BIG TREES, BIG FALL: LARGE-DIAMETER TREES AND THE FATE OF CARBON STOCKS IN ATLANTIC FOREST REMNANTS

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Abstract: Large trees (*i.e.* ≥ 50 cm diameter) are known for being more susceptible to stress, and its decline in fragmented forests have been reported as an important cause of carbon loss associated with forest degradation. In this paper, we investigate along edge-interior gradients (0-250 m) the distribution, biomass and health indicators (trunk and branch breaks, partial crown death, fungi infection, termites and liana infestation) of live and standing dead Large Trees (LTs; diameter ≥ 50 cm), in five seasonal Atlantic Forests (34 to 690 ha) of northern Paraná state, Brazil. We sampled 118 live LTs in the five forest fragments, where aboveground biomass (AGB) ranged from 22 to 78 Mg/ha, living tree abundance ranged from 8 to 25.6 trees/ha, and standing dead trees ranged from 1 to 4 trees/ha. Larger forest fragments presented more living LTs, which were healthier and contain more biomass than LTs in small forest fragments. We found that edge effects were stronger than size effects for standing dead LT abundance; we found more dead trees up to 200 m from forest edge, independently of fragment size. Almost all living LTs had some health problem. The most frequent health problems were partial crown death, liana and termite infestation. Results suggests also that hard-wooded, slow-growing species tend to be replaced by soft-wooded, gap specialist species, coupled with an overall decline in LT density in small forest fragments, as well as in forests that suffered logging. Long-term prospects for these big carbon stocks are not encouraging, and given the low abundance of LTs in more degraded sites, strategies of intervention in large, century-old individual trees should be considered, such as liana infestation control and edge effect mitigation, in order to avoid further biodiversity and carbon losses.

Keyword: aboveground biomass; tree health; edge effects; fragmentation.

INTRODUCTION

Trees are among the larger organisms of the world; thus, they are also the bigger organisms found in any forest (Clark & Clark 1996, Lindenmayer *et al.* 2012). Definition of large trees (LTs) usually is based in diameter and height, sometimes following habitat-specific basis (Lindenmayer & Laurance 2016). In the Brazilian Atlantic Forest trees with diameter at breast height (DBH) above 50 cm have been considered "large" (Alves *et al.* 2010, D´Albertas *et al.* 2018).

These trees play several ecological roles in forest ecosystems, including influence in hydrological regime (Laurance *et al.* 2000), nutrient (Lindenmayer & Laurance 2016) and carbon storage (Clark & Clark 1996, Slik *et al* 2013, Lindenmayer & Laurance 2016). These trees occur in lower abundance (Clark & Clark 1996) but hold most of the carbon in tropical forests (Lutz *et al.*

2018). In South America, trees with DBH larger than 70 cm may store about 25 % of aboveground biomass, although represent only 1.5 % of trees (Slik *et al.* 2013).

In highly fragmented landscapes LTs suffer higher mortality rate (Laurance *et al.* 2000), for several reasons, including drought, wind turbulence, fire and pathogens, all influenced both by landscape fragmentation and climate change (Lindenmayer & Laurance 2016). LTs are tall and less flexible; thus, they are more prone to breaks at forest edges (Laurance *et al.* 2000). Another consequence of habitat fragmentation is liana overabundance, which can also contribute to the increase in mortality of LTs, manly in the forest edges (Putz 1984, D´Angelo *et al.* 2004, Laurance *et al.* 2008). Liana infestation increases wind drag, mechanical strain and crown breakage (Putz 1984), leading to an increase in the fall of living LTs. In addition, these trees are also more prone to commercial logging, because of higher lumber yield (Lindenmayer *et al.* 2012).

The Brazilian Atlantic Forest is extremely fragmented and the majority of its forest remnants have less than 50 ha (Ribeiro *et al.* 2009). In the lower end of fragment size range, edge effects can be exacerbated, due to a combination of fragmentation effects and increased human disturbance, in many cases leading to lower abundance or absence of LTs (D' Albertas *et al.* 2018). As consequence of all these threats to LTs, biomass loss in the tropical forests may be higher than predicted by models based on vegetation cover only. Research have been suggesting that edge-related carbon loss in Atlantic Forest remnants can reach up to 69 Tg C within ten years (Pütz *et al.* 2014). Assessing liana infestation and other conditions that are related to tree breakage, fall or death, such as the presence of fungi rot or termites, could help to predict tree fate and the extent of future losses (Lindenmayer & Laurance 2016), and help to develop management actions, such as liana control, edge effect mitigation, etc.

In this study, we estimated the abundance and aboveground biomass and recorded health conditions of living LTs in five Atlantic Forest fragments of different sizes and at different edge distances; additionally we recorded the abundance of standing dead LTs, as an indicator of recent mortality. Our goal was to record both the present contribution of LTs to forest structure as well as to foresee, through health diagnosis, future losses. Here we presented a single-time assessment of abundance, biomass and health, tested its relationships with forest fragment size and edge distance, and provided a first record for long term monitoring under the long term ecological research site PELD-MANP. We attempt to answer the following questions: *(i)* Are aboveground biomass and abundance of living LTs lower near fragment edge and in small fragments? *(ii)* Can factors known to threat the health of LTs be more intense at forest edge and in small forest fragments?

MATERIAL AND METHODS

Study site

The study was conducted from January to May 2019 in five seasonal semidecidual forest fragments ranging from 34 to 690 ha, in northern Paraná state, Brazil (Table 1), including a conservation unit, the Mata dos Godoy State Park (PEMG) and four Legal Reserves, situated on private properties. With the exception of PEMG all the other forest fragments suffered some logging in the 1970´s (according field observations and interviews with farmers). In forest fragment sampling was done in permanent plots of the Mata Atlântica do Norte do Paraná (PELD-MANP), a site belonging to the long-term ecological research network of the Brazilian science foundation (CNPq - Programa de Pesquisa Ecológica de Longa Duração - PELD). Altitudes at the studied sites range from 334 to 700 m a.s.l. The Köppen climate classification is humid subtropical (Cfa), the average annual rainfall range from 1400 mm to 1600 mm, and the mean annual temperature ranges from 21 ºC to 22 ºC (Nitche *et al.* 2019). Soils are originated from basalt rock, mostly eutroferric latosols and nitosols (Bhering *et al.* 2007).

Sampling design

Sampling was performed once, continuously along 250 m long, border-to-interior permanent transects. The present sampling is the first record for the planned long term monitoring by PELD-MANP staff. This length of transect was chosen following edge effect reports as being generally up to 100 m from forest edge in Atlantic Forests,

Table 1: Features of seasonal semidecidual Atlantic Forest fragments, in northern Parana state, Brazil. The number of transects are proportional to the fragment size. The PEMG accounting with total area of 2.800 ha when included other fragments and capoeira around.

Site	site code	altitude	fragment size (ha)	transect number (sampled area)	Coordinates
Mata dos Godoy State Park	PEMG	640	670*	5 (2.5 ha)	23°26'46"S, 51°14'46"W
Fazenda Bule	BUL	700	288	3 (1.5 ha)	23°24'19"S, 51°19'31"W
Fazenda Alvorada	ALV	340	128	2 (1.0 ha)	22°49'04"S, 51°11'25"W
Fazenda Congonhas	CGH	340	108	2 (1.0 ha)	22°59'52"S, 50°56'30"W
Fazenda Santo Antônio	SAN	340	34	1 (0.5 ha)	22°56'26"S, 50°57'10"W

thus the adopted transect length likely exceeds the edge-affected region by 150 %. The number of transects distributed in each forest fragment was adjusted to the fragment size, following a design widely used in assessment of tree abundance and biomass (Wekesa *et al.* 2019), totalizing 13 transects. Given differences in the shape of forest fragments, transects were installed in mostly straight edge sections or inside concave angles, avoiding proximity of convex fragment corners were edge effects could be increased by additive effects. All transects were installed in edges adjacent to soybean plantations, avoiding neighborhood of other land uses (Supplementary material, Figure S1). Sampling was done within 10 m from transect central line, resulting in a sample of 5000 m² (0.5 ha) per transect. Transects were divided, for data analysis, into five 1000 m², 50 x 20 m segments, or plots (0-50, 50-100, 100-150, 150-200, 200-250 m), resulting in 65 plots.

All trees (living or standing dead) with diameter at breast height (DBH, at 1.3 m) ≥ 50 cm were individually marked, identified and geo-referenced. When species identification was not possible in the field samples were collected and identified by means of herbarium material and the aid of specialists. For each marked tree both DBH (using a flexible, 1mm precision measuring tape) and total height (using a measuring pole or a Haglöf® clinometer) were estimated. For all tree measures, the Pearson *et al.* (2005) inventory protocol was used.

Aboveground biomass estimation

The aboveground biomass (AGB) of LTs was estimated using DBH, total height and the specific wood density (WD). Wood specific density for 20 species come from an author's locally produced database (Arcanjo 2017) that used wood cores following procedures suggested by Chave *et al.* (2006). For the remaining seven species, data from Global Wood Density Database (Chave *et al.* 2009) were used. For non-identified species, we considered the botanical family average WD (two trees from two morpho-species) or average site WD (three trees from unknown families).

We used the equation from Chave *et al.* (2005) for dry-forest stands, recommended for DBH ranging from 5 to 156 cm:

$$\text{tree biomass} = \exp(-2.187 + 0.916 \times \ln(WD \times DBH^2 \times H)),$$

where *exp* is the *e* constant (approximately 2.7183), ln is the natural logarithm, WD is the specific wood density, DBH (cm) the trunk diameter at breast height, and H (m) is the tree total height.

Tree health assessment

Tree health was assessed using a protocol adapted from protocols developed for urban trees, selecting a total of 11 variables (liana infestation in the crown, partial crown death, liana in stem, stem rot, fungus in stem, stem cavity, termites, roots rot, fungus in roots, cavity in roots and galls) that can affect health or cause death (Tattar 1989, Auer 1996, Schomaker *et al.* 2007).

Each indicator was recorded for each tree using an ordinal score, from zero (absence) to three (maximum indicator score). An "inverted health index (IHI)" was composed by the sum of the scores, ranging from zero to 33; the trees with higher index had worse health conditions. We call this "IHI" because all indicators represent threats to the health, instead of health itself.

Data analysis

We used generalized linear models (GLMs) to test the relationships between fragment size and edge distance (considered as factors) and the response variables: AGB, living and dead tree density (using plots as sampling units) and IHI (using trees as sampling units). We run four models for each response variable: (i) fragment size (FS) and edge distance (ED), (ii) FS and ED with interaction, (iii) FS only and (iv) ED only. Gaussian distribution was used for modelling AGB, and negative binomial was used for living LT density and IHI, which showed overdispersed data, and the Poisson distribution was used for the dead LT density (Zuur *et al.* 2009). Overdispersion was tested using the AER package (Christian & Achim 2008). Given the different number of transects among fragments, transects were included in all models by means of the offset function in the R script, in order to counterbalance such difference (Zuur et al. 2009). Models were selected by means of the Akaike information criterion (AIC). We considered the models with $\Delta AIC \leq 2$ as equally informative (Zuur *et al.* 2009).

Spearman rank correlation was used to evaluate the relationships among the IHI variables. All analyses were carried out in R environment version 3.6.0 (R Core Team 2019). The GLM was done using the package *MASS* (Venables & Ripley 2002).

RESULTS

The aboveground biomass (AGB) ranged from 22 to 78 Mg/ha, averaging 42 Mg/ha among fragments; the higher figure found for an individual plot (transect segment) was 278 Mg/ha and the smaller was zero (supplementary material, Table S1). A total of 118 living trees (DBH ≥ 50 cm), distributed in 32 species from 19 families, were sampled across the five fragments. The abundance of living trees ranged from 8 to 25.6 trees/ha, and of standing dead trees ranged from 1 to 4 trees/ha among transects in the fragments (supplementary material, Table S2). The average fragment Inverse Health Index (IHI) ranged from 4.8 (PEMG) to 9.2 (SAN). DBH for living trees ranged from 50 cm to 149 cm and dead trees ranged from 50 to 76 cm. The AGB and abundance of both living and dead trees increased, and IHI decreased with fragment size; abundance of living trees increased and standing dead tree abundance decreased with edge distance (Figure 1, Table 2).

Most trees had at least one signal of health threat. The average IHI for all trees was 5.2; the lower score was zero and the higher was 14 (out of 33 maximum). The most common health threat observed in the living trees were the partial crown death, and liana infestation, occurring mostly in association. The majority of the standing dead trees (10 of 13 individuals) also had liana infestation. Some of the health indicators were highly correlated, such as stem liana infestation, partial crown death, presence of stem cavity, rotten stem and presence of root cavity (Table 3).

DISCUSSION

We found higher storage of AGB in LTs, higher living LTs abundance and lower IHI in larger fragments. However, we found slightly higher standing dead LTs abundance in large fragments. We also observed more standing dead LTs abundance up to 200 m of edge forest, independently of fragment size. AGB in the four forest fragments that suffered logging decades ago (~ 40 years) was almost constant and were smaller than in PEMG, a protected area never subjected to logging. However, living LT abundance increased almost linearly with fragment size, as well as with forest edge distance.

Logging quickly and strongly decrease tropical forest AGB by removing most LTs (Sist *et al.* 2014). Moreover, logging activities are concentrated in hardwoods, which show higher contribution to AGB than softwoods (Shimamoto *et al.* 2014). Hardwoods are generally long-lived, slow growth and shade tolerant species, the opposite of softwoods (Wright *et al.* 2003, Shimamoto *et al.* 2014). Some fast-growing, soft wooded species can reach large sizes in short time. Thus, several years
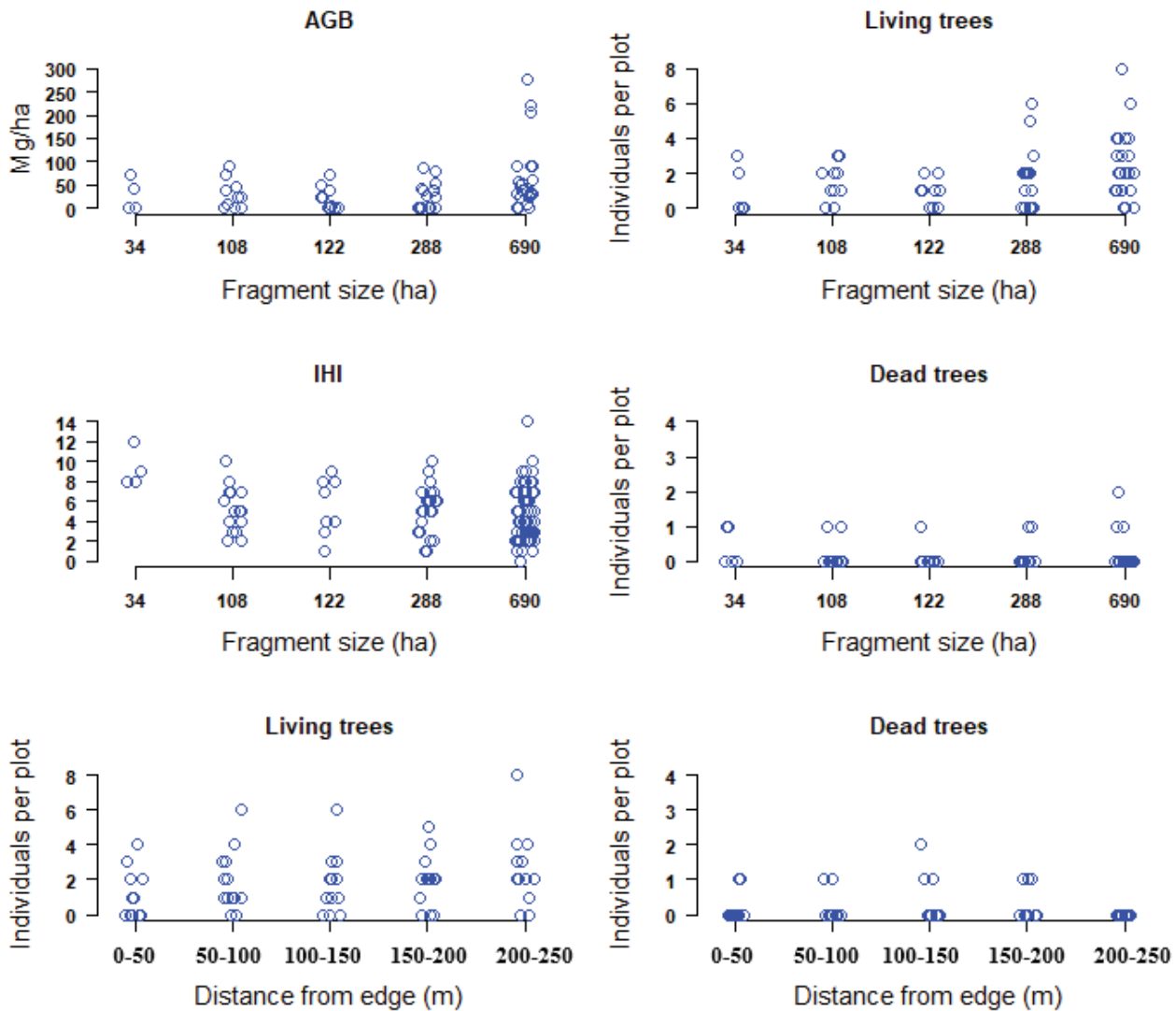
Figure 1. Relationship of aboveground biomass (AGB), living large tree abundance, standing dead tree abundance and inverted health index (IHI) with forest fragment size, living large tree abundance and standing dead tree abundance and distance from forest edge, in 13 0-250 m transects in five seasonal semidecidual Atlantic Forest fragments, northern Paraná State, Brazil. Both AGB (Mg/ha) and tree abundance (number of trees) are values for 500 m² plots (N = 65); IHI is unitless, range from zero to 33, and is reported for each sampled tree (N = 118).

later, logging effect was partially compensated by an increase in density and size of soft wooded, fast-growing species, which replaced the harvested slow-growing, late succession species. However, our results suggest that this same process (recruitment of fast-growing LTs) did not allowed a full recovery of LT abundance in forest fragment edges.

Indeed, the most common species in logged forests and at edges was *Gallesia integrifolia*, which is a shade-intolerant, gap specialist, softwood specie (Wright *et al.* 2003). In another study in the same region, *G. integrifolia* contributed with

several large trees in forest fragments that suffered selective logging decades before (Bianchini *et al.* 2010); it also was observed in higher abundance in the smaller diameter classes (≥ 20 cm) in some of these same forest fragments (ALV, CGH and SAN) (Arcanjo 2017).

However, these soft-for-hard wooded species turnover did not compensate for the effect of fragment size, in neither LT density nor AGB. Records of large (DBH ≥ 50 cm) and very large trees (DBH > 100 cm), regardless of species, has been increasingly rare in small fragments or in forests under strong anthropogenic disturbance (Oliveira

Table 2. Generalized linear models (GLM) built for prediction of the aboveground biomass, abundance of living and dead trees and inverse health condition index for large trees (DBH ≥ 50 cm) along forest edge-interior gradients (from 0 to 250 meters) in five seasonal semidecidual Atlantic Forest fragments, northern Paraná state, Brazil. All models with ∆AIC ≤ 2 were considered equally informative (presented in bold; please see text). FS = Fragment size and ED = Distance from edge.

Variables	∆AIC	df	Weight
Above-ground biomass (Mg/ha)			
~FS	**0.001**	**3**	**0.79**
~FS+ED	2.9	7	0.18
~ED	8.4	6	0.01
~FS *ED	10.0	11	0.01
Living trees abundance			
~FS	**0.001**	**3**	**0.53**
~ED	**0.4**	**6**	**0.43**
~FS*ED	5.4	10	0.03
~FS+ED	14.9	10	0.001
Dead trees abundance			
~ED	**0.001**	**5**	**0.36**
~FS	**0.2**	**2**	**0.32**
~FS+ED	**0.3**	**6**	**0.30**
~FS*ED	6.5	10	0.01
Inverse health condition index			
~FS	**0.001**	**3**	**0.85**
~FS+ED	3.6	7	0.13
~FS*ED	8.0	11	0.02
~ED	30.9	6	0.001

Table 3. Spearman rank correlation coefficient among variables that represent health threats for large trees (DBH ≥ 50 cm) in the seasonal semidecidual Atlantic Forest fragments, northern Paraná state, Brazil. The right and above diagonal contains the p-values and left and lower the value Spearman correlation. See text for variable description. (*) p ≤ 0.05.

Spearman Correlation / p value	Liana in crown	Partial crown death	Liana in stem	Stem rotten	Fungus in stem	Cavity in stem	Termites	Roots rotten	Fungus in roots	Cavity in roots	Galls
Liana in crown	-	0.00*	0.00*	0.10	0.37	0.44	0.27	0.14	0.59	0.63	0.77
Partial crown death	0.55	-	0.00*	0.74	0.17	0.21	0.14	0.55	0.96	0.31	0.62
Liana in stem	0.45	0.43	-	0.46	0.54	0.40	0.24	0.77	0.60	0.05	0.21
Stem rotten	-0.15	-0.03	-0.07	-	0.00*	0.59	0.80	0.06	0.79	0.32	0.04*
Fungus in stem	0.08	0.13	0.06	0.36	-	0.00*	0.00*	0.66	0.80	0.25	0.45
Cavity in stem	0.07	0.12	0.08	0.05	0.33	-	0.02*	0.09	0.77	0.00*	0.37
Termites	0.10	0.14	0.11	0.02	0.27	0.22	-	0.56	0.74	0.05	0.87
Roots rotten	-0.14	-0.06	0.03	0.17	-0.04	0.16	-0.05	-	0.87	0.00*	0.62
Fungus in roots	0.05	0.00	-0.05	-0.02	-0.02	-0.03	-0.03	-0.01	-	0.82	0.00*
Cavity in roots	-0.05	0.09	0.18	0.09	0.11	0.51	0.17	0.45	-0.02	-	0.47
Galls	-0.03	0.05	-0.12	0.19	0.07	-0.08	0.01	-0.04	0.32	-0.07	-

et al. 2008, D´Albertas *et al.* 2018). Moreover, smaller forest fragments show more species and more trees of fast growing, low WD species (see supplementary material Table S3), as reported elsewhere because of fragmentation (Tabarelli *et al.* 1999).

AGB decreased with fragment size, but showed a weak, non-significant positive relationship with distance from edge. As pointed above, this can be explained by the late effect of selective logging, which alter canopy structure and lead to the predominance of early secondary species (Villela *et al.* 2006), decreasing biomass (Philips *et al.* 2019) in the whole forest, rather than only near edges. Indeed, removing PEMG data (where logging never occurred) from the analysis, AGB relationship with forest edge distance is even more weak (data not shown).

Standing dead LT abundance responded to edge distance and to fragment size. Apparently, the main cause of LT death seems to be proliferation of lianas, crown breakage, or both; liana tangles can increase wind drag, causing more breaks, which is corroborated by the high correlation between liana infestation and crown death (r = 0.55, p < 0.001). However, significant edge effects in standing dead LT (DBH ≥ 60 cm) were not reported for forests in Amazonia, where the main cause of death in edges was uprooting of live trees (D' Angelo *et al.* 2004). Nevertheless, the higher abundance of standing dead LT up to 200 m of forest edges reported here might be associated with highest intensity of liana infestation, causing crown death. Only long-term monitoring will ensure proper determination of death causes.

Threats to the health of living LTs were stronger in the smaller forest fragments. Microclimatic stress, wind damage (Laurance *et al.* 2000) and liana infestation (Putz 1984) are the head factors linked to diseases and death of the LTs in tropical forests, especially at the edges (Laurance *et al.* 2000, D' Angelo *et al.* 2004, Smith *et al.* 2018). In the sampled forest fragments liana infestation seems to be the main cause of tree damage. Prolonged liana infestation is known to cause AGB loss (Laurance *et al.* 2013).

Health conditions of living trees at smaller fragments points to further losses in the number of LTs and in forest AGB in the future, and suggests that specific management may be necessary (Lindenmayer & Laurance 2016). Nonetheless, there is a need for long term monitoring of forest trees, and LTs in particular, in order to identify key threats and develop ecosystem management options, aiming to minimize or avoid further losses on these organisms. There are several other organisms that depends on LT, such as orchids and insects that feed on them (Zotarelli *et al.* 2018). Thus, beyond biomass loss, threats to these trees may imply in cascade effects on other species.

AGB and living LTs abundance depended on forest fragment size. Lack of relationship between AGB and edge distance may be related with the past logging events in most of our sampling sites. Threats to the health of LTs was more intense in smaller forest fragments, but not at forest edges. Standing dead large trees, however, was more abundant at edges, which points to the need of long term monitoring to determinate causes of tree death and reconcile such patterns. Long-term prospects for these big carbon stocks are not encouraging, and given the low abundance of LTs in more degraded sites, strategies of intervention in large, century-old individual trees should be considered, such as liana infestation control and edge effect mitigation, in order to avoid further biodiversity and carbon losses.

Here we presented the first large trees inventory and the first tree health assessment for PELD-MANP sites, creating a framework for long-term monitoring. The challenge for next decades, given the joint influences of past logging activities, forest fragmentation and climate change is to use monitoring information to develop management alternatives.

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Supplementary Material: Figure S1. Google Earth image cutouts showing transect allocation (red circles show approximate plot center), in each of the five seasonal semidecidual Atlantic Forest fragments sampled.

Supplementary Material: Figure S2. Residuals analyze plot of diagnostic of glm (generalized linear models) for aboveground biomass, living trees abundance, standing dead trees abundance and inverse health condition index versus fragment size (FS) and distance from edge (ED), with interaction (*) and without (+).

Supplementary Material: Table S1. Aboveground biomass (AGB) of trees with DBH ≥ 50 cm, sampled along edge-interior gradient (250 m), in seasonal Atlantic Forest fragments, Paraná state, Brazil. DBH = diameter at breast height.

Supplementary Material: Table S2. Abundance of living and dead trees (DBH ≥ 50 cm) sampled along edge-interior gradients (250 m) in seasonal Atlantic Forest fragments, Paraná state, Brazil. DBH = diameter at breast height.

Supplementary Material: Table S3. Abundance of tree species with DBH ≥ 50 cm sampled in seasonal Atlantic Forest fragments, Paraná state, Brazil. DBH = diameter at breast height.