

Interactions between repeated fire, nutrients, and insect herbivores affect the recovery of diversity in the southern Amazon

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Abstract Surface fires burn extensive areas of tropical forests each year, altering resource availability, biotic interactions, and, ultimately, plant diversity. In transitional forest between the Brazilian cerrado (savanna) and high stature Amazon forest, we took advantage of a long-term fire experiment to establish a factorial study of the interactions between fire, nutrient availability, and herbivory on early plant regeneration. Overall, five annual burns reduced the number and diversity of regenerating stems. Community composition changed substantially after repeated fires, and species common in the cerrado became more abundant.

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The number of recruits and their diversity were reduced in the burned area, but burned plots closed to herbivores with nitrogen additions had a 14 % increase in recruitment. Diversity of recruits also increased up to 50 % in burned plots when nitrogen was added. Phosphorus additions were related to an increase in species evenness in burned plots open to herbivores. Herbivory reduced seedling survival overall and increased diversity in burned plots when nutrients were added. This last result supports our hypothesis that positive relationships between herbivore presence and diversity would be strongest in treatments that favor herbivory—in this case herbivory was higher in burned plots which were initially lower in diversity. Regenerating seedlings in less diverse plots were likely more apparent to herbivores, enabling increased herbivory and a stronger signal of negative density dependence.

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In contrast, herbivores generally decreased diversity in more species rich unburned plots. Although this study documents complex interactions between repeated burns, nutrients, and herbivory, it is clear that fire initiates a shift in the factors that are most important in determining the diversity and number of recruits. This change may have long-lasting effects as the forest progresses through succession.

Keywords Community composition · Disturbance · Regeneration · Resource availability · Species evenness

Introduction

Tropical forests are extremely rich ecosystems in terms of their biodiversity and carbon stocks (Strassburg et al. 2010). They are, however, increasingly encroached upon by agriculture and pasture (Fearnside 2001; Morton et al. 2006), conversions that could represent a loss of 40 % of the Amazon forest by 2050 (Soares-Filho et al. 2006). At the southern edge of the Amazon, reduced precipitation, more frequent fires, and acidic, well-drained soils create a boundary between tropical forest and cerrado (savanna; Oliveira and Marquis 2002). Fire is naturally important in this region as it helps to maintain the cerrado ecosystem, but, with increasing demands for agricultural and pasture land, fire is used as a tool to remove unwanted biomass, and it often degrades forests via escaped fires (Nepstad et al. 1999). This has increased fire frequencies in the southern Amazon above historical levels, particularly during drought years (Bush et al. 2008). These human impacts and a lack of protected areas combine to place the seasonally-dry forest of the southern Amazon among the most vulnerable of the Amazonian ecoregions (Soares-Filho et al. 2006), particularly because predictions for this area suggest temperature will increase and precipitation will decrease—a combination that will result in increased fire potential (Malhi et al. 2008). The capacity for forest recovery after fire is therefore an important topic for research and conservation in this area.

Multiple studies from other tropical forests have examined the effects of fire on the recovery of species composition and plant biomass, and they converge on the finding that tree diversity is negatively affected by fire (Cleary and Priadjati 2005; Dezzeo et al. 2008). For example, in the eastern Amazon, recurrent fires reduced the numbers of saplings and lianas while increasing the colonization rates of pioneer species, altering forest structure and composition (Cochrane and Schulze 1999; Barlow and Peres 2008). In addition, fire can reduce

species diversity and alter the species composition in favor of root-sprouting species (Saha and Howe 2003). Densities of sapling and adult stems can recover relatively quickly after fire (within 6.5 years in Indonesia), but stand biomass and species evenness are less resilient because of the early dominance of pioneer species (Slik et al. 2008). So, although stem density can start to recover within a few years of fire, species composition lags behind, and a loss of diversity or change in species composition can affect forest integrity and function (Lewis 2009). Repeated fires can have even more dramatic effects on the composition of the regenerating plant community. For example, after two el Niño-related fires in the Amazon, species relying on abiotic seed dispersal were more common than in once burned forest, which had more species with seeds commonly dispersed by birds (Barlow and Peres 2004).

Here, we examine how repeated annual fires and associated ecosystem changes interact to affect this decline in tree and liana diversity. Among the many factors hypothesized to affect plant diversity, in this work we investigate the roles of disturbance (fire; Grime 1973; Bongers et al. 2009), resource availability (soil nutrients; Stevens and Carson 2002; Bartels and Chen 2010), and herbivory (Janzen 1970; Connell 1971; reviewed in Carson et al. 2008). This is the first experiment that jointly analyzes all these factors to evaluate how human-created disturbance, an annual fire regime, interacts with nutrient availability and herbivory to affect the diversity and species composition of seedling regeneration.

