

Article

# The Influence of Urbanization and Fire Disturbance on Plant-floral Visitor Mutualistic Networks

Mariana Beal-Neves <sup>1,\*</sup> , Cleusa Vogel Ely <sup>2</sup>, Marjorie Westerhofer Esteves <sup>1</sup>, Betina Blochtein <sup>3</sup> , Regis Alexandre Lahm <sup>4</sup>, Everton L.L. Quadros <sup>4</sup>  and Pedro Maria Abreu Ferreira <sup>1</sup> 

<sup>1</sup> Laboratório de Ecologia de Interações, prédio 12, bloco C, sala 111, Pontifícia Universidade Católica do Rio Grande do Sul, PUCRS, Av. Ipiranga 6681, Porto Alegre, RS 90619-900, Brasil; marjorie.esteves@acad.pucrs.br (M.W.E.); pedro.abreu@pucrs.br (P.M.A.F.)

<sup>2</sup> Laboratório de Estudos em Vegetação Campestre/Grassland Vegetation Lab—UFRGS, Av. Bento Gonçalves, 9500, prédio 43432, Sala 109, Porto Alegre, RS 90650-970, Brasil; cleusavely@gmail.com

<sup>3</sup> Laboratório de Entomologia—Ecologia de Abelhas, sala 123, Museu de Ciências e Tecnologia, PUCRS, Porto Alegre, RS 90619-900, Brasil; betinabl@pucrs.br

<sup>4</sup> Laboratório de Tratamento de Imagens e Geoprocessamento (L.T.I.G), Pontifícia Universidade Católica do Rio Grande do Sul, PUCRS, Av. Ipiranga 6681, Porto Alegre, RS 90619-900, Brasil; lahm@pucrs.br (R.A.L.); everton.quadros@pucrs.br (E.L.L.Q.)

\* Correspondence: mariana.beal@edu.pucrs.br; Tel.: +55-513-353-4063

Received: 10 February 2020; Accepted: 18 March 2020; Published: 3 April 2020



**Abstract:** The biodiversity loss resulting from rising levels of human impacts on ecosystems has been extensively discussed over the last years. The expansion of urban areas promotes drastic ecological changes, especially through fragmentation of natural areas. Natural grassland remnants surrounded by an urban matrix are more likely to undergo disturbance events. Since grassland ecosystems are closely related to disturbances such as fire and grazing, grassland plant communities, pollinators, and their interaction networks may be especially sensitive to urban expansion, because it promotes habitat fragmentation and modifies disturbance regimes. This work evaluated the effect of the level of urbanization and recent history of fire disturbance on grassland plants communities and plant-floral visitor mutualistic networks. We sampled plant communities and floral visitors in 12 grassland sites with different levels of urbanization and time since the last fire event. Sites with higher levels of urbanization showed higher values for plant species richness, floral visitor richness, and network asymmetry. All sampled networks were significantly nested (with one exception), asymmetric, and specialized. In addition, all networks presented more modules than expected by chance. The frequency of fire disturbance events increased with the level of urbanization. Since grassland ecosystems depend on disturbances to maintain their structure and diversity, we inferred that the history of fire disturbance was the mechanism behind the relationship between urbanization and our biological descriptors. Our findings highlight the importance of small and isolated grassland remnants as conservation assets within urban areas, and that the disturbance events that such sites are submitted to may in fact be what maintains their diversity on multiple levels.

**Keywords:** community ecology; habitat structure; landscape context; interaction networks; arthropods; pollination

## 1. Introduction

Studies on the impact of human activities over pollinating faunas have increased in the past few years, mostly focusing on the decrease in abundance and diversity of pollinators [1,2]. Human activities, such as the expansion of urban areas and agricultural frontiers, lead to extensive changes in natural landscapes [3]. Urbanization is likely to grow even further in the future, following the increase

in human population [4]. Habitat reduction and fragmentation is often followed by species loss. The loss of pollinators affects plant–pollinator interactions, promoting cascading effects: once a species is lost, there is a tendency for other species (from the same or other trophic levels) to be lost as well, and the loss of mutualistic interactions leads to serious consequences in ecological and evolutionary processes [5,6].

The mutualistic relationships between plants and animals play a critical role in the dynamics and diversity of communities [7,8]. These relationships can be summarized as complex mutualistic networks of interacting organisms, which present recurrent structural patterns, provide valuable multitaxonomic information, and can be used to unravel ecological patterns and to plan conservation efforts [5,9]. Studies analyzing the structure and dynamics of plant–pollinator networks allowed for novel insights on ecological theory, for example in coevolutionary processes [10,11], and network resilience to disruptions [12,13]. Most mutualistic networks, especially those involving plants and pollinators, show a nested structure, i.e., the more specialist species tend to interact with subsets of species cohesively connected (generalists). In other words, plants with few interactions will only be associated with generalist animals; and, specialized animals will only be found associated to plants with many links [14]. This mechanism reinforces the linkage asymmetry and the persistence of the entire network. If one or few links are lost, the network resists without much modification, which may be an evolutionary advantage [15]. A different (and not mutually exclusive [16]) pattern seen in mutualistic networks is related to the presence of modules of species that interact more (or exclusively) among themselves [17]. In addition, the frequency of interactions (i.e., the degree) of a given species may be largely determined by that given species' abundance in the community [18,19]. In this context, abundant species tend to be generalists, showing a higher degree in comparison with rare species [18,20]. For example, a highly abundant plant species could show a high number of interacting partners because it is easier to find in the landscape, in comparison with a rare plant. However, considering disturbance-prone ecosystems such as grasslands, empirical data that support these assumptions at the community level are still scarce, especially considering the potential role of disturbance as a mediator of the abundance–degree relationship, which is the relationship between the number of species found in the community and the degree of importance that each one presents in the network.

One of the major causes of fragmentation worldwide is urban expansion. It promotes drastic ecological modifications (e.g., conversion of native environments and consequent fragmentation, disruption of ecological systems, and introduction of exotic species) [21,22]. For example, it has been shown that plant–bird networks from urbanized forest areas are structurally different in comparison with natural forests, and are dominated by stronger interactions shaped by invasive species [23,24]. In addition, natural grassland fragments close to urban areas are more likely to undergo frequent disturbance events, such as human-caused fires, in comparison with fragments surrounded by natural vegetation [25,26]. However, grassland ecosystems are deeply linked to disturbance regimes that include grazing and fire [27]. These regimes maintain the high plant species richness and physiognomy of these systems [28,29], which is likely to scale-up in a bottom-up effect of attracting more (or different) interacting partners [30,31]. In fact, plant–pollinator assemblages can be drastically affected by fire events [32], and can show high resilience after fire events [33–36]. There is empirical evidence supporting that the frequency of fire disturbance in natural grasslands is higher in fragments inserted in more urbanized areas in comparison with grasslands surrounded by natural vegetation [37,38]. However, to our knowledge there is no study addressing the influence of these two phenomena (fire and urbanization) on grassland plant–pollinator ecological networks.

Most studies of plant–animal mutualistic networks are concentrated in tropical regions, or in temperate regions from the northern hemisphere [39]. Even with all the diversity of ecosystems present in Brazil, the majority of research on ecological networks comes from forest ecosystems. To our knowledge, only three previous works focused on community-wide, plant–pollinator mutualistic networks from grassland ecosystems in Brazil, none of which were carried out in urban

