino et al. 2010; Aizen et al. 2012)

Among the threats to tropical forests, hydroelectric dams constitute an increasing risk because many governments regard them as the solution to the growing demand for energy. Over the next 20 years, for instance, 151 dams are planned in the Andean Amazon and 118 are planned in the Brazilian Amazon lowlands (MME/EPE 2011; Finer & Jenkins 2012). It is estimated that river impoundment by hydroelectric dams in the Brazilian Amazon will cause the direct loss of more than 10 million ha (Fearnside 2006). The affected area may be much larger because the novel water landscape can generate strong edge effects and act as a barrier to the movement and dispersal of terrestrial organisms (Prevedello & Vieira 2010). Furthermore, where topography is relatively flat, the lower areas are flooded, whereas on hilltops forest islands of different sizes, shapes, and level of isolation remain. The effect of dams on the structure of ecological networks is poorly known. In Western Amazon, dam fragmentation caused a cascade effect through ecological interactions due to the loss of top predators, an increase of herbivores, and a decrease in plant regeneration (Terborgh et al. 2001; Feeley & Terborgh 2008).

We examined the effects of the largest landscape disturbance ever caused by a dam in Central Amazon

on the mutualistic network between myrmecophytes and their specialized ants. Myrmecophytes are domatia-bearing plants that provide nesting space to their specialized ant partners (Janzen 1966; Benson 1985). They are dependent on their associated ants for defense, growth, survival, and reproduction (e.g., Vasconcelos 1991; Fonseca 1994; Bruna et al. 2004). Most specialized ants associated with myrmecophytes are also highly dependent on their partners because they nest exclusively on one or a few myrmecophyte species, colony density is constrained by the density of hosts in the local community, and colony size depends on availability of domatia space provided by the plant (Fonseca & Ganade 1996; Fonseca 1993, 1999). Specialized mutualistic plant ants and their myrmecophytes have a close evolutionary history; there are many documented cases of cospeciation, phylogenetic diversification, coevolved traits, and strong biogeographic matching (e.g., Janzen 1966; Benson 1985; Ward & Downie 2005). However, in disturbed environments myrmecophytes are sometimes used opportunistically by ants that normally nest on the ground or on hollow twigs or built carton nests. Such opportunistic ants lack the territorial and defensive behaviors that characterize the specialized plant-ants and fail to provide benefits to the plants. Sometimes these opportunistic ants are characterized as parasites of the mutualism (Janzen 1975).

Ant-myrmecophyte networks are organized in small, well-defined compartments that give this system one of the lowest connectance levels and one of the highest compartmentalization values known in mutualistic interactions, which elucidate the tight coevolutionary history between partners (Fonseca & Ganade 1996; Guimarães et al. 2006). The stability of the system is ensured by the fact that a given mutualistic ant species is frequently able to use a few phylogenetically related host species and a given myrmecophyte can be associated with a few mutualistic ant partners. Therefore, each ant-myrmecophyte

compartment is a coevolutionary unit whose conservation is essential if the aim is to preserve mutualistic processes, interacting species, and evolutionary trajectories. The structure of ant–myrmecophyte networks strongly differs from that of many other mutualistic networks, such as the interactions between plants and their pollinators, seed dispersers, and extrafloral-nectary visiting ants, in which generalist species are key elements that connect subgroups of more specialized species and configure a nested network structure (Guimarães et al. 2006; Guimarães et al. 2007). In these mutualistic systems, higher values of interaction nestedness and connectance can enhance network robustness to disturbance, promote community stability, and, consequently, improve conservation status (Thébault & Fontaine 2010; but see Heleno et al. 2012). In the ant–myrmecophyte mutualism, however, a stable and well-preserved community is expected to have low connectance, high levels of compartmentalization, and a non-nested structure.

We tested how size, structure, and robustness of ant–myrmecophyte mutualistic networks are affected by landscape fragmentation caused by dams. We compared networks in undisturbed sites with those on lake edges and water-isolated forest islands. We also tested how island area, shape, neighborhood (proportion of land and water surrounding the island), and isolation affect network properties. We expected that increasing levels of disturbance would lead to smaller network sizes due to loss of whole compartments with extinctions and co-extinctions, loss of mutualistic interactions, gain of new interactions due to colonization by opportunistic ants that could alter connectance levels, and decreases in network robustness.

Methods

Study Area and Sampling Design

The study was conducted in undisturbed and disturbed submontane dense rainforest sites surrounding the Balbina Dam in Central Amazon (Fig. 1). Balbina began operation in 1989, 16 months after the impoundment of the Uatumã River, a major tributary of the Amazon. Due to the shallow topography and poor drainage of the Uatumã River basin, 3129 km² of pristine forest were flooded, a 4437 km² lake was formed, and more than 3500 forest islands were isolated in a lake that is 210 km long (Fearnside 1989). Our study sites were located in undisturbed forest, at lake edges, and on water-isolated forest islands surrounding Balbina Dam. We randomly selected 6 undisturbed forest sites as controls in the Uatumã Biological Reserve. Location of control sites was constrained by logistic access to undisturbed forest; thus, the relative proximity among them could lead to an underestimate

of the ant–myrmecophyte regional community richness, although we believe it would not change the general network structure. We established 5 potentially disturbed lake-edge sites along Balbina Lake, starting on average 330 m from the lake edge. Finally, we established one site in each of the 20 selected forest-island fragments that differed in area, isolation, shape, and neighborhood and represented the range of island sizes in Balbina Lake: <15 ha, <50 ha, <200 ha, <500 ha, and <2000 ha.

For each island site, we estimated area (\log_{10} transformed), isolation (shortest Euclidean distance between island and lake edge), shape (residual of linear regression between perimeter and area, both \log_{10} transformed), and neighborhood (residual of linear regression between area and PROX—an index used to estimate fragment isolation that takes into consideration area and distance among all fragments in a given area around a fragment [i.e., buffer zone]) (McGarigal et al. 2002). For neighborhood analyses, we used a 1-km buffer due to the dispersal range limitation of plant-ants and myrmecophytes. These island metrics were not significantly correlated ($-0.278 \leq r \leq 0.562$).

From December 2009 to July 2010, we surveyed a plot of 600 m × 5 m in each study site. On islands, plots were at least 100 m from the edge, when possible. Each plot was intensively surveyed, without time constraint, by 2 or 3 trained people until all myrmecophytes and associated ants were recorded. Unoccupied myrmecophytes were also recorded. We classified ant species as plant-ants (species classified by taxonomists or in the literature as domatia inhabiting specialists and the ant morphotypes colonizing domatia in undisturbed forest) or opportunistic ants (species classified by taxonomists or in the literature as habitat generalists, ground, carton, or hollow-twig nesting species and the morphotypes recorded exclusively on disturbed sites). Ant and plant specimens were deposited at the Instituto Nacional de Pesquisas da Amazônia and Instituto Federal de Educação, Ciência e Tecnologia do Amazonas, respectively.

Spatial Nestedness

We performed spatial nestedness analyses to determine whether ant–myrmecophyte communities on fragmented islands and at lake edges were nested subgroups of the undisturbed forest. We built 3 binary matrices that represented the presence (1) or absence (0) of plant species, ant species, and ant–myrmecophyte interactions (lines in the matrices) on each one of the 31 studied sites (columns in the matrices). We measured nestedness with NODF index and tested its statistical significance against 1000 iterations with a CE null model available in the Aninhado Software (Guimarães & Guimarães 2006; Almeida-Neto et al. 2008).

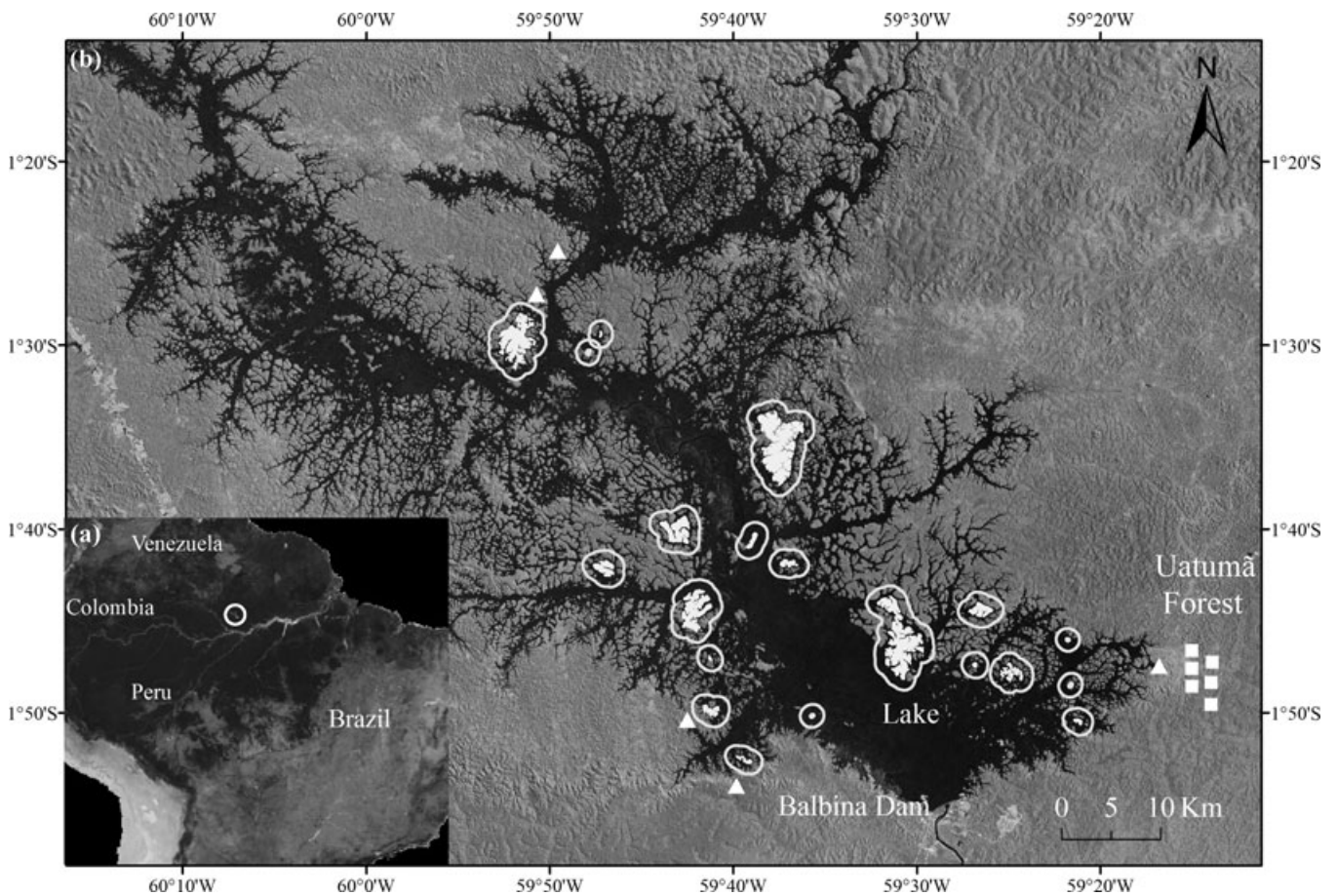


Figure 1. Study area (a) location within the Central Amazon (white circle, study area; light color within circle, water) and (b) at a regional scale (black, water [primarily inundation caused by Balbina Dam]; gray around the lake, disturbed and undisturbed tropical forest; white, dam-induced forest islands selected for this study among 3500 others; circles around islands, 1-km buffer zones within which we estimated the effects of adjacent land or water on ant–myrmecophyte networks; triangles, sites at the lake edge; squares, undisturbed Amazonian forest sites at the Uatumã Biological Reserve).

Network Parameters

Ant–myrmecophyte networks were organized as a bipartite $m \times n$ quantitative matrix, with m plant species and n ant species. The elements in the matrix represented the number of plants of a given myrmecophyte species that were used as a nesting site by colonies of a given ant species. Because ant–myrmecophyte networks were very small in some disturbed sites and some analyses were sensitive to network size, we performed data analyses on 2 levels: habitat networks (site data pooled to create one single network per habitat) and site networks (each site kept as a replicate of the respective habitat). Ant–myrmecophyte networks were described on the basis of size, connectance, compartmentalization, interaction nestedness, and robustness. We based estimates of network size on species richness and species density (number of plant individuals or ant colonies per 3000 m²). We estimated overall changes in quantitative species interactions on the basis of weighted connectance (WCon),

which we calculated as the linkage density divided by the number of species in the network (Tylianakis et al. 2007). Due to the small size of most networks, mainly on islands, WCon could not be run at site level. Thus, we estimated connectance with the community allometry approach (Fonseca & John 1996) by fitting a power function between the number of realized interactions and the number of possible interactions ($I_r = aI_p^b$), a and b being empirical parameters. Then, we used the power function residuals as a measure of connectance, which is fully independent of community size (hereafter standardized connectance).

We estimated compartmentalization with the modularity maximization index (M) (Guimerà & Amaral 2005), which is widely used to identify compartments in ecological networks (Bascompte & Jordano 2007; Thébaud & Fontaine 2010). Here, we characterized one compartment on the basis of a sub-group of ant and plant species that were more strongly connected among themselves than with species outside the compartment. We used the

program Netcarto (kindly made available by R. Guimerá) that produces M values that vary from 0 (nonmodular) to 1 (highly modular). We built a bipartite null model to test the significance of M for each habitat network by randomly reallocating the interactions on the $m \times n$ matrix, while maintaining marginal totals, and to produce a set of simulated M_{rand} values (100 iterations).

We estimated interaction nestedness with the quantitative index WNODF (NODF-program [Almeida-Neto & Ulrich 2011]). We sorted each network by species interaction frequency, and obtained WNODF statistical significance with the null-model rc in the NODF program (1000 iterations). The WNODF index ranges from zero (non-nested) to 100 (highly nested). At the site-network level, when ant-plant interactions were absent or only one interaction was recorded in a single site, we assumed WNODF equaled zero.

We estimated robustness (R) of the 3 large-scale habitat networks as the number of secondary extinctions when plants, ants, or both were removed from the community (i.e., primary extinctions) under the least-to-most abundant scenario (Burgos et al. 2007; Dormann et al. 2009). Robustness estimates the area below the extinction curve, which represents the proportion of species that still exist after primary extinctions. A robustness index of 0.5 meant that for each species removed, one secondary extinction (i.e., species that disappeared from the community due to the lack of interactions with its associated partners following primary extinctions) occurred. Thus, R values close to 1 indicate a robust system, whereas R values close to 0 point toward a fragile network structure. We compared across-network robustness with relative robustness (R^*) (Bascompte et al. 2003), defined as $R^* = (R - R_r)/R_r$, where R and R_r are the robustness of observed data and the average robustness of the null model, respectively.

Statistical Analyses

At the habitat-network level, we used z scores to compare the significance of the observed results of WCon and robustness with 1000 simulated values generated by the null model proposed by Vazquez et al. (2007). The null model was conservative because it constrained connectance and marginal totals with values from the original matrix. At the site-network level, we used sites as replicates in all analyses. We applied analyses of deviance with binomial errors to estimate the proportion of unoccupied plants and opportunistic ants among habitats. We tested differences among habitats in species richness, species density, connectance, modularity, and interaction nestedness with one-way analysis of variance. Where appropriate, species richness and density were included as covariants. We tested landscape metric effects on modularity, connectance, species richness, and density with multiple regressions. We performed statistical analyses

in Systat 11 and R (R Development Core Team 2012). Moran's I test did not detect significant spatial autocorrelation among island metrics, density, and richness of mutualistic partners and network parameters.

Results

Network Size

At the landscape level, 13 myrmecophytes, 16 mutualistic ant species, and 11 opportunistic ant species were recorded in the 31 sites and formed a general network containing 42 different ant-plant interactions. This was the product of an intense sampling effort in which 424 interactions, 519 host plants, and 424 ant colonies were identified. Plants without an ant colony made up 16.7% of the records.

Myrmecophyte and ant species richness was higher in undisturbed forest than at lake edges and on islands (Table 1). Overall, islands had 8 of the 11 myrmecophytes and 7 of the 15 mutualistic ant species recorded in the forest. At lake edge and island sites 3 and 8 opportunistic ant species were found, respectively. Mean richness of ants and plants was lower in disturbed habitats than in undisturbed forest. Similarly, density of ant colonies and host plants was lower in disturbed habitats than in undisturbed forest (Fig. 2). The proportion of unoccupied plants on islands (34.4%) was almost double that in undisturbed forests (18%) and much higher than on lake edges (6.6%; $F = 13.94$, $p < 0.001$). Despite the presence of the opportunistic ant species, the ant-myrmecophyte community in disturbed areas remained as nested subsets of the larger networks naturally found on undisturbed forest (plants: NODF = 62.66, $p < 0.01$; ants: 45.54, $p < 0.01$; ant-plant interactions: 32.37, $p < 0.01$).

Network Structure

The ant-myrmecophyte network of the undisturbed forest was, as expected, highly compartmentalized ($z = 3.5$, $p < 0.05$); there were 6 well-defined compartments completely isolated from each other (Fig. 3a). The lake-edge network lost the compartmentalized structure ($z = 1$, not significant) due to the loss of mutualistic partners and the presence of opportunistic ants that act as network connectors (Fig. 3b). The island network had the lowest compartmentalization value ($z = 1.5$, not significant) (Fig. 3c). At the site level, compartmentalization was also lower on islands than at lake edges and in undisturbed forests ($F_{2,27} = 3.84$, $p = 0.03$) (Table 1).

No sign of interaction nestedness was detected in the ant-myrmecophyte networks. For all habitats, the observed WNODF values were at least 5 times lower than expected by the null models (Table 1). At the site level,

Table 1. Ant–myrmecophyte network structure in undisturbed forest, at lake edges, and on islands.

	Habitat			Site ^a		
	forest	lake edge	island	forest (n = 5)	lake edge (n = 6)	island (n = 20)
No. myrmecophyte species	11	10	8	6.0 (1.07)a	5.0 (0.84)a	2.8 (0.32)b
No. mutualistic ant species	15	8	7	5.67 (1.02)a	4.0 (0.76)ab	2.56 (0.31)bc
No. opportunistic ant species	0	3	8	0	0.8 (0.37)	0.58 (0.22)
Unoccupied plants (%)	18	6.6	34.4	0.55 (1.29)a	0.8 (0.37)b	2.45 (0.56)b
No. ant–plant interactions	189	97	138	31.5 (6.8)a	19.4 (7.24)a	6.9 (1.31)b
Interaction diversity	21	19	25	7.67 (1.43)a	6.8 (1.85)ab	3.25 (0.55)bc
Connectance ^b	0.12 (0.09 [0.02])	0.16 (0.12 [0.14])	0.13 (0.14 [0.01])	-0.31 (0.39)	0.14 (0.42)	-0.02 (0.13)
Compartmentalization (<i>M</i>) ^c	0.78 (0.71[0.02])	0.64 (0.62 [0.02])	0.55 (0.52 [0.02])	0.64 (0.04)ab	0.57 (0.05)bc	0.37 (0.06)c
Number of compartments	6	4	4	3.5 (0.49)a	3.6 (0.25)a	2.1 (0.29)b
Interaction nestedness WNODF ^d	5.62 (40.02)	7.5 (36.66)	7.77 (33.89)	5.72 (1.89)ab	10.65 (5.87)b	1.21 (0.87)ac

^aValues in site columns are the mean and SE of collected data. Means followed by different letters in the same line are significantly different, according to Tukey post hoc test ($p < 0.05$).

^bValues in parentheses in habitat columns are mean and SD, respectively, of the null model.

^cIndex of Modularity, which is used to estimate whether the network is organized in distinct subgroups of interacting species (compartments). Values in parentheses in habitat columns are mean and SD, respectively, of the null models.

^dValues in parentheses in habitat columns are the mean of the null model.