Nutrient availability is important to study in conjunction with fire because fire changes soil nutrient content; even low intensity fires volatilize large amounts of nitrogen, the least stable nutrient in tropical dystrophic soils. Less phosphorus, potassium, and calcium are lost with fire, and most of these nutrients are found in ash after a burn (Certini 2005). Nutrient depletion does occur with repeated fires, however, and it is thought that these losses diminish carbon accumulation in sites of forest re-growth (Zarin et al. 2005). Nutrients also affect biodiversity; for example, increased nitrogen availability can lead to decreased species diversity as a few species take advantage of higher resource levels and out-compete their neighbors (Bobbink et al. 2010; Siddique et al. 2010).

Effects of fire can also cascade up to affect herbivory in accordance with the plant vigor hypothesis (Price 1991), which suggests that plants with stronger growth are more attractive to herbivores. For example, increased growth after fire in a cerrado tree species was correlated with a significant increase in gall midge densities (Vieira et al. 1996), and similar results were found for willow resprouts and their grasshopper herbivores after fire (Stein et al.

1992). In the present study site, fire resulted in higher abundances of leaf-cutter ant colonies (*Atta* spp.; Santana Carvalho 2008), potentially leading to an increase in herbivory on regenerating plants.

Interactions between fire, nutrient availability, and herbivory can therefore affect the growth and diversity of regenerating plants. The effect of herbivores on plant diversity is formalized in the Janzen–Connell hypothesis (Janzen 1970; Connell 1971), which suggests that densities of conspecific individuals are limited by specialist herbivores and pathogens that increase diversity by preventing a high concentration of intraspecific regeneration (negative density dependence). Between 1970 and 2003, 53 studies addressed the Janzen–Connell hypothesis, but 40 % of these were on or near Barro Colorado Island, Panamá, and only 7 of the studies considered diversity as a response variable (Carson et al. 2008). Based on these 7 studies, support of the Janzen–Connell hypothesis is equivocal. Recent studies show evidence of negative density dependence (Comita and Hubbell 2009; Chen et al. 2010; Comita et al. 2010), but more studies involving multiple species are needed (Carson et al. 2008; Swamy and Terborgh 2010). Fire can be expected to influence the strength of these herbivore–plant interactions by decreasing plant diversity, effectively making regenerating seedlings more apparent to herbivores. In addition, fire could alter the composition of species in favor of faster-growing pioneers that invest weakly in antiherbivore defenses and are more vulnerable to herbivory. Both these changes, reduced diversity and an increase in less defended seedlings, could result in a strengthening of Janzen–Connell effects in burned forest.

We tested these multiple factors, fire, nutrient availability, and herbivory, in a fully-crossed experiment to examine the interacting effects of these variables on the success and diversity of early regeneration through one dry season. We addressed two sets of hypotheses.

1. In burned plots, nutrient additions will increase survival, the number of recruits, and their diversity due to an amelioration of depleted resources. In contrast, the effects of nutrient additions will have little effect on recruit survival, number of recruits, and diversity in the unburned plots because light may be more limiting than nutrients.
2. Herbivores will decrease survival and the number of recruits, yet increase diversity in both the unburned and burned plots due to negative density dependence (Janzen 1970). We anticipated the effect would be most pronounced in burned plots with nutrient additions because fire and additional nitrogen and/or phosphorus may result in increased plant growth and apparency, thereby favoring herbivores.

Materials and methods

Study site

This work took place on the Fazenda Tanguro (13°04′35.39″S, 52°23′08.85″W), an area of transitional dry forest between the cerrado and Amazon wet forest (sensu Holdridge 1967) in Mato Grosso, Brazil. The Fazenda is a private soy plantation that also contains 50,000 ha of forest. The native forest is lower in stature and diversity than tropical wet forest. Soils are Oxisols, and the water table is at 12–15 m (Balch et al. 2008). Fire naturally maintains the cerrado ecosystem (Coutinho 1982) that lies about 20 km south of the Fazenda Tanguro, and fire is used extensively in regional agriculture as well as to clear forests. Many fires escape into the forest, where they tend to burn the surface litter layer (i.e. they do not become crown fires) and cause tree mortality by exposing the base of the tree to a low-intensity fireline (Brando et al. 2011).

The region experiences a dry season between May and September (Nimer 1989), and more than 95 % of the 1,900 mm annual precipitation falls during the rainy season (F. Bäse, personal communication). The majority of species set fruit between November and January (Carvalho 2011). In 2010, the year this experiment took place, there was an unusually strong drought, and only 1,420 mm of precipitation fell with just 147 mm of precipitation during the dry season. The drought was also accompanied by an increase in fire in the region (Instituto Nacional de Pesquisas Espaciais <http://sigma.cptec.inpe.br/queimadas/queimamensaltotal1.html?id=mm>).