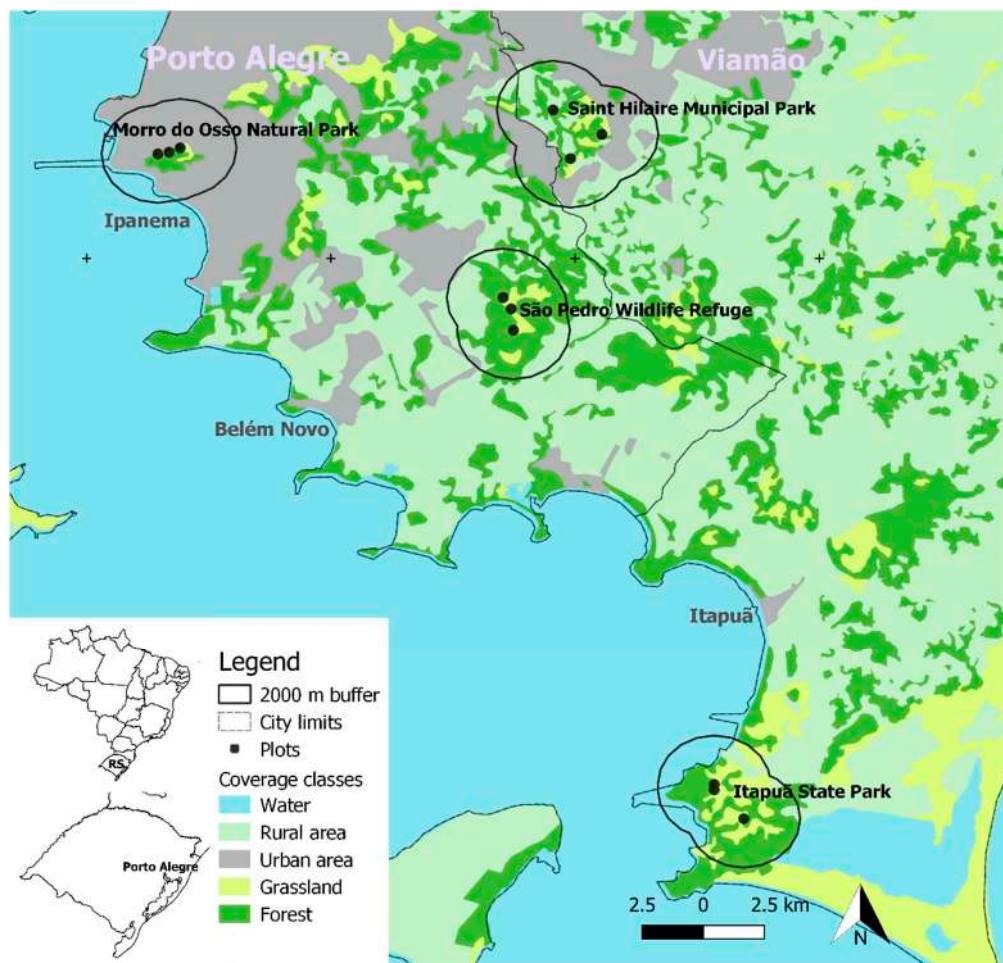
landscapes [40–42]. The South Brazilian grasslands (*Campos Sulinos* grasslands) are a promising system to study animal–plant relationships due to their high diversity and richness in both taxonomic levels [41,43]. Moreover, this ecosystem is not adequately protected under current conservation policies [43], and when protected areas include grassland systems, these policies are usually guided towards the complete removal of disturbances [44].

Here we use natural grassland remnants in an urban matrix as a study system to evaluate the influence of the level of urbanization on grassland plant communities and animal-plant mutualistic networks. We hypothesized that fragments under higher levels of urbanization undergo more frequent fire events, because proximity to urban areas affects susceptibility to this disturbance. By testing this hypothesis, we inferred if the effect of urbanization on biological descriptors was mediated by fire disturbance history. Then, we hypothesized that increasing urbanization (i) negatively influences plant community descriptors (species richness and diversity), (ii) negatively influences species richness of both trophic levels in the networks, (iii) promotes shifts in network metrics (network size, nestedness, modularity, asymmetry, and others; see methods), and (iv) increases the importance of a super-generalist invasive floral visitor in the networks. Additionally, we sampled plant community structure independently from the sampling of interactions, and hypothesized that (v) the number of interactions (degree) of a given plant species is predicted by the overall abundance of the species in the community, and that this prediction pattern is dependent on the level of urbanization.

## 2. Materials and Methods

### 2.1. Sampling Sites

Sampling was conducted from September 2016 to March 2017 in 12 natural grassland fragments (hereafter ‘sites’), in four hills inserted into the urban matrix of the metropolitan region of Porto Alegre, Rio Grande do Sul, Brazil. Each site was composed of a sampling buffer with a 100 m radius (1-hectare). These sites are inserted into four formally protected areas: Morro do Osso Natural Park (MO), Saint’Hilaire Municipal Park (SH), São Pedro Wildlife Refuge (SP), and Itapuã State Park (PI; Figure 1, see Table S1 for UTM coordinates of sites).



**Figure 1.** Sampling sites. Location of the 12 grassland sites/plots (black dots) in each hill: Morro do Osso Natural Park (MO), Saint Hilaire Municipal Park (SH), São Pedro Wildlife Refuge (SP), and Itapua State Park (PI). Black circles enclose 2 km buffer zones used to measure land cover classes (see methods). Coverage classes are presented as water (light blue), rural area (light green), urban area (grey), grassland sites (gold), and forest (dark green).

These sites are inserted into a chain of granitic hills that surrounds the city. This geological unit was shaped in the same event ca. 800 million years ago, and it is known by the presence of several rare and endemic plant species [45,46]. These hills have a long evolutionary history associated with local plant endemism, because they functioned as island refuges during ocean transgression times in the Cenozoic [47,48]. Today, these sites are almost completely isolated by the urban matrix, representing the last remnants of natural vegetation cover in the region. Additionally, all sites have a historical disturbance regime, which comprised cattle grazing until the late 1980s and fire until the present. In the last decades, fire disturbance was frequent, but highly variable between the sites. We estimated the time elapsed since the last fire event (hereafter ‘time since fire’) before our sampling took place for each fragment using satellite images and validating the estimation with personal communication with managers of the conservation units (Table 1).

**Table 1.** Land cover categories and fragment fire history. Land cover estimated in a buffer of 2000 m radius and the time, in months, since the last fire disturbance prior to 2017, in each fragment. Sites ordered by increasing levels of urbanization. (MO) Morro do Osso Natural Park, (SH) Saint’Hilaire Municipal Park, (SP) São Pedro Wildlife Refuge, and (PI) Itapua State Park.

Land Cover Classes	MO1	MO2	MO3	SH3	SH2	SH1	SP3	SP1	SP2	PI2	PI3	PI1
Urban	63.3	58.8	51.6	37.2	35.5	23.0	4.4	3.7	2.7	2.1	2.0	1.5
Grassland	2.9	2.6	2.3	17.7	17.6	17.5	28.6	26.7	25.7	15.6	15.9	18.8
Forest	31.5	30.4	28.9	44.6	46.4	53.6	66.4	68.3	71.1	53.3	54.6	74.0
Water	2.3	8.2	17.3	0.3	0.4	5.9	0.6	1.4	0.6	29.0	27.4	5.7
Plantations	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Time since fire	9	120	3	36	19	120	15	120	17	300	300	300

## 2.2. Plant Community Sampling

In each site, a quali-quantitative sampling of the vegetation was carried out. All plant species present in 1 m × 1 m plots (10 per site, systematically allocated to sample the grassland site evenly) were identified and had their cover value estimated with Londo’s scale [49–51]. Plant community data were pooled in community matrices (one per site), and community descriptors were calculated (see the Statistical Analyses section). All plant species were identified to the species level, and collected specimens were deposited in Museum of Science and Technology of Pontificia Universidade Católica do Rio Grande do Sul (MCT-PUCRS).

## 2.3. Interaction Sampling

Sampling of invertebrate floral visitors was conducted in 1-hectare circular plots in each fragment (200 m minimum distance between plots; license number 53690—ICMBio). Data were collected for at least 2 days per month, during 7 months, in each site, alternating the sampling events between sites of the same hill during the day. We sampled floral visitors in all plant species showing fully opened flowers within each site, using the focal method (10 min of observation per plant; total sampling time 207 h). Floral visitors were collected with entomological nets and kept in bottles with ethyl acetate. All collected specimens were deposited in the MCT-PUCRS. We only included potential pollinators in the analyses (i.e., species that consume floral resources or floral parts and spend time in contact with floral parts). This criterion restricted visitors to the following orders: Hymenoptera, Coleoptera, Diptera, Lepidoptera, Orthoptera, Phasmatodea, and Thysanoptera [52–56]. All floral visitors were separated in morphospecies within each order, and identified to the highest possible taxonomic level using the literature and consulting specialists.

## 2.4. Urbanization Level and Fire Disturbance History

The degree of isolation of each fragment in the urban matrix was calculated as the total urbanized area in a buffer of a 2000 m radius. We also estimated other coverage categories: grassland, forest, water, and plantation (Table 1).

Land cover classes were estimated based on the land use data of the municipality of Porto Alegre available at the Laboratory of Image Treatment and Geoprocessing (LTIG-PUCRS), in which spatial analyses were carried out. For these geoprocessing analyses and map construction, we used QGIS software [57]. We evaluated the collinearity between land cover categories using Pearson’s product-moment correlation. Urban, forest, and grassland land cover showed significant collinearity (urbanization–grassland:  $\rho = -0.694$ ,  $p = 0.012$ ; urbanization–forest:  $\rho = -0.855$ ,  $p < 0.001$ ). Therefore, we only included urban cover (log-transformed values) as the independent variable in the analyses that followed.

## 2.5. Statistical Analyses

All analyses were carried out using the R environment [58]. We calculated plant species diversity (Simpson's 1-D), richness (S) and Chao 1 estimator [59] for plant communities in each site. We also calculated plant species diversity for each site with multiple diversity indexes using the Hill series, which were plotted in diversity profiles [60]. We tested the influence of the level of urbanization on plant community composition using a multivariate analysis of variance with permutation (PERMANOVA: Bray–Curtis distance, with 10,000 permutations restricted within hills) with 'vegan' package [61]. We calculated the relative cover value (RC) of each plant community species, which were also sampled in the interaction networks. These values were used as a measure of species abundance, and were used in tests as predictors of species degree observed in each network.