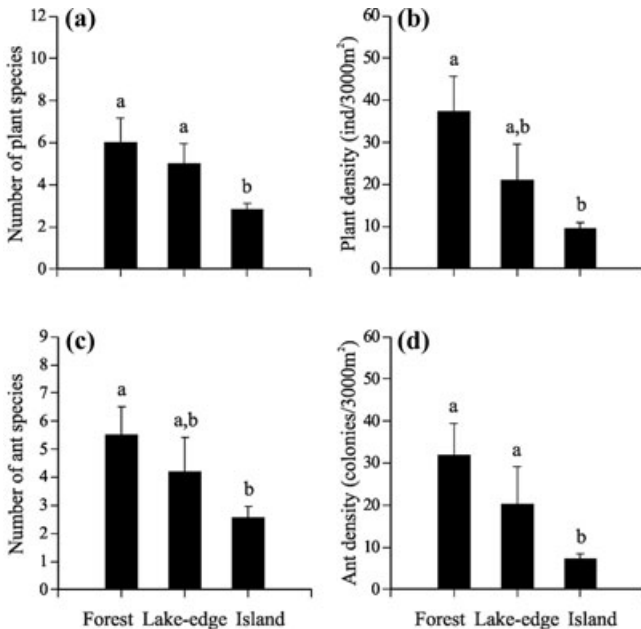


Figure 2. Differences in (a) plant species richness, (b) plant density, (c) ant species richness, and (d) ant density among networks located in undisturbed forests, at lake edges and on forest islands created by the Balbina Dam (vertical lines, 1 SE; different letters indicate significant differences among means [Tukey test, $\alpha = 0.05$]).

island networks were less nested than lake edges and undisturbed forest ($F_{2,27} = 4.88$, $p = 0.02$).

WCon was very low and similar among habitat networks (0.12 – 0.16) and did not differ from what was expected by chance ($z_{\text{forest}} = 1.5$; $z_{\text{edge}} = 0.29$; $z_{\text{islands}} = -1$, all nonsignificant) (Table 1). Similarly, at the site

level, standardized connectance among habitats did not differ ($F_{2,28} = 0.57$, $p = 0.58$). Lack of variation in connectance among habitats is explained by the compensatory effect produced by the presence in the disturbed networks of a number of opportunistic ant species. On lake edges, opportunistic ant species were responsible for 15.8% of the interaction diversity and 4.1% of the total interactions. On islands, opportunistic ant species were responsible for 46% of the interaction diversity and 19.7% of the total realized interactions ($F = 26.59$, $p < 0.001$).

Network Island Biogeography

Plant richness was positively related to area and negatively related to isolation ($F_{4,15} = 3.06$, $p = 0.005$, $r^2 = 0.45$). However, these variables were not related when we controlled for plant density, which in turn was strongly determined by area, isolation, and neighborhood ($F_{4,14} = 21.44$, $p < 0.001$, $r^2 = 0.85$). Ant richness was positively affected by area and negatively by isolation ($F_{4,15} = 7.31$, $p = 0.002$, $r^2 = 0.66$). Again, this effect was mediated by ant density, which was in turn affected by area, isolation, and neighborhood ($F_{4,15} = 31.26$, $p < 0.001$, $r^2 = 0.89$). Smaller islands had a higher proportion of unoccupied plants ($t = -3.26$, $p = 0.004$).

Compartmentalization was higher on larger islands than on smaller islands ($t = 4.16$, $p = 0.001$) (Fig. 4a) and higher on less isolated islands than on more isolated islands ($t = -2.73$, $p = 0.015$) (Fig. 4b). Area and isolation were the main determinants of compartmentalization across sites ($F_{2,16} = 12.61$, $p < 0.001$, $r^2 = 0.61$). Connectance, however, was not related to any island metrics.

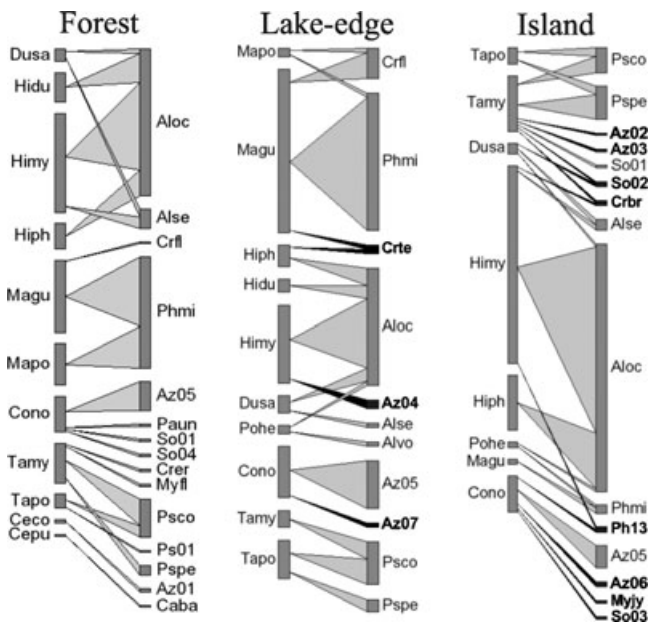


Figure 3. Ant-mycorrhizal networks in the complex of Balbina Dam and Uatumã Biological Reserve pooled over all study sites within the gradient of disturbance: (a) forest, (b) lake edge, and (c) dam-induced forest island (left side of network diagrams, plant species; right side, ant species; bar height, individual species frequency; triangular link widths, frequency of interaction between species; black triangular links and bars, interactions with opportunistic ants; bold, opportunistic ant species; Himy, *Hirtella myrmecophila*; Hiph, *Hirtella physophora*; Hidu, *Hirtella duckei*; Dusa, *Duroia saccifera*; Cono, *Cordia nodosa*; Tamy, *Tachigali myrmecophila*; Tapo, *Tachigali polyphylla*; Magu, *Maieta guianensis*; Mapo, *Maieta poeppigii*; Ceco, *Cecropia concolor*; Cepu, *Cecropia purpurascens*; Pohe, *Pourouma heterophylla*; Aloc, *Allomerus octoarticulatus*; Alse, *Allomerus septemarticulatus*; Alvo, *Allomerus vogeli*; Azt, *Azteca*; Caba, *Camponotus balzanii*; Crbr, *Crematogaster brasiliensis*; Crfl, *Crematogaster flavosensitiva*; Crte, *Crematogaster tenuicula*; Myfl, *Myrmelachista flavocotea*; Myjy, *Myrmelachista cf. joycei*; Paun, - *Pachycondila unidentata*; Phmi, *Pheidole minutula*; Ph13, *Pheidole* sp. 13; Psco, *Pseudomyrmex concolor*; Psni, *Pseudomyrmex nigrescens*; Ps01, *Pseudomyrmex* sp. 1; So01, *Solenopsis* sp. 1; So02, *Solenopsis* sp. 2; So03, *Solenopsis* sp. 3; So04, *Solenopsis* sp. 4).

Network Robustness

Overall, robustness of the ant-mycorrhizal networks varied from $R = 0.56$, when both ants and plants were removed from islands, to $R = 0.82$ when ants were eliminated from undisturbed forest. All habitat networks were

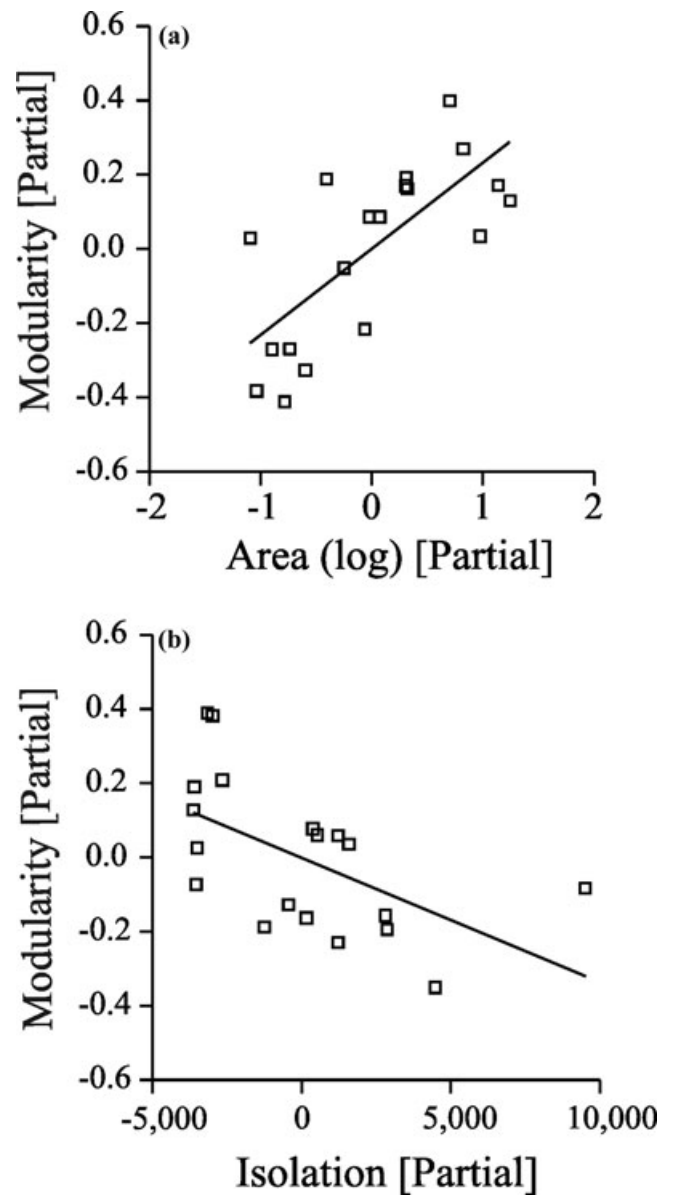


Figure 4. Partial regressions of the effect of (a) log area and (b) isolation on modularity, a measure of network compartmentalization of the ant-mycorrhizal networks found on dam-induced forest islands.

more sensitive to secondary extinctions when plants were eliminated first (Fig. 5). The island network was significantly less robust than the null model when only plants ($z = -4.01$, $p < 0.001$) or only ants were removed ($z = -3.51$, $p < 0.001$), but showed no statistical difference when both were eliminated ($z = -1.27$, $p = 0.102$). In contrast, the networks of undisturbed forest and lake edges were as robust as the simulated networks (Fig. 5).

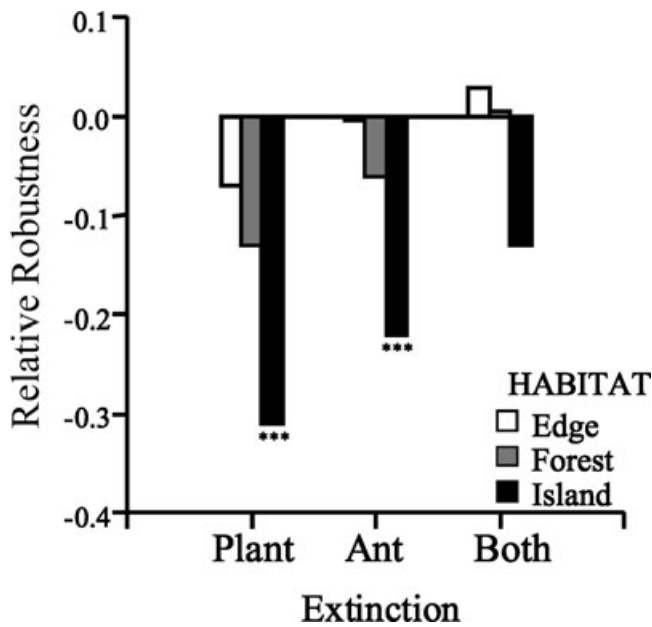


Figure 5. Relative robustness of the ant-myrmecophyte networks across a gradient of disturbance (undisturbed forest, lake edge, and dam-induced forest islands) in the complex of Balbina Dam and Uatumã Biological Reserve (bars, relative robustness of networks when plants, ants, or both were eliminated randomly in simulations; asterisks, significant difference between observed and random networks [$p < 0.0001$]).

Discussion

The Balbina Dam produced a cascade effect in the ant-myrmecophyte mutualistic networks. The structure of networks at disturbed lake edges and on islands differed from network structure in undisturbed forest due to local extinctions of plants and ants, coextinctions, loss of mutualistic interactions, and invasion of opportunistic non-symbiotic ants. Island and lake-edge networks became depauperate nested subsets of undisturbed Amazonian forest networks. Richness and abundance of both plant and mutualistic ants were lower on islands than at lake edges, which suggests a disturbance gradient. Ant-plant interactions were accordingly lower in terms of diversity and quantity. This downsizing situation had a cascade effect on the network structure.

The highly compartmentalized structure of the ant-myrmecophyte network in undisturbed forests vanished from lake edges and forest islands with the dam-induced landscape fragmentation. This structural change occurred mainly due to the loss of compartments and interactions, an increase of unoccupied plants, and domatia colonization by opportunistic ants, which acted as compartment connectors. Loss of compartments occurred due to the local extinction of plants and their

associated mutualistic ant partners. For instance, the inundation of the lowlands caused the local coextinction of *Maieta* populations and their main ant partner, *Pheidole minutula*. The absence of mutualistic ant colonies in many myrmecophytes is likely to have reduced their defenses against herbivores, making their populations more prone to decline, which can be seen as an extinction debt (Kuussaari et al. 2009). Furthermore, the lack of mutualistic ants facilitated colonization by opportunist ant species that, due to their generalist nature, contributed to link previously unconnected compartments.

No sign of nested structure was detected in the ant-myrmecophyte networks. Lack of nested structure is rare in mutualistic interactions; most documented mutualisms are highly nested due to the presence of generalist species connecting the entire community, as with plant-pollinator systems or ants associated with plants bearing extrafloral nectaries (Guimarães et al. 2006; Bascompte & Jordano 2007). In ant-myrmecophyte systems, however, strong ant-ant competition for nesting space leads to the exclusion of generalists (Fonseca 1993, 1999). The high compartmentalization we found in ant-myrmecophyte networks is unusual in small mutualistic communities, considering that it occurs primarily in larger networks with more than one guild, as in plant-seed disperser networks (Mello et al. 2011). The compartmentalization found in ant-myrmecophyte communities, however, is produced by niche conservatism because ants frequently nest on cogenetic host plants (Fonseca & Ganade 1996). Lack of interaction nestedness and high compartmentalization point to coevolutionary processes structuring the ant-myrmecophyte system, which we believe should be conserved.

Connectance was the only network parameter not affected by the dam. We attributed this result to the novel interactions established by the opportunistic ants in the disturbed networks. These ants numerically replaced the extirpated specialized ants. On islands, for instance, 8 specialist ants were extirpated from the system and 8 opportunistic ants were present in the network. However, this substitution was purely numerical because the functional role of the 2 ant guilds is expected to be rather different and may affect the evolution and ecology of the system (Kiers et al. 2010). The presence of novel species has been reported in plant-pollinator networks in fragmented and modified landscapes, where, analogous to our opportunistic ants, highly generalist non-native species entered the system and increased the number of interactions and modified network architecture (Sabatino et al. 2010; Sugiura 2010). Most opportunistic ants entering disturbed networks are probably parasites of mutualisms (Janzen 1975), and their presence can further reduce the chances of colonization by specialized ants due to competition for domatia nesting space, which negatively affects plant fitness in disturbed areas and

thus contributes to population declines of both ants and plants.

The robustness of ant–myrmecophyte networks was affected by the dam-induced disturbance. This effect was apparent even with the conservative approach of the null model selected and the small size of the networks. Islands were particularly fragile to secondary extinctions when only plants or only ants were eliminated. The functional redundancy of ants observed within compartments, where a single plant species usually had higher link diversity than a single ant species, made the system more robust to the loss of ants, as expected due to the asymmetric relation of the ant–myrmecophyte interactions (Fonseca & Ganade 1996). The model in which we assumed random extinction of species produced less-pronounced effects on robustness than the models in which we assumed the removal of plants or ants, but the stronger effect was still on islands.

Thus, one can consider that the compartmentalized structure and the low connectance of the ant–myrmecophyte network conferred some degree of stability to the system. The local extinction scenario from least to most abundant species eliminates, at first, the redundancy within compartments (for instance, rare ant species), and this disturbance does not affect other compartments. In this situation, our results contradict Thébault and Fontaine's (2010) model, which predicts that high compartmentalization and low connectance make mutualistic systems unstable.

Network Island Biogeography

The principles of the theory of island biogeography (MacArthur & Wilson 1967), where area and isolation are the main determinants of colonization and extinction rates in islands, are also consistent with the ant–myrmecophyte network responses to the landscape fragmentation associated with Balbina Dam. Network compartmentalization decreased as island area decreased and isolation increased; the same pattern was observed for ant and plant species richness and density. Our findings are consistent with the recently demonstrated relation between fragment area and species interactions in mutualistic networks of plant-pollinators and extrafloral nectary-bearing plants with its symbiotic ants, after fragmentation and species invasion (Sugiura 2010; Aizen et al. 2012).

The spatially nested communities of myrmecophytes, plant-ants, and interactions at the site level followed similar patterns in which smaller islands constituted subcommunities of the larger islands. Edge-effects, which affect species composition and survival, tended to be more pronounced as fragmentation increases (smaller areas, more isolation) (Laurance et al. 2010). Furthermore, the positive effect of landscape neighborhood on both plant and ant density suggests that island-to-island dispersal is

also important to the colonization-extinction equilibrium in ant–myrmecophyte communities.

Ecological Mechanisms

The impoverishment of the ant–myrmecophyte community on islands seems to be largely a product of dispersal limitation and low survival rates. Most myrmecophytes are small-seeded, ornithochorous, understory plants dispersed by small-bodied secretive birds that may be reluctant to cross large water bodies. The only canopy tree in the community (*Tachigali* sp.) has anemochoric single-seeded fruits that can disperse up to 40 m, but this is still insufficient to reach most Balbina islands (Kitagima & Augspurger 1989). Bat-dispersed *Cecropia* spp. propagules could reach distant islands, although *Cecropia* were completely absent from islands, which may be due to the absence of the mutualistic services provided by their ant partners.

Plants and their protective ant partners are failing to disperse and maintain viable populations in fragmented landscapes, as illustrated by the fact that one-third of the myrmecophytes on islands were unoccupied. Moreover, smaller islands had more unoccupied plants than larger ones. Although we lacked good information on plant-ant dispersal, evidence suggests that queen dispersal can be fairly limited (Vasconcelos 1993; Bruna et al. 2011). Colonization failure may negatively affect plant populations; experimental evidence shows that many Amazonian myrmecophytes depend on their ant partners for growth and reproduction (e.g., Vasconcelos 1991; Fonseca 1994; Bruna et al. 2004). For instance, unoccupied *Maieta guianensis* produced 45 times fewer fruits than ant-attended plants (Vasconcelos 1991). Therefore, the absence of protective ants probably led to reduction of plant fitness and population decline in disturbed areas, perhaps generating a cascade effect by disturbing the population of associated pollinators and an increase in herbivore biomass that may benefit from the increase in food availability.

Overall Dam Effects and Conservation of Species Interactions

Direct effect of habitat loss caused by the Balbina Dam can be estimated by multiplying the mean density of myrmecophytes per hectare in undisturbed sites (124.3 plants/ha) by the flooded area (3129 km²). This calculation yields an estimated loss of 38.9 million ant-plant mutualistic systems due to habitat loss. In addition, effects of dams can reach hundreds of meters into the surrounding forest. In Balbina the lake-edge sites differed greatly from undisturbed forest in terms of structure and were located, on average, from 330 to 742 m from the lake margin. This suggests that edge effects created by the artificial lake, such as higher wind incidence and dryness, go farther than reported into fragments with terrestrial matrix (Laurance et al. 2010). Our results

are not consistent with a previous study on ant–myrmecophyte interactions in Central Amazon, where comparisons between 4, 25-years-old 1-ha fragments surrounded by old fields and 4 nearby areas of continuous forests failed to detect significant differences in density and richness of ants and myrmecophytes (Bruna et al. 2005). Although Balbina fragments were more isolated from continuous forests than those from Bruna et al. (2005), such differences in results indicate that fragments surrounded by water bodies are more prone to fragmentation effects than fragments immersed in a terrestrial landscape.

Local fragmentation in Balbina created a complex mosaic of coevolution (Thompson 2005). Each island became a different evolutionary arena, where the number and composition of mutualistic partners was altered. Some populations went without their mutualistic partners, whereas others interacted with new partners or with species whose behavior affected them negatively, which may further shift mutualistic evolutionary trajectories toward antagonisms (Kiers et al. 2010). The maintenance of coevolved ant–myrmecophyte mutualisms depends primarily on the integrity of their natural habitats and surrounding landscape. If high rates of fragmentation in the Amazon remain unchallenged, we predict drastic changes in evolutionary trajectories with accentuated deterioration of coevolved mutualistic networks.

We found that the effect of dams extended over a much larger area than is normally assumed. Landscape fragmentation associated with the dams has a strong potential to eliminate species, disrupt ecological networks, and alter unique evolutionary and coevolutionary pathways. Consequently, at a time when there are proposals to build hundreds of large hydroelectric dams in the Amazon (Fearnside 2006; Finer & Jenkins 2012), we believe governments should design landscape plans and dam mitigation efforts that are based on estimates of habitat loss and that account for the wider spatiotemporal effects of dams we found in our study.

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