Experimental design

The experimental area is composed of three adjacent 500 × 1,000 m plots running north–south with soy fields bordering the north side. One plot serves as a control, the second has been experimentally burned at the end of the dry season (late August) every 3 years since 2004, and the third has been burned at the end of the dry season every year since 2004, with the exception of 2008. The experiment described here took place in the unburned and the annual burn plots. The annual burn plot had been burned five times when this experiment was initiated. The burn regime was maintained to examine the resilience of the forest to repeated fire and potential savannization in the area (see Balch et al. 2008 for a complete description of the fire treatment). The scale of the burn treatment prohibited plot level replication of the fire, a common limitation of fire studies (van Mantgem et al. 2001; Balch et al. 2008). We dealt with this as carefully as possible with comparisons to the neighboring unburned control plot and by using split-split plot tests to analyze our data.

A fertilization experiment was nested within the fire experiment. In March 2009, 24 plots, each 30×40 m, were established along two rows 500 and 750 m from the forest edge in the large experimental plots, with 12 plots in the unburned area and 12 in the annually burned area. Included in each set of 12 plots were four levels of the fertilization treatment (additions of N, P, N + P, and a control), with three replicate plots for each fertilizer by fire treatment combination (24 plots total). The plots were fertilized with 100 kg N ha^{-1} (urea) and/or 50 kg P ha^{-1} (superphosphate) in April 2009 and October 2009. In February 2010, at the end of the fruiting season, two adjacent 2×1 m plots were established within each of the fertilizer by fire treatment plots (48 plots). One plot of each pair was enclosed with 75-cm-high walls of durable, clear plastic buried 10 cm into the soil and painted with a stripe of glue (*Brunonia Raupenleim Grün*, Hennstedt, Germany) to exclude non-flying herbivores. Light was measured in the herbivore enclosure plots and in the plots open to herbivores with a LI-COR 250 light meter, and the plot walls did not affect the amount of light in the enclosure plots (paired *t* test, $P = 0.3$).

Field methods

Seedlings (including sprouts) less than 2 mm in basal diameter of every woody species in the plots were tagged and identified to species (89.9 % of individuals) or morphospecies (10.1 % of individuals). These new seedlings and sprouts are termed ‘recruits’ throughout the paper. Leaf area and herbivory of each seedling were measured at the time of plot establishment (932 seedlings), and after 1 month (March 2010; 1,058 seedlings), 3 months (May 2010; 902 seedlings), and 6 months (August 2010; 861 seedlings). Leaf area and herbivory were measured with a transparent plastic grid with 0.25-cm^2 cells. For plants with more than 30 leaves, 10 leaves were measured, and their average size was multiplied by the total number of leaves. Percent herbivory was the amount of leaf area consumed by herbivores divided by what the total leaf area would be (including that lost to herbivores) multiplied by 100. When entire leaves were missing but the petiole was still on the stem, the leaf was considered to be consumed by herbivores because most leaves that drop naturally fall with the petiole attached. In these cases, the size of the leaf was estimated based on the size of neighboring intact leaves and was included in percent herbivory calculations. Mortalities were recorded, and the cause of death was noted when possible. Plants with multiple stems were counted as a single individual. Newly regenerating seedlings were tagged and measured at each measurement period. The time period of this study was dictated by the experimental fire regime, and although the study period does not allow

for conclusions regarding long-term changes in diversity, it does allow for the examination of ecological factors acting on early seedling survival.

Data analysis

Seedling herbivory

Log-transformed percent herbivory values at 1 month, 3 months, and 6 months after the experiment was initiated were analyzed at the plot level using split-split-plot repeated measures ANOVA (SAS proc mixed) with fire, fertilizer, the herbivore enclosure treatments, and time as predictors. Time was also included in the ‘repeated’ statement. The fertilizer treatment and the herbivore enclosure treatment nested within the fertilizer treatment were included as random effects. The covariates were the differences between the treatment level means in the burned or unburned areas and plot level means at the beginning of the experiment for height, basal diameter, and leaf area. Models were run using a backward elimination maximum likelihood procedure decreasing in complexity from fully crossed models. Terms were sequentially removed until a significant model was reached. The significance of each model in the hierarchy was determined by subtracting $(-2 \times \log \text{likelihood})_{\text{model b}}$ from $(-2 \times \log \text{likelihood})_{\text{model a}}$; the significance of the result was determined based on a χ^2 distribution with $df = df_{\text{model a}} - df_{\text{model b}}$. The term removed from the model at each step was that which had the lowest *F* value. Interactions between the covariates (initial sizes) and main effects were non-significant, indicating the slopes of the covariates and the common slope were equal to zero throughout the different treatment levels, permitting the use of ANCOVA (following Littell et al. 1996). Analyses were run using proc mixed (SAS/STAT® 9.1), and the code is available as supplementary information. An entrance to an *Atta* sp. nest emerged inside one of the plots closed to herbivores, so this plot was omitted from analyses.

Seedling survival

Survival of individual seedlings after 6 months was tested using stepwise selection logistic regression with survival as a binomial response. Explanatory variables significant at the 0.05 level were kept in the model. Akaike information criteria were used to determine the best fit model, and the Hosmer–Lemeshow goodness of fit test describes the final model fit. Predictor variables entered in the models were fire, fertilizer, herbivore enclosure, seedling height and diameter in February (the beginning of the experiment), herbivory at each measurement period, species richness, and the number of regenerating stems in February. These

variables were included to test the effects of diversity and potential crowding on survival.

Diversity and number of recruits

Recruitment diversity (based on the Shannon–Wiener diversity index, H' ; Simpson's index, D ; and species richness) and the number of recruits 6 months after implementing the herbivore exclosure treatments were analyzed with each plot as a replicate. The Shannon–Wiener index is the most commonly used index in ecological studies, and it describes both abundance and richness and is sensitive to rare species. Simpson's index describes dominance and is less sensitive to rare species (Rad et al. 2009). Values for the Shannon–Wiener index were normally distributed, and the other variables were log-transformed for normality. Split-split-plot analyses of covariance (ANCOVA) were performed following the procedure described above. Mean initial diversity values and the number of recruits at the level of the burn treatment were subtracted from initial plot level diversity or recruit count data for use as covariates (Simpson's index and the numbers of recruits were log-transformed for normality). Interactions between the covariates and the treatments were non-significant except for the initial Simpson's index with fertilizer and the initial number of recruits with fire. These interactions indicate treatment effects from nutrients and fire were impacting Simpson's index and recruitment before the herbivore exclosure treatment was added, so a detailed examination of how diversity and recruitment change with time is presented in the results. The plot closed to herbivores in which the *Atta* sp. nest entrance appeared was excluded from analyses because two individuals died due to *Atta* sp. herbivory, affecting diversity values. All analyses were performed with SAS/STAT[®] 9.1. The split-split-plot analyses for herbivory and diversity were also run as standard ANCOVA with each plot treatment as a main effect, and the results were very similar. We present results of the split-split-plot analyses, however, because they are more appropriate for the experimental design.

Community similarity indices were used to compare community composition between the treatment levels. For these analyses, morphospecies were excluded because they were differentiated only at the plot level, and between plots some morphospecies may be the same. This removed 53 of 628 individuals from the unburned plots, 21 of 98 individuals from the burned plots, 32 of 363 individuals from the plots open to herbivores, and 42 of 384 individuals from the herbivore exclosure plots in August. Because morphospecies had to be removed from the dataset, the adjusted Jaccard abundance index was calculated to compare burned and unburned plots as well as plots with and without herbivore exclosures. This index is based on

species abundance and takes into account rare and unseen species by bootstrapping data 200 times (see Chao et al. 2005a, b, for an explanation of the equations). The Morisita index was calculated to compare community similarity among the four levels of the nutrient treatment because it is designed for comparison of more than two communities. This index gives a global similarity value as well as pairwise comparisons and also accounts for rare and unseen species by bootstrapping 200 times (Chao et al. 2008). These calculations were performed with SPADE software (Chao and Shen 2010).

Results

Regeneration herbivory

Herbivory was higher in the burned plots, and it was effectively limited in the herbivore exclosure plots (fire $F = 7.2$, $df = 1/43.2$, $P = 0.01$; herbivore exclosures $F = 10.2$, $df = 1/42.9$, $P = 0.003$; overall model -2 log likelihood 272.5, AIC 292.5). These effects changed with time because of variation in the plots open to herbivores (herbivory treatment \times time $F = 3.3$, $df = 2/71.6$, $P = 0.04$). In March and August, herbivory in plots open to herbivores was 17.5 and 15.5 %, respectively. In May, however, herbivory in open plots dropped to 10.1 %. In plots closed to herbivores, herbivory averaged 7.4 % through time, ranging from just 6.3 % in May to 8.6 % in August. In the unburned plots, herbivory decreased between March and May, but it was constant in the burned plots. Herbivory increased in both the unburned and the burned plots in August. Averaged over time, herbivory in the plots open to herbivores was 14.4 ± 12.2 % SD, and inside the herbivore exclosures it was 7.4 ± 6.0 % SD. Herbivory in the burn treatment was on average 13.5 ± 10.5 %, and it was 8.9 ± 9.6 % in the unburned area. Nutrient additions did not have significant effects on herbivory. These data are important for understanding the following results regarding the role of herbivores in survival and diversity.

Regeneration survival

Seedling and resprout survival was affected by initial size, herbivory, and plot richness. Smaller seedlings were less likely to survive, as initial height was a strong predictor of mortality at 6 months (maximum likelihood estimate -0.2 , $P < 0.0001$). Herbivory negatively affected survival, and both early herbivory (at the beginning of the experiment; maximum likelihood estimate 0.01, $P = 0.02$) and herbivory measured at 3 months (maximum likelihood

estimate 0.02, $P = 0.01$) increased the probability of mortality at 6 months. Individuals in plots with greater species richness at the beginning of the experiment were less likely to die than individuals in less diverse plots (maximum likelihood estimate -0.08 , $P = 0.006$). The herbivore exclusions were the only significant treatment (maximum likelihood estimate for closed plots 0.4, $P = 0.003$), but the direction of this effect was contrary to expectations. Although the exclusions served to limit herbivory, and herbivory limited survival, the overall result of exclusions was to increase mortality (Hosmer and Lemeshow Goodness-of-Fit Test $\chi^2 = 3.1$, $df = 8$, $P = 0.93$).

Number of recruits and diversity

Overall, the number of recruits and their diversity were significantly reduced by fire. Unburned plots had more than five times the number of seedlings (median 22, ranging from 3 to 97) as burned plots (median 4, ranging from 0 to 15). The interaction between fire, nutrients, and herbivores had important effects on diversity, and the presence of herbivores increased diversity in burned plots with nutrient additions (Fig. 1b, d). The following paragraphs describe these interactions in detail and present results of the Shannon–Wiener index, Simpson's index, species richness, and numbers of regenerating individuals.

Diversity (as described by the Shannon–Wiener index, H') was affected by the four-way interaction between the treatments and the covariate (Table 1). In both burned and unburned areas, H' increased over the course of the experiment when herbivores were excluded and no nutrients were added (significant herbivore exclusion \times nutrient \times initial H' covariate interaction; $\Delta H'$ final–initial burned 0.12, $\Delta H'$ final–initial unburned 0.16; Fig. 1a, b). In contrast, there were large decreases in H' in paired plots open to herbivores ($\Delta H'$ burned -0.30 , $\Delta H'$ unburned -0.29 ; Fig. 1a, b). The greatest decrease in H' ($\Delta H' = -0.54$) was in unburned plots with phosphorus open to herbivores (Fig. 1a, b). This effect was driven by a single plot with a high abundance and survival of the liana, *Hippocratea* sp., and the higher mortality of other species. The lowest diversity at the end of the experiment was in burned plots with N + P (plots open to herbivores: $H' = 0$, $n = 1$ plot with recruits; plots closed to herbivores: $H' = 0.32 \pm 0.26$), and the highest diversity was in unburned plots without herbivores or nutrient additions ($H' = 2.4 \pm 0.16$). H' decreased the least in plots with nitrogen, and it actually increased in burned plots with nitrogen that were open to herbivores ($\Delta H' 0.26$, Fig. 1a, b).

Simpson's index (D) was also affected by interactions between the treatments and initial D (Table 1); lower values of D indicate higher diversity (evenness). Similar to H' , the greatest diversity by this metric was in unburned plots without nutrients or herbivores ($D = 0.08 \pm 0.04$;

Fig. 1d). Also similar to H' , the largest decrease in D (indicating an increase in evenness) was in burned plots open to herbivores with nitrogen additions (ΔD final–initial -0.15 ; Fig. 1c, d). In contrast, in burned plots open to herbivores without nutrients, D increased (evenness declined, $\Delta D 0.11$; Fig. 1c, d). The largest increase in D (decline in evenness) was in unburned plots open to herbivores with nitrogen additions ($\Delta D 0.15$; Fig. 1c, d). Overall then, nitrogen and herbivores together had positive effects on evenness in burned but not unburned plots (significant fire \times herbivore exclusion \times nutrient interaction; Table 1; Fig. 1c, d).

Species richness was affected by the four-way interaction between the treatments and initial species richness (Table 1). Contrary to results of H' and D , herbivores did not increase richness in burned plots (all plots Δ richness final–initial ≤ 0.67 ; Fig. 1e, f). Similar to the other two indices, however, richness decreased more in the presence of herbivores in unburned plots (Δ richness -2.33) than in the burned plots (Δ richness -0.5 , significant fire \times herbivory \times initial richness interaction; Table 1; Fig. 1e, f).

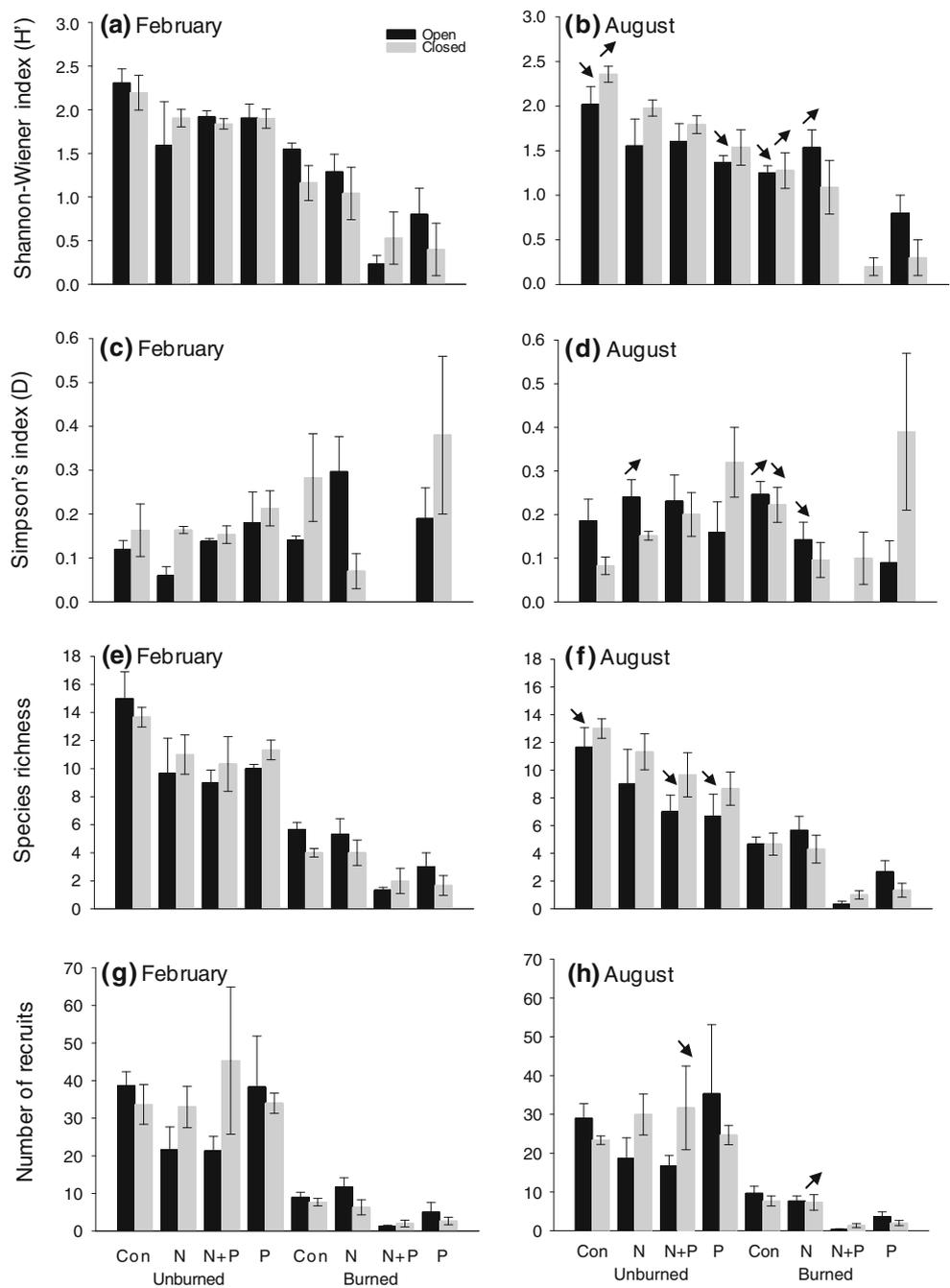
Lastly, the number of recruits was likewise influenced by interactions between the treatments and the initial number of seedlings (Table 1). The number of regenerating individuals decreased the most over time in unburned plots with N + P and without herbivores (survival was significantly reduced in plots closed to herbivores; Fig. 1g, h).

Community composition

The composition of species was most similar between plots with and without herbivores (adjusted Jaccard abundance index 0.93 ± 0.04). The total relative abundance of species present in both levels of the herbivore treatment was almost the same between the plots with herbivores (0.95 ± 0.02) and without herbivores (0.97 ± 0.04).

The adjusted Jaccard abundance index for burned versus unburned communities was 0.68 ± 0.08 . The total relative abundance of species shared between the burned and unburned areas was greater in the burned plots (0.86 ± 0.06), indicating greater richness in the unburned plots (0.76 ± 0.09). Burned plots had 25 species that could be identified beyond 'morphospecies,' and unburned plots had 38 identified species. In the burned plots, the species comprising 5 % or more of the total recruits were *Pouteria ramiflora* (Mart.) Radlk. (Sapotaceae, 5 %), *Myrcia silvatica* (Aubl.) (Myrtaceae, 6 %), *Myrcia multiflora* (Lam.) DC. (8 %), *N. cuspidata* (17 %), and *Hippocratea* sp. (33 %). In the unburned plots, there were only two species making up more than 5 % of the total number of recruits: *N. cuspidata* (12 %) and *Hippocratea* sp. (33 %). *Myrcia multiflora* was only 4 % of the total, *P. ramiflora* was 3 %, and *M. silvatica* was just 0.5 %.

Fig. 1 Treatment effects on various measures of species diversity. Shown are values at the beginning (February) and end (August) of the experiment at the scale of the herbivore exclusion plots: **a, b** Shannon–Weiner index; **c, d** Simpson’s index; **e, f** species richness; **g, h** the number of recruits. The herbivore exclusion treatment was just established in February and is shown for comparison of changes from initial to final values. Bars are means (\pm SE); arrows indicate the direction of changes between initial and final values. Treatment codes are: *Con* control, *N* nitrogen addition, *N + P* nitrogen and phosphorus addition, *P* phosphorus addition. See text for details



The Morisita similarity index for multiple communities was calculated to compare community similarity between plots with different nutrient additions. The overall index was 0.71 ± 0.04 , and the average pairwise comparison between levels was 0.74 . Plots with no nutrients added and plots with nitrogen were the most similar (pairwise comparison 0.96 ± 0.04), and plots with phosphorus or with N + P differed the most (pairwise comparison 0.53 ± 0.08). In plots with no nutrients or with nitrogen, *Hippocratea* sp., *Protium guianense* (Aubl.) (Bursuraceae), and *N. cuspidata* were the most abundant species, but

P. guianense was much less common when phosphorus was added to plots.

Discussion

We hypothesized that interactions among fire, nutrients, and herbivores would affect the number of recruits and the diversity and composition of regeneration, and we found that, while fire had the strongest effect on changes in recruitment, complex interactions between all three

Table 1 ANCOVA results for responses of diversity and recruitment after 6 months to the experimental treatments with initial values as covariates

Treatment	Shannon–Wiener index AIC = 47.4 –2 log likelihood = –12.6			Simpson index AIC = 42.4 –2 log likelihood = –15.6			Species richness AIC = –38.2 –2 log likelihood = –106.2			Number of recruits AIC = 45.9 –2 log likelihood = –10.1		
	F	df	P	F	df	P	F	df	P	F	df	P
Burn	9.7	1/39.8	0.003	1.2	1/40.3	0.3	116.8	1/31.5	<0.0001	5.4	1/47	0.02
Nutrient addition	11.7	3/25	<0.0001	9.0	3/20.2	0.0005	3.4	3/23.8	0.03	23.7	3/47	<0.0001
Herbivore exclosure	27.3	1/38.3	<0.0001	0.2	1/27.8	0.6	2.9	1/21.2	0.1	8.0	1/47	0.007
Burn × nutrients	11.9	2/33.6	0.0001	11.1	2/32.6	0.0002	4.1	3/23.8	0.02	20.8	3/47	<0.0001
Burn × herbivores	16.7	1/38.2	0.0002	20.7	1/35.4	<0.0001	4.6	1/21.2	0.04	12.0	1/47	0.001
Nutrient × herbivores	10.3	3/24.2	0.0001	16.4	3/29.2	<0.0001	5.0	3/16.3	0.01	5.2	3/47	0.003
Burn × nutrients × herbivores	13.4	2/26.5	<0.0001	4.9	3/20	0.01	5.9	3/16.3	0.006	5.3	3/47	0.003
Initial value (plot mean–burn treatment mean)	8.0	1/40.7	0.007	38.5	1/22.8	<0.0001	28.6	1/47	<0.0001	27.6	1/47	<0.0001
Burn × initial value	38.2	1/35.3	<0.0001	1.6	1/40.5	0.2	0.2	1/47	0.6	2.8	1/47	0.1
Nutrients × initial value	9.8	3/36.3	<0.0001	8.8	3/19.7	0.0007	7.1	3/43.8	0.0005	23.7	3/47	<0.0001
Herbivores × initial value	28.1	1/38.1	<0.0001	0.2	1/27.8	0.6	0.08	1/30.2	0.8	8.2	1/47	0.006
Burn × nutrients × initial value	15.4	2/37.6	<0.0001	11.8	2/32.1	0.0001	5.6	3/43.8	0.002	21.1	3/47	<0.0001
Burn × herbivores × initial value	33.2	1/39.5	<0.0001	20.1	1/35.2	<0.0001	6.2	1/30.2	0.02	12.0	1/47	0.001
Nutrients × herbivores × initial value	15.3	3/28.5	<0.0001	16.6	3/29.3	<0.0001	5.4	3/25.8	0.005			
Burn × nutrients × herbivores × initial value	15.2	2/39.5	<0.0001				5.1	3/25.8	0.007			

For the Shannon–Wiener and Simpson's indices and species richness, the split-plot effect of the nutrient treatment was included in the model as a random effect. The split-split-plot herbivory treatment did not improve the model. For the number of recruits, the split-split-plot effect of the herbivore treatment was included in the model as a random effect; the split-plot nutrient treatment did not improve the model

variables structured patterns of regeneration. Negative effects of fire on diversity or community composition are well established in the Amazon, particularly after repeated burns (Cochrane and Schulze 1999; Barlow and Peres 2008), but this is the first study to evaluate how fire interacts with other important abiotic and biotic factors to shape early recruitment. The factorial nature of this experiment allows for understanding how multiple variables that have long been hypothesized to influence diversity in isolation interact to affect forest recovery. While many other variables remain to be studied, as a first step, our results show fire, nutrient availability, and herbivory have strong, interconnected relationships. For example, there were on average five times more individuals and over three times more species in the unburned as compared to the burned area, but the addition of nitrogen ameliorated the negative effect of fire on diversity, and interactions between nitrogen and herbivores were also positive for diversity in the burned area (see results for H' and D).

Species composition was affected by fire and nutrients, and fire increased the number of individuals resprouting as opposed to germinating from seeds (unpublished data). In addition, two of the species most commonly recruiting from seeds in the burned plots are also common in the cerrado, *P. ramiflora* and *M. multiflora* (Oliveira Silva et al. 2002). This suggests that species typically found in the cerrado may regenerate more successfully after repeated fires than forest species. Grasses were not detected in any experimental plots. Plots with nitrogen were more similar to control plots than were plots with phosphorus or N + P. Nitrogen-fertilized plots also had higher species richness than plots with phosphorus or N + P, and diversity increased the most in burned plots when nitrogen was added and herbivores were present. Nitrogen therefore seems to be an important limiting factor in forest recovery after fire, although the effects of nutrients may change with site-specific conditions and growth requirements of individual species (Ceccon et al. 2003).

A major question addressed in this work was the impact of herbivores on plant diversity after fire. We hypothesized herbivores would increase diversity in accordance with Janzen's negative density dependence prediction (1970) and that this result would be strongest in burned plots with nutrient additions because these conditions might favor herbivore host-searching. Negative effects of herbivores and pathogens on recruitment are required to support the Janzen–Connell hypothesis, and such effects have been demonstrated multiple times (Coley 1983; Clark and Clark 1985; Norghauer et al. 2006), although rarely at the community level (Swamy and Terborgh 2010; Alvarez-Loayza and Terborgh 2011). Our application of the Janzen–Connell hypothesis is nontraditional in that we considered effects of all herbivores rather than specialists alone, but

recent work has shown that even generalist herbivores can have important positive effects on diversity, resulting in Janzen–Connell patterns (Dyer et al. 2010). We found herbivory did increase the probability of seedling mortality, and our hypothesis was supported in that herbivores were related to higher diversity in burned plots with nitrogen (higher H' , lower D) or phosphorus additions (lower D). This suggests Janzen–Connell effects may be strongest in disturbed sites, perhaps due to greater herbivore activity or lower initial plant species evenness and diversity in these areas (conditions that could favor specialist herbivores; Barbosa et al. 2009). Abundant nutrients also seem necessary for Janzen–Connell effects to become apparent. These interactions show forest recovery after fire is influenced by changes in both biotic and abiotic factors associated with burning. In the present study, we tested the role of herbivores and nutrients, but future work by this research team will explore the effects of seed availability, canopy opening, and water availability on regeneration.

In conclusion, fire alters the composition and diversity of regeneration, potentially changing the forest even after biomass recovers. Areas undergoing secondary succession in general become more diverse with time, but species composition is slow to recover or converge between sites, even those that are close together (Capers et al. 2005). In our study, diversity and the number of current year recruits decreased over the dry season; for diversity, this pattern was more pronounced in burned plots. We also found species common in the cerrado established more frequently in the forest after fire, demonstrating what may be early stages of savannization if frequent fires continue. Phosphorus and N + P additions may limit the recovery of forest diversity at this site, although nitrogen enhanced diversity. Herbivore damage increased diversity after fire in nitrogen-fertilized plots. This finding supports the hypothesis that herbivores can lead to higher local diversity, and is one of few studies to do so at the community level. Our results describe seedling regeneration through just one fruiting and dry season after five annual fires, and it will be informative to monitor the development of these early patterns over a longer time period. Nonetheless, these data capture a critical period in seedling establishment and describe early processes which influence survival among a common cohort of recruits. Although the importance of these factors may change interannually, it is likely that diversity and community composition of forests with a frequent fire history will be slow to recover.

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