For network analyses we used packages 'igraph' [62] and 'bipartite' [9,63,64]. We constructed quantitative bipartite animal–plant interaction matrices and calculated independent network metrics for each fragment. We evaluated the following parameters for each network: network size, number of animal and plant species, number of visits, number of links, plant species degree, connectance, network asymmetry, specialization (H2), interaction evenness, nestedness, and the degree of *Apis mellifera*. We evaluated the degree of *A. mellifera* separately, and estimated its relationship with urbanization and network size, because it is an invasive species that may also contribute to the decrease of native pollinators [3], and because it remains unclear how urbanization may influence the impact of this species on plant–pollinator networks [65]. Species degree is number of different species a certain species interacts with; in other words, it is the total number of links of each species in the network [5]. Connectance is the proportion of possible links that were actually observed [9,66]. Network specialization (H2) is a measure based on the deviation of a species' realized number of interactions from what is expected based on the total number of interactions [9,67]. A network is nested when specialists interact with subsets of more generalist species [68]. Interaction evenness is Shannon's evenness index, where links correspond to species and interaction values to their abundance [9]. Network asymmetry is the balance between the numbers of partners in both trophic levels [69]. Modularity is related to the presence of modules, i.e., subsets of species interacting more among themselves than with other species from the same network. We calculated modularity based on the QuanBiMo algorithm [70]. Since this is an optimization algorithm, modularity values may be slightly distinct between single runs, so we considered the observed modularity for each network as the average value obtained from ten runs.

We estimated the significance of each network metric based on comparisons with null models. Based on each of the 12 observed networks (one per fragment), we created 1000 random networks using the 'vaznull' algorithm [19], which reshuffles interactions while maintaining the observed matrix dimensions and connectance. Then we calculated each metric for the set of random networks, generating 95% confidence intervals. Observed values that departed from the confidence interval were considered significant. Since most network metrics are sensible to network dimensions, which make pairwise comparisons difficult, we standardized the observed metrics by calculating the difference between the observed value and the mean value that resulted from the null model, or z-scores, as follows:

$$m_z = \left( \frac{m_{obs} - m_{null}}{\sigma m_{null}} \right) \quad (1)$$

in which  $m_z$  is the corrected metric value,  $m_{obs}$  is the observed value,  $m_{null}$  is the mean value derived from the null model, and  $\sigma m_{null}$  is the standard deviation of the null model [71].

We used linear models (or generalized linear models when data did not meet the assumptions for linear models) to test if the level of urbanization predicted time since fire, vegetation community descriptors (plant species richness and diversity), and network metrics (network size, number of visitors and plant species, connectance, network asymmetry, H2, interaction evenness, nestedness, and *Apis mellifera* network degree). Finally, we tested if the frequency of interactions (species degree) of

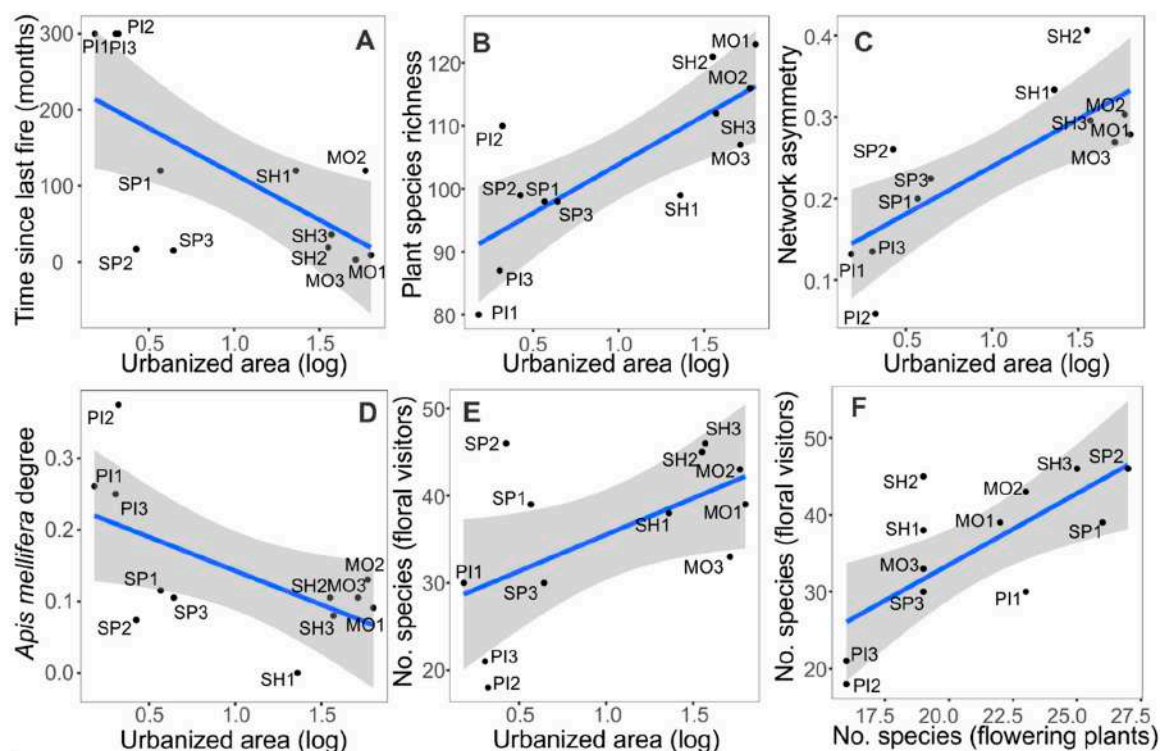
each plant species from the networks was predicted by that given species' abundance in the community, and if this relationship was related to the level of urbanization.

Since the sites we sampled were nested within four isolated hills (three sites per hill; Figure 1), we tested if our results regarding plant communities and network metrics showed spatial autocorrelation using Mantel tests (package 'SYNCSA'; [72]), restricting permutations within hills. For these analyses we constructed a matrix **S** of sites described by UTM coordinates, a matrix **P** of sites described by relative cover of plant species, and a matrix **N** of sites described by standardized network metrics (H2, nestedness, connectance, interaction evenness, and network asymmetry). We removed the remaining metrics from this analysis due to collinearity. Then we computed separate Mantel correlations  $\rho(\mathbf{SP})$ ,  $\rho(\mathbf{SN})$  to test for the congruence between space and vegetation and space and network descriptors.

### 3. Results

#### 3.1. Spatial Autocorrelation, Urbanization, and Fire

We found no significant correlation between space (sites described by UTM coordinates) and plant community descriptors (MANTEL  $\rho = 0.3195$ ,  $p = 0.5281$ ) or between space and network metrics ( $\rho = -0.0280$ ,  $p = 0.9265$ ). The time since fire (i.e., time elapsed since the last fire event prior to our sampling) was predicted by the level of urbanization (GLM  $\beta = -1.244$ ,  $SE = 0.054$ ,  $t = -22.630$ ,  $z = -22.63$ ,  $p < 0.0001$ ; Figure 2A). The level of urbanization and time since fire ranged from 1.5% to 63.3%, and from 3 to 300 months since the last fire event, respectively (Table 1).



**Figure 2.** Relationships between level of urbanization, fire disturbance history, and biological descriptors. (A) Urbanized area as a predictor of fire disturbance history; (B–E) urbanized area as a predictor of the (B) plant species richness, (C) network asymmetry, (D) *Apis mellifera* normalized degree, and (E) number of flower visitors species in the networks, and (F) species richness of flowering plant species as a predictor of species richness of floral visitors.

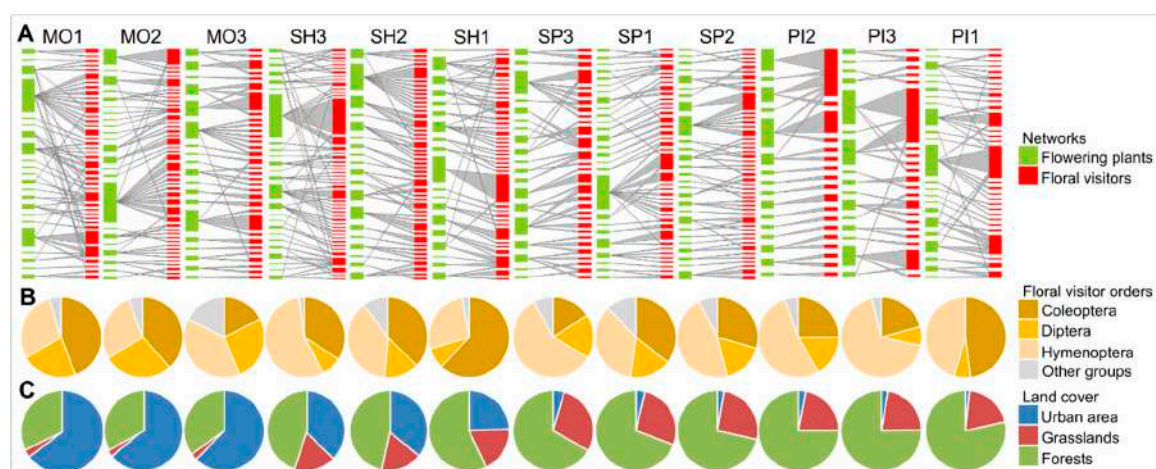
### 3.2. Plant Communities

Plant species richness was predicted by the level of urbanization (LM  $\beta = 15.503$ ,  $t = 3.907$ ,  $p = 0.002$ ,  $R^2 = 0.564$ ; Figure 2B), interestingly in a direct relationship. The level of urbanization showed no significant influence over plant community composition (MANOVA  $R^2 = 0.101$ ,  $F = 1.128$ ,  $p = 0.309$ ), or plant species diversity (LM  $\beta = -0.129$ ,  $t = -1.101$ ,  $p = 0.297$ ,  $R^2 = 0.018$ ).

Plant community sampling resulted in 436 plant taxa, distributed in 53 plant families (see Table S2 for a complete list of plant species and their presence/absence across sites). The only species common to all 12 sites were *Vernonanthura nudiflora* (Asteraceae), *Paspalum plicatulum* (Poaceae), *Evolvulus sericeus* (Convolvulaceae), and *Tibouchina gracilis* (Melastomataceae). Flowering species with the largest contribution to each fragment in terms of relative cover are presented in Table S3. The estimated values for plant species richness (Chao 1) for each sampling site were similar to the observed values, which indicate that our sampling was sufficient (Table S4). The diversity profiles revealed a general pattern of higher diversity in sites with a shorter time since fire, regardless of the diversity index considered (Figure S1).

### 3.3. Interaction Networks

Using the focal method, we sampled 1040 plant individuals distributed in 105 species and 29 families. The total abundance of floral visitors was also 1040 individuals (439 Hymenoptera (42.21%), 383 Coleoptera (36.82%), 157 Diptera (15.09%), 25 Thysanoptera (2.40%), 14 Orthoptera (1.34%), 12 Lepidoptera (1.15%), and 10 Phasmatodea (0.96%)). The relative contribution of insect groups varied between sites. However, the most representative orders were Hymenoptera, Coleoptera, and Diptera in all sites (Figure 3B). See Table S5 for the complete dataset of plant-visitor networks.



**Figure 3.** Structure of mutualistic networks, contribution of flower visitor orders, and land cover in twelve grassland sites. (A) Bipartite plant-floral visitor networks. (B) Relative contribution of orders of floral visitors. (C) Land cover classes in each site. Sites ordered by decreasing levels of urbanization.

The twelve mutualistic networks varied from 16 to 27 plant species, 18 to 46 floral visitor species, 23 to 72 links, and 36 to 70 total visits. Overall network topology varied between sites. All networks showed a nested topology, i.e., presented nestedness values higher than expected by chance, with the exception of SP3 (Figure 3A and Table 2). Networks from all sites were sparsely connected, with modularity and specialization also higher than expected by chance (Figure 3A and Figures S2–S13 and Table 2 for detailed information on the species that pertain to the modules in each network). The observed values of network size, nestedness, connectance, specialization, and modularity were not predicted by the level of urbanization. All networks were also highly asymmetric, and the level of asymmetry was predicted by the level of urbanization (LM  $\beta = 0.116$ ,  $t = 4.041$ ,  $p = 0.002$ ,  $R^2 = 0.582$ ;



Figure 2C and Table 2). The degree of *A. mellifera* showed an inverse relationship with level of urbanization (LM  $\beta = -0.094$ ,  $t = -2.4$ ,  $p = 0.037$ ,  $R^2 = 0.302$ ; Figure 2D and Table 2) and with network size (LM  $\beta = -1905.8$ ,  $t = -2.753$ ,  $p = 0.020$ ,  $R^2 = 0.374$ ; Table 2). Finally, the richness of floral visitors was predicted by the level of urbanization (LM  $\beta = 8.363$ ,  $t = 2.264$ ,  $p = 0.047$ ,  $R^2 = 0.272$ ; Figure 2E) and by the number of flowering plants (LM  $\beta = 1.857$ ,  $t = 3.381$ ,  $p = 0.006$ ,  $R^2 = 0.486$ ; Figure 2F).

Plant species degree (i.e., frequency of interactions) was predicted by species cover in the community (i.e., a proxy for abundance) in only four of the 12 grassland sites (LM MO3  $\beta = 10.417$ ,  $t = 6.759$ ,  $p < 0.0001$ ,  $R^2 = 0.712$ ; SH3  $\beta = 19.976$ ,  $t = 3.413$ ,  $p = 0.002$ ,  $R^2 = 0.307$ ; SP1  $\beta = 45.936$ ,  $t = 3.493$ ,  $p = 0.002$ , Adjusted  $R^2 = 0.309$ ; and SP2  $\beta = 30.744$ ,  $t = 3.567$ ,  $p = 0.002$ ,  $R^2 = 0.310$ ). Sites in which abundance predicted network degree included different levels of urbanization (high urbanization in MO3 and SH3, low urbanization in SP1 and SP2; Table 1), suggesting that the abundance–degree relationship was independent from urbanization.

**Table 2.** Network metrics. Metrics of plant–pollinator networks from 12 natural grassland sites. Values that departed from 95% confidence intervals (CI) are shown in bold. Number of visits represents the sum of interactions in each network. Network size is the total cells of the matrix (the multiplication between the dimensions of the two trophic levels). See Figures S2–S13 for species distributions in the network modules. Sites ordered by decreasing levels of urbanization.

Network Metric	MO1	MO2	MO3	SH3	SH2	SH1	SP3	SP1	SP2	PI2	PI3	PI1
N plant species (richness)	22	23	19	25	19	19	19	26	27	16	16	23
N visitor species (richness)	39	43	33	46	45	38	30	39	46	18	21	30
N visits	102	107	57	170	70	108	63	90	78	36	53	106
N links	62	52	38	72	52	52	41	50	51	23	27	45
Network size	858	989	627	1150	855	722	570	1014	1242	288	336	690
Connectance	0.07	0.05	0.06	0.06	0.06	0.07	0.07	0.05	0.04	0.08	0.08	0.07
Network asymmetry	0.28	0.30	0.27	0.33	0.41	0.30	0.20	0.26	0.22	0.13	0.06	0.14
<i>A. mellifera</i> degree	0.09	0.13	0.11	0.08	0.11	0	0.11	0.12	0.07	0.38	0.25	0.26
Nestedness	<b>7.98</b>	<b>7.19</b>	<b>12.58</b>	<b>7.80</b>	<b>11.22</b>	<b>12.57</b>	11.29	<b>8.49</b>	<b>11.34</b>	<b>17.85</b>	<b>22.00</b>	<b>11.49</b>
high CI	7.88	5.68	10.48	4.51	9.83	10.42	11.78	6.04	6.75	12.68	10.47	6.29
low CI	7.78	5.62	10.36	4.45	9.73	10.29	11.64	5.96	6.67	12.51	10.33	6.21
Nestedness (z-score)	0.06	1.63	1.15	3.63	0.90	1.05	−0.19	2.13	3.76	1.97	5.62	4.05
Specialization (H2)	<b>0.57</b>	<b>0.83</b>	<b>0.78</b>	<b>0.74</b>	<b>0.71</b>	<b>0.76</b>	<b>0.61</b>	<b>0.66</b>	<b>0.75</b>	<b>0.74</b>	<b>0.77</b>	<b>0.57</b>
high CI	0.49	0.66	0.53	0.36	0.62	0.58	0.58	0.55	0.48	0.38	0.48	0.55
low CI	0.49	0.66	0.52	0.35	0.62	0.57	0.57	0.54	0.47	0.37	0.47	0.54
Specialization (H2; z-score)	2.02	2.55	2.86	5.93	1.32	2.99	0.83	2.16	3.37	3.28	2.84	1.64
Interaction evenness	0.58	0.52	0.53	0.47	0.56	0.53	0.56	0.52	0.52	0.50	0.48	0.50
high CI	0.58	0.54	0.57	0.52	0.57	0.55	0.56	0.54	0.56	0.57	0.55	0.52
low CI	0.58	0.54	0.56	0.52	0.57	0.55	0.56	0.54	0.56	0.57	0.55	0.52
Inter. evenness (z-score)	−1.31	−2.39	−3.93	−3.81	−2.05	−2.15	−0.23	−1.79	−4.54	−4.45	−3.93	−1.42
Modularity	<b>0.68</b>	<b>0.78</b>	<b>0.83</b>	<b>0.83</b>	<b>0.83</b>	<b>0.83</b>	<b>0.83</b>	<b>0.83</b>	<b>0.83</b>	<b>0.75</b>	<b>0.70</b>	<b>0.70</b>
high CI	0.63	0.68	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.73	0.64	0.66
low CI	0.59	0.63	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.68	0.60	0.61
Modularity (z-score)	1.72	2.01	0.91	0.91	0.91	0.92	0.92	0.90	0.91	0.93	1.02	0.86
N Modules	15	13	14	12	13	13	9	17	13	14	9	14

## 4. Discussion

In this paper, we evaluated the influence of the level of urbanization surrounding grassland sites on fire disturbance history, plant communities, and on plant-floral visitor mutualistic networks. In our first hypothesis, we tested if sites under higher levels of urbanization underwent more frequent fire disturbance events. We corroborated this hypothesis, since we found a positive relationship between urbanization and time since fire, meaning that more urbanized areas had more recent (therefore more frequent) fire events, which is in accordance with previous findings [37,38]. Land-use changes have global impacts on biodiversity [73], and habitat fragmentation is a major driver of negative effects on biodiversity [74] (but see [75]). However, disturbance, or susceptibility to disturbance, is seldom (if ever) included as an intermediary between fragmentation (in our case, the level of urbanization surrounding natural grassland sites) and biodiversity descriptors. Differently from forest ecosystems, grasslands are deeply linked to disturbances such as fire [23]. Fire disturbances promote high species richness and maintain the physiognomy in grassland plant communities [28,29], which in turn support more higher-level interactions [30,31]. Grassland plant–pollinator networks also show high resilience after fire disturbance events [33–36]. Therefore, our results suggest that fire disturbance history, here measured as time elapsed since the last fire event, was the driving factor behind the relationship between the level of urbanization and biodiversity descriptors. Below we discuss in three separate sections the relationships between the level of fragment urbanization and our biological descriptors: plants, floral visitors, and their interacting networks. In this discussion, we considered that the effects of urbanization on biodiversity were indirect, and mediated by fire disturbance history (although we were aware that the level of urbanization could influence biodiversity descriptors through other mechanisms, which we did not consider in this paper).

### 4.1. Plant Community

We hypothesized that urbanization would negatively affect plant community descriptors, which was not corroborated by our results. On the contrary, plant species richness increased with the level of urbanization (Figure 2B), differently to what would be intuitively expected. However, the level of urbanization showed no significant influence over plant community composition, and no clear relationship with plant species diversity. Therefore, heavily urbanized grassland sites encompassed similar plant community composition and diversity when compared to less urbanized (and presumably better-preserved) sites. The direct relationship between urbanization and plant species richness may be explained by the varying susceptibility of the study sites to human-driven disturbances, in our case, specifically fire. However, “disturbance” in this case cannot be included in the vast array of potential problems posed by habitat fragmentation, because grassland ecosystems are evolutionary related to disturbance regimes that include grazing and fire. These regimes, under appropriate intensity and frequency, maintain the high species richness, diversity, and physiognomy of these systems [28,29]. In our study, all grassland sites were inserted into protected areas, which should minimize anthropogenic influences, prevent fire events, and ultimately affect the disturbance regime as a whole. When this happens, grassland communities change towards a simplified structure with few dominant species, predominantly tussock grasses and shrubs [76–78], coupled with the accumulation of flammable biomass and the colonization of ligneous species [44]. Even though they are all inserted into protected areas, the protection of these sites regarding human intervention was directly linked to their surroundings. While study areas more deeply inserted into the urban matrix (e.g., MO and SH) have recent (<2 years) records of burning events, areas under milder urban pressure such as PI have not recorded a single fire event for 25 years (Table 1). Moreover, sites from the PI area were the only ones that presented a native forest species with relatively high coverage (*Dodonaea viscosa*; see Table S2 for complete flowering species list and also Table S3 for flowering species list with the largest contribution to each fragment), even though these sites had a high proportion of surrounding grassland cover (Figure 3C, Table 1). This is a clear indication that the suppression of disturbance for such a long time is promoting not only grassland biodiversity loss, but also the beginning of a shift towards a different

system, which starts with the encroachment by ligneous species [78]. Conversely, sites that have been subjected to more frequent disturbances due to their proximity to urban areas presented higher plant species richness in comparison with sites that, although surrounded by natural vegetation, had their disturbance regimes alleviated or completely stopped.

#### 4.2. Floral Visitors

We hypothesized that urbanization would negatively affect the richness of floral visitors, which again was not supported by our data. On the contrary, the richness of floral visitors increased with the level of urbanization (Figure 2E, Table 2), and was predicted by the richness of flowering plants in the networks (Figure 2F). Considering our data, the richness of flowering plants is a proxy for the availability of resources in the community when the sampling of floral visitors took place. Grassland sites with less urbanization in their surroundings, which are less prone to human-driven disturbance, showed lower plant species richness, produced fewer plant resources (i.e., less flowering plant species), and, consequently, attracted less species of floral visitors. This pattern may be related to the intermediate disturbance hypothesis [79], which has been extensively used to explain patterns in grassland ecosystems [80,81]. In this sense, fire disturbance maintain plant community richness, which scales-up to the upper trophic level via bottom-up mechanisms [30,31]. Conversely, areas that have been longer without any disturbance lose plant species (as discussed above), which is followed by losses in floral visitors by similar mechanisms.

#### 4.3. Interaction Networks

With the exception of network asymmetry, we rejected the hypothesis that urbanization and the associated fire disturbance history would promote shifts in network metrics. Below we discuss the general structural patterns that these networks presented.

The most representative orders of floral visitors across all networks were Hymenoptera, Coleoptera, and Diptera (Figure 3B), which is in agreement with previous findings considering community networks of floral visitors in grassland ecosystems [41]. Nearly all of our sampled networks presented a nested structure, which also agrees with the recurrent topological pattern described for mutualistic (especially plant–pollinator) networks [17]. In addition, all networks presented modules (Figure 3A and Figures S2–S13 and Table 2), in which subsets of species interacted more with themselves than with other species from the same network [42,70]. However, none of these network metrics was predicted by the level of site urbanization. This indicates that the level of urbanization (and the consequent habitat fragmentation and varying susceptibility to disturbance) does not seem to influence these metrics considering grassland mutualistic plant–pollinator networks, at least regarding the productive South Brazilian grasslands. However, there is evidence that disturbance can influence network metrics in similar, disturbance-prone (although less productive) systems. In plant–pollinator networks from xeric environments in Argentina, freshly-burnt sites showed lower network modularity and presented higher abundances of generalists, in comparison with sites with a longer time since fire [36]. In networks from forest ecosystems, nestedness may increase with urbanization, although network size, modularity, and interaction evenness decrease as landscapes surrounding forests become more urbanized [23]. There is evidence that disturbance (coupled with seasonality) influences metrics in ant–plant networks from disturbance-prone ecosystems in Brazil [82]. Disturbances (including urbanization and fire events) promote different effects on networks from disturbance-prone systems, such as grasslands and xeric environments, in comparison with forests. Our results indicate that network-level metrics of plant–pollinator networks from productive grassland ecosystems may be resistant to the effects of urbanization, as well as to the relaxation of management that we found in less urbanized areas. However, further studies are needed to experimentally test the direct effect of fire disturbance in these metrics.

All networks were highly specialized (Table 2). Assuming that the network-level specialization (H2) is unaffected by network size and shape, and that it depicts biologically meaningful system-specific

differences [83], species from both trophic levels showed high selectiveness regarding their interactions with the opposite trophic level. However, as for the previously discussed metrics, the level of specialization was not explained by the level of fragment urbanization, suggesting that this partner selectiveness, although high in all networks, was independent from the urbanization context and, consequently, of disturbance susceptibility and frequency. High specialization at the network level indicates that interactions are more likely explained by matching of biological traits (or non-matching due to forbidden links; [84]), rather than random partner coupling [67,85], and closer coevolutionary processes in comparison with more nested networks [17,86]. It has been shown that mutualistic networks from temperate zones have higher specialization in comparison with tropical areas [87], which could partly explain our results. In addition, this pattern may also be the product of the geologic and evolutionary isolation of the granitic hills in which the grassland sites are inserted [46,48]. A long history of cyclic isolation, originally due to natural phenomena such as ocean transgression events, artificially maintained today by human-driver factors such as urbanization, could lead to closer evolutionary bonds between interacting pairs of species, in comparison with interaction networks in which there is no such history of isolation. A promising next step is to evaluate the role of functional trait matching in these networks, to unravel the specific mechanisms underlying network specialization.

Interaction networks in forests under higher levels of urbanization present increased nestedness and lower modularity in comparison with less urbanized forests [23]. These authors point out that this difference in network-level metrics are due to the fact that generalist species with invasive behavior become the most connected and central nodes in more urbanized networks. We are not aware of any previous work focused on similar questions in grassland systems. Therefore, our results bring novel evidence that grassland networks may respond in a different manner to urbanization (and the associated disturbances), in comparison with forest ecosystems. Although all but one of our networks presented a nested structure, they also presented modularity values higher than expected by chance. Moreover, the variation of both metrics was not explained by the level of urbanization. As discussed above, urbanization leads to habitat fragmentation, while also increasing fragment susceptibility to disturbances [25,26,37,38]. Although these phenomena (especially disturbances) may promote negative effects in forest systems, they do not seem to drive similar patterns in grassland systems. This contrasting response in comparison with forests is again probably related to the link between the urbanization level and disturbance. Grasslands can show a high resilience to different disturbances [28,43,88]. Moreover, it has been pointed out that grassland systems worldwide may be dependent on anthropogenic disturbances in the long run [81]. All of our sampling sites were inserted into protected areas, in which one of the main conservation goals is to 'protect' the area from disturbances such as fire and grazing [43,44]. However, as we have shown, the proximity to more urbanized areas is related to increased frequency of disturbance by fire. Our results indicate that this increased fire frequency has no influence over most network descriptors as it does in other ecological systems. Moreover, the relaxation of management in the protected areas is actually promoting negative effects over biodiversity (reduced plant and floral visitor species richness in comparison with more recently burned areas).

We found an inverse relationship between the level of urbanization and the importance (network degree) of the invasive European honeybee *Apis mellifera*, i.e., the species is more important in less urbanized sites, and loses importance in heavily urbanized ones, which is the opposite of what we had hypothesized. In addition, the degree of *A. mellifera* was higher in smaller networks. In networks from forest ecosystems, it has been reported that, in urbanized areas, invasive species increase in importance, and can reduce the network size [23,24]. Our results for grassland networks suggest a similar relationship between the degree of an invasive species and network size. It has been shown that *A. mellifera* can promote shifts in the structure of interaction networks, playing a different role in comparison with other (native) equally generalist species [89], and that its introduction may contribute to the overall decrease of native pollinators [3]. Although *A. mellifera* is a widespread species, present in most ecosystems including urban areas [90,91], the relationship between the level

of urbanization/fragmentation of a given natural area and the potential impacts of the species on mutualistic networks remains unclear. We provide evidence that the relative importance of *A. mellifera* in mutualistic networks from grassland ecosystems may be dependent on the level of urbanization, on the directly related disturbance regime, and may influence network size. Sites in which *A. mellifera* showed the highest degree were also the ones where plant diversity descriptors presented the lowest values, which can pose additional threats to these communities due to competitive exclusion of native pollinators. These results emphasize the need for studies addressing how the level of urbanization and disturbance in grassland sites can mediate the influence of invasive species on plant–pollinator networks.

In bipartite networks, asymmetry represents the balance between both trophic levels. In our case, the higher the values for this metric, the higher the floral visitor species/plant species ratio was [69]. As expected, all networks showed positive values for asymmetry, i.e., more floral visitor species in comparison with flowering plant species. Interestingly, network asymmetry was the only network-level metric predicted by the level of urbanization (Figure 2C). This relationship indicates that, the higher the urbanization level of a given site, the higher the asymmetry towards the higher trophic level in that given network. Although species richness of floral visitor was predicted by flowering plant species richness (Figure 2F), neither of these descriptors was independently predicted by level of urbanization. However, a pattern arose when richness of both trophic levels was standardized in the network asymmetry index, suggesting that visitor and plant species richness increase at different rates along the gradient of urbanization (Figure S14). Specialization in mutualistic networks may arise as a response of the higher trophic level to low plant diversity in temperate regions (in comparison with tropical regions), which would also render these systems less tolerant to extinctions [69]. Plant species in mutualistic networks are strongly dependent on animal species, whereas animal species are less dependent on plant species [92]. We provide evidence that these relationships of interdependence between trophic levels are mediated by the level of urbanization and susceptibility to disturbance in productive grassland ecosystems.

Finally, we found that plant species degrees in the networks were not predicted by species abundance in the community for most networks, and that the level of urbanization did not play a role in that relationship. Flowering phenology may be highly variable between and within species. Since plants compete for pollinators, asynchronous flowering may arise to reduce competition [93]. Our results suggest that resource availability (i.e., which individuals presented resources in a given time window), rather than the overall abundance in the community, determined plant species degree for most networks.

## 5. Conclusions

The level of urbanization of grassland sites is an important factor in the assembly of plant–floral visitor networks, because it influences not only plant species richness, but also how many potential pollinator species a given fragment may harbor. Most network metrics were not sensitive to urbanization, as opposed to what has been reported for other ecosystems. The mechanism behind the effect (or the absence of effect) of urbanization on our biological descriptors was related to fire disturbance frequency. Fire disturbance leads to a short-term increase in plant species richness and diversity in grasslands [28,94–96], and these effects scale up to interaction networks descriptors, such as the number of floral visitors and network asymmetry. However, our data showed that most network-level metrics were not responsive to varying frequencies of fire disturbance. Furthermore, the relative importance of *A. mellifera* in mutualistic networks from grassland ecosystems may be dependent on the level of urbanization and on the directly related disturbance regime. We obviously do not aim to use our results to defend that urban expansion has positive effects on plants, floral visitors, and the interaction networks they shape together. However, we did find out that lower levels of urbanization, which related to lower disturbance frequency, presented reduced species richness of plants and floral visitors in comparison with more urbanized and fire disturbance-prone sites. All of our sampling sites were inserted into protected areas, in which management is deliberately removed from all systems,

including grasslands. The unwanted effect of increased fire frequency due to urban proximity in these protected areas showed that management removal or alleviation do not have the expected positive effect, at least not for plants and their floral visitors. Therefore, our results bring further evidence that conservation efforts that target grassland ecosystems in Brazil need to include the disturbance regime in management planning.

Although our data showed a clear relationship between level of urbanization and fire disturbance history, we emphasize the need for future works to assess the role of both drivers independently in order to estimate their relative influence on network features. In addition, we cannot rule out other factors related to habitat fragmentation that could be potential drivers of these patterns, but that we did not take into account here. Finally, our findings highlight the importance of small and isolated grassland remnants as potential conservation assets. Although large continuous areas may be the optimal choice for biodiversity conservation [97,98], smaller areas should also be valued [99], especially in urban environments in which small and isolated natural grassland fragments are the last remnants of the local wildlife. The disturbance events that such sites are submitted to due to the proximity with urban areas, which are often seen as a problem for conservation, in fact seem to be what maintains their diversity on multiple levels, at least considering productive grassland systems.

**Supplementary Materials:** The following are available online at <http://www.mdpi.com/1424-2818/12/4/141/s1>, Figure S1. Diversity profiles of plant communities. It includes the diversity profiles (plant species diversity) with Hill's series for 12 grassland sites with varying time since the last fire event (Time Since Fire), Figures S2–S13. Species distribution of network modules in all sampled networks. It includes two lists, one of plants and one with flower visitors species complete name and the respective code in the modules. Site legends: Morro do Osso Natural Park (MO), Saint'Hilaire Municipal Park (SH), São Pedro Wildlife Refuge (SP) and Itapuã State Park (PI), Figure S14. Relationship between site urbanization, network asymmetry and number of plant and flower visitor species in the networks. Equation parameters: number of flowering plants ( $\beta = 0.351$ ,  $t = 0.197$ ,  $p = 0.848$ ,  $R^2 = -0.095$ ); number of flower visitors ( $\beta = 8.363$ ,  $t = 2.264$ ,  $p = 0.047$ ,  $R^2 = 0.272$ ); network asymmetry ( $\beta = 0.116$ ,  $t = 4.041$ ,  $p = 0.002$ ,  $R^2 = 0.582$ ), Table S1. UTM coordinates of grassland sites sampled, Table S2. Incidence matrix of plant species per grassland site. Sites are ordered by the level of urbanization. Legend of acronyms: NA—not applicable, MO—Morro do Osso Natural Park, SH—Saint'Hilaire Municipal Park, SP—São Pedro Wildlife Refuge, PI—Itapuã State Park. The names of the species were checked through the TNRS (Taxonomic Name Resolution Service) and Flora do Brasil 2020 websites. Boyle, B. et al. 2013. The taxonomic name resolution service: an online tool for automated standardization of plant names. Available in: <http://floradobrasil.jbrj.gov.br/>. Accessed: 11 Sep. 2019, Table S3. Plant species with the largest contribution to each site in terms of relative coverage, Table S4. Observed and estimated (Chao 1) plant species richness, as well as the standard error for the estimation, Table S5. Plant-visitor networks dataset. Interaction data collected in 12 grassland sites ('site') distributed in four hills ('hill'). Each line represents the interaction events (indicated by 'month', 'year', and 'time') between a plant (identity provided in 'plant\_family', 'plant\_species', and 'plant\_code') and a floral visitor (identity provided in 'vis\_order', 'vis\_family', 'vis\_species', and 'vis\_code'). Frequency of the interaction indicated by 'freq'.

**Author Contributions:** Conceptualization and design of the experiment: M.B.-N., P.M.A.F., and B.B.; Project administration: M.B.-N.; Data collection and curation: M.B.-N., P.M.A.F., C.V.E., M.W.E., B.B., E.L.L.Q., and R.A.L.; Investigation (plants): C.V.E. and M.B.-N.; Investigation (arthropods): M.B.-N., M.W.E., and B.B.; Formal analysis: P.M.A.F. and M.B.-N.; Visualization: M.B.-N. and P.M.A.F.; Writing—original draft: M.B.-N. and P.M.A.F.; Writing—review and editing: All authors. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding. The APC was funded by the Pontifícia Universidade Católica do Rio Grande do Sul—PUCRS.

**Acknowledgments:** We gratefully acknowledge CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) and the postgraduate program for the scholarship and financial support to the first author—Finance Code 001. We also acknowledge park managers and rangers who gave all the support they could, despite the operational limitations. The members of Grassland Vegetation Laboratory of UFRGS (Universidade Federal do Rio Grande do Sul), which identified the plant species. Luciano Moura who identified the species and morphospecies of Coleoptera. Rosana Halinski who reviewed the species and morphospecies of bees. Nicole Garcia and Kássia Ramos who assisted in field and laboratory work.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Biesmeijer, J.C.; Roberts, S.P.M.; Reemer, M.; Ohlemüller, R.; Edwards, M.; Peeters, T.; Schaffers, A.P.; Potts, S.G.; Kleukers, R.; Thomas, C.D.; et al. Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. *Science* **2006**, *313*, 351–354. [[CrossRef](#)] [[PubMed](#)]
2. Burkle, L.A.; Marlin, J.C.; Knight, T.M. Plant-pollinator interactions over 120 years: Loss of species, co-occurrence, and function. *Science* **2013**, *339*, 1611–1615. [[CrossRef](#)] [[PubMed](#)]
3. Ahrné, K.; Bengtsson, J.; Elmqvist, T. Bumble bees (*Bombus* spp.) along a gradient of increasing urbanization. *PLoS ONE* **2009**, *4*, e5574. [[CrossRef](#)]
4. McKinney, M.L. Urbanization as a major cause of biotic homogenization. *Biol. Conserv.* **2006**, *127*, 247–260. [[CrossRef](#)]
5. Bascompte, J.; Jordano, P. Plant-animal mutualistic networks: The architecture of biodiversity. *Annu. Rev. Ecol. Evol. Syst.* **2007**, *38*, 567–593. [[CrossRef](#)]
6. Tylianakis, J.M.; Didham, R.K.; Bascompte, J.; Wardle, D.A. Global change and species interactions in terrestrial ecosystems. *Ecol. Lett.* **2008**, *11*, 1351–1363. [[CrossRef](#)]
7. Nabhan, G.P.; Buchmann, S.L. Services provided by pollinators. In *Nature's Services*; Daily, G., Ed.; Island Press: Washington, DC, USA, 1979; pp. 133–150.
8. Jordano, P. Fruits and frugivory. In *Seeds: The Ecology of Regeneration in Natural Plant Communities*; Cabi Publication: Wallingfor, UK, 2000; pp. 125–166.
9. Dormann, C.F.; Fründ, J.; Blüthgen, N.; Gruber, B. Indices, graphs and null models: Analyzing bipartite ecological networks. *Open Ecol. J.* **2009**, *2*, 7–24. [[CrossRef](#)]
10. Armbruster, W.S. Phylogeny and the evolution of plant-animal interactions. *Bioscience* **1992**, *42*, 12–20. [[CrossRef](#)]
11. Fenster, C.B.; Armbruster, W.S.; Wilson, P.; Dudash, M.R.; Thomson, J.D. Pollination syndromes and floral specialization. *Annu. Rev. Ecol. Evol. Syst.* **2004**, *35*, 375–403. [[CrossRef](#)]
12. Bond, W. Do mutualisms matter? Assessing the impact of pollinator and disperser disruption on plant extinction. *Philos. Trans. R. Soc. London. Ser. B Biol. Sci.* **1994**, *344*, 83–90.
13. Traveset, A.; Richardson, D.M. Biological invasions as disruptors of plant reproductive mutualisms. *Trends Ecol. Evol.* **2006**, *21*, 208–216. [[CrossRef](#)] [[PubMed](#)]
14. Lewinsohn, T.M.; Ina, P.; Prado, P.I. Structure in plant-animal interaction assemblages. *Oikos* **2006**, *113*, 1–11. [[CrossRef](#)]
15. Strona, G.; Galli, P.; Fattorini, S. Fish parasites resolve the paradox of missing coextinctions. *Nat. Commun.* **2013**, *4*, 1–5. [[CrossRef](#)] [[PubMed](#)]
16. Lewinsohn, T.M.; Inácio Prado, P.; Jordano, P.; Bascompte, J.M.; Olesen, J. Structure in plant-animal interaction assemblages. *Oikos* **2006**, *113*, 174–184. [[CrossRef](#)]
17. Bascompte, J.; Jordano, P.; Melián, C.J.; Olesen, J.M. The nested assembly of plant-animal mutualistic networks. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 9383–9387. [[CrossRef](#)] [[PubMed](#)]
18. Vázquez, D.P.; Aizen, M.A. Null model analyses of specialization in plant-pollinator interactions. *Ecology* **2003**, *84*, 2493–2501. [[CrossRef](#)]
19. Vázquez, D.P.; Melián, C.J.; Williams, N.M.; Blüthgen, N.; Krasnov, B.R.; Poulin, R. Species abundance and asymmetric interaction strength in ecological networks. *Oikos* **2007**, *116*, 1120–1127. [[CrossRef](#)]
20. Dupont, Y.L.; Hansen, D.M.; Olesen, J.M. Structure of a plant-flower-visitor network in the high-altitude sub-alpine desert of Tenerife, Canary Islands. *Ecography* **2003**, *26*, 301–310. [[CrossRef](#)]
21. Shochat, E.; Warren, P.S.; Faeth, S.H.; McIntyre, N.E.; Hope, D. From patterns to emerging processes in mechanistic urban ecology. *Trends Ecol. Evol.* **2006**, *21*, 186–191. [[CrossRef](#)]
22. Goddard, M.A.; Dougill, A.J.; Benton, T.G. Scaling up from gardens: Biodiversity conservation in urban environments. *Trends Ecol. Evol.* **2010**, *25*, 90–98. [[CrossRef](#)]
23. Rodewald, A.D.; Rohr, R.P.; Fortuna, M.A.; Bascompte, J. Community-level demographic consequences of urbanization: An ecological network approach. *J. Anim. Ecol.* **2014**, *83*, 1409–1417. [[CrossRef](#)] [[PubMed](#)]
24. Rodewald, A.D.; Rohr, R.P.; Fortuna, M.A.; Bascompte, J. Does removal of invasives restore ecological networks? An experimental approach. *Biol. Invasions* **2015**, *17*, 2139–2146. [[CrossRef](#)]



25. Lambin, E.F.; Turner, B.L.; Geist, H.J.; Agbola, S.B.; Angelsen, A.; Bruce, J.W.; Coomes, O.T.; Dirzo, R.; Fischer, G.; Folke, C.; et al. The causes of land-use and land-cover change: Moving beyond the myths. *Glob. Environ. Chang.* **2001**, *11*, 261–269. [[CrossRef](#)]
26. Deák, B.; Valkó, O.; Török, P.; Tóthmérész, B. Factors threatening grassland specialist plants—A multi-proxy study on the vegetation of isolated grasslands. *Biol. Conserv.* **2016**, *204*, 255–262. [[CrossRef](#)]
27. White, R.P.; Murray, S.; Rohweder, M. *Grassland Ecosystems*; World Resources Institute: Washington, DC, USA, 2000.
28. Overbeck, G.E.; Müller, S.C.; Pillar, V.D.; Pfadenhauer, J. Fine-scale post-fire dynamics in southern Brazilian subtropical grassland. *J. Veg. Sci.* **2005**, *16*, 655–664. [[CrossRef](#)]
29. Cordova, C. Grasslands and grassland ecology by Gibson David J. *J. Veg. Sci.* **2009**, *20*, 1191. [[CrossRef](#)]
30. Hunter, M.D.; Price, P.W. Playing chutes and ladders: Heterogeneity and the relative roles of bottom-up and top-down forces in natural communities. *Ecology* **1992**, *73*, 724–732.
31. Terborgh, J.W. Toward a trophic theory of species diversity. *Proc. Natl. Acad. Sci. USA.* **2015**, *112*, 11415–11422. [[CrossRef](#)]
32. Potts, S.G.; Vulliamy, B.; Dafni, A.; Ne’eman, G.; O’Toole, C.; Roberts, S.; Willmer, P. Response of plant-pollinator communities to fire: Changes in diversity, abundance and floral reward structure. *Oikos* **2003**, *101*, 103–112. [[CrossRef](#)]
33. Capitano, R.; Carcaillet, C. Post-fire Mediterranean vegetation dynamics and diversity: A discussion of succession models. *For. Ecol. Manag.* **2008**, *255*, 431–439. [[CrossRef](#)]
34. Turner, M.G.; Romme, W.H.; Tinker, D.B. Surprises and lessons from the 1988 Yellowstone fires. *Front. Ecol. Environ.* **2003**, *1*, 351–358. [[CrossRef](#)]
35. Van Nuland, M.E.; Haag, E.N.; Bryant, J.A.M.; Read, Q.D.; Klein, R.N.; Douglas, M.J.; Gorman, C.E.; Greenwell, T.D.; Busby, M.W.; Collins, J.; et al. Fire promotes pollinator visitation: Implications for ameliorating declines of pollination services. *PLoS ONE* **2013**, *8*. [[CrossRef](#)] [[PubMed](#)]
36. Peralta, G.; Stevani, E.L.; Chacoff, N.P.; Dorado, J.; Vázquez, D.P. Fire influences the structure of plant-bee networks. *J. Anim. Ecol.* **2017**, *86*, 1372–1379. [[CrossRef](#)] [[PubMed](#)]
37. Syphard, A.D.; Radeloff, V.C.; Keeley, J.E.; Hawbaker, T.J.; Clayton, M.K.; Stewart, S.I.; Hammer, R.B. Human influence on California fire regimes. *Ecol. Appl.* **2007**, *17*, 1388–1402. [[CrossRef](#)] [[PubMed](#)]
38. Syphard, A.D.; Radeloff, V.C.; Hawbaker, T.J.; Stewart, S.I. Conservation threats due to human-caused increases in fire frequency in Mediterranean-climate ecosystems. *Conserv. Biol.* **2009**, *23*, 758–769. [[CrossRef](#)] [[PubMed](#)]
39. Vizenin-Bugoni, J.; Maruyama, P.K.; de Souza, C.S.; Ollerton, J.; Rech, A.R.; Sazima, M. Plant-pollinator networks in the tropics: A review. In *Ecological Networks in the Tropics*; Springer: Cham, Switzerland, 2018; pp. 73–91.
40. Danieli-Silva, A.; de Souza, J.M.T.; Donatti, A.J.; Campos, R.P.; Vicente-Silva, J.; Freitas, L.; Varassin, I.G. Do pollination syndromes cause modularity and predict interactions in a pollination network in tropical high-altitude grasslands? *Oikos* **2012**, *121*, 35–43. [[CrossRef](#)]
41. Oleques, S.S.; Overbeck, G.E.; Avia, R.S. Flowering phenology and plant-pollinator interactions in a grassland community of Southern Brazil. *Flora* **2017**, *229*, 141–146. [[CrossRef](#)]
42. Carstensen, D.W.; Sabatino, M.; Morellato, L.P.C. Modularity, pollination systems, and interaction turnover in plant-pollinator networks across space. *Ecology* **2016**, *97*, 1298–1306. [[CrossRef](#)]
43. Overbeck, G.E.; Müller, S.C.; Fidelis, A.; Pfadenhauer, J.; Pillar, V.D.; Blanco, C.C.; Boldrini, I.I.; Both, R.; Forneck, E.D. Brazil’s neglected biome: The south brazilian Campos. *Perspect. Plant Ecol. Evol. Syst.* **2007**, *9*, 101–116. [[CrossRef](#)]
44. De Patta Pillar, V.; Vélez, E. Extinção dos Campos Sulinos em unidades de conservação: Um fenômeno natural ou um problema ético? *Nat. Conserv.* **2010**, *8*, 84–86. [[CrossRef](#)]
45. Boldrini, I.I.; Miotto, S.T.S.; Longhi-Wagner, H.M.; Pillar, V.D.P.; Marzall, K. Aspectos florísticos e ecológicos da vegetação campestre do Morro da Polícia, Porto Alegre, RS, Brasil. *Acta Bot. Brasilica* **1998**, *12*, 89–100. [[CrossRef](#)]
46. Rambo, B. Análise histórica da flora de Porto Alegre. *Sellowia* **1954**, *6*, 9–111.
47. Setubal, R.; Boldrini, I.I.; Ferreira, P.M.A. *Campos Dos Morros de Porto Alegre*; Igré—Associação Sócio-Ambientalista: Porto Alegre, Brazil, 2011.

48. Menegat, R.; Porto, M.L.; Carraro, C.C.; Fernandes, L.A. *Atlas ambiental de Porto Alegre*; Universidade Federal do Rio Grande do Sul: Porto Alegre, Brazil, 1999.
49. Mueller-Dombois, D.; Ellenberg, H. *Aims and Methods of Vegetation Ecology*; John Wiley & Sons: New York, NY, USA, 1974.
50. Pielou, E.C. *Ecological Diversity*; John Wiley & Sons: New York, NY, USA, 1975; ISBN 0471689254.
51. Londo, G. The decimal scale for relevés of permanent quadrats. *Vegetatio* **1976**, *33*, 61–64. [[CrossRef](#)]
52. Cruden, R.; Hermann-Parker, S.M. Butterfly pollination of *Caesalpinia pulcherrima*, with observations on a psychophilous syndrome. *J. Ecol.* **1979**, *67*, 155–168. [[CrossRef](#)]
53. Gottsberger, G. Some aspects of beetle pollination in the evolution of flowering plants. In *Flowering Plants*; Springer: Vienna, Austria, 1977; pp. 211–226.
54. Sakai, S. Thrips pollination of androdioecious *Castilla elastica* (Moraceae) in a seasonal tropical forest. *Am. J. Bot.* **2001**, *88*, 1527–1534. [[CrossRef](#)] [[PubMed](#)]
55. Micheneau, C.; Fournel, J.; Warren, B.H.; Hugel, S.; Gauvin-Bialecki, A.; Paillet, T.; Strasberg, D.; Chase, M.W. Orthoptera, a new order of pollinator. *Ann. Bot.* **2010**, *105*, 355–364. [[CrossRef](#)]
56. Somavilla, A.; Köhler, A. Preferência floral de vespas (Hymenoptera, Vespidae) no Rio Grande do Sul, Brasil. *EntomoBrasilis* **2012**, *5*, 21–28. [[CrossRef](#)]
57. Geographic Information System Development Team. *QGIS Geographic Information System*; Geographic Information System Development Team: Bonn, Germany, 2019.
58. R Development Core Team. *R: A Language and Environment for Statistical Computing*; R Development Core Team: Vienna, Austria, 2019.
59. Chao, A.; Gotelli, N.J.; Hsieh, T.C.; Sander, E.L.; Ma, K.H.; Colwell, R.K.; Ellison, A.M. Rarefaction and extrapolation with Hill numbers: A framework for sampling and estimation in species diversity studies. *Ecol. Monogr.* **2014**, *84*, 45–67. [[CrossRef](#)]
60. Hill, M.O. Diversity and Evenness: A Unifying Notation and Its Consequences. *Ecology* **1973**, *54*, 427–432. [[CrossRef](#)]
61. Oksanen, J.; Blanchet, F.G.; Friendly, M.; Kindt, R.; Legendre, P.; McGlinn, D.; Minchin, P.R.; O'Hara, R.B.; Simpson, G.L.; Solymos, P.; et al. The Package Vegan. *Commun. Ecol. Package* **2013**, *2*, 1–295.
62. Csárdi, G.; Nepusz, T. The igraph software package for complex network research. *Int. J. Complex Syst.* **2006**, *1695*, 1–9.
63. Dormann, C.F.; Gruber, B.; Fründ, J. Introducing the bipartite package: Analysing ecological networks. *R. News* **2008**, *8*, 8–11.
64. Dormann, C.F. How to be a specialist? Quantifying specialisation in pollination networks. *Netw. Biol.* **2011**, *1*, 1–20.
65. Geslin, B.; Gauzens, B.; Thébault, E.; Dajoz, I. Plant pollinator networks along a gradient of urbanisation. *PLoS ONE* **2013**, *8*, e63421. [[CrossRef](#)]
66. Dunne, J.A.; Williams, R.J.; Martinez, N.D. Network structure and biodiversity loss in food webs: Robustness increases with connectance. *Ecol. Lett.* **2002**, *5*, 558–567. [[CrossRef](#)]
67. Blüthgen, N.; Menzel, F.; Blüthgen, N. Measuring specialization in species interaction networks. *BMC Ecol.* **2006**, *6*, 9. [[CrossRef](#)]
68. Almeida-Neto, M.; Ulrich, W. A straightforward computational approach for measuring nestedness using quantitative matrices. *Environ. Model. Softw.* **2011**, *26*, 173–178. [[CrossRef](#)]
69. Blüthgen, N.; Menzel, F.; Hovestadt, T.; Fiala, B.; Blüthgen, N. Specialization, constraints, and conflicting interests in mutualistic networks. *Curr. Biol.* **2007**, *17*, 341–346. [[CrossRef](#)]
70. Dormann, C.F.; Strauss, R. A method for detecting modules in quantitative bipartite networks. *Methods Ecol. Evol.* **2014**, *5*, 90–98. [[CrossRef](#)]
71. Dalsgaard, B.; Schleuning, M.; Maruyama, P.K.; Dehling, D.M.; Sonne, J.; Vizentin-Bugoni, J.; Zanata, T.B.; Fjeldså, J.; Böhning-Gaese, K.; Rahbek, C. Opposed latitudinal patterns of network-derived and dietary specialization in avian plant-frugivore interaction systems. *Ecography* **2017**, *40*, 1395–1401. [[CrossRef](#)]
72. Debastiani, V.J.; Pillar, V.D. SYNCSA-R tool for analysis of metacommunities based on functional traits and phylogeny of the community components. *Bioinformatics* **2012**, *28*, 2067–2068. [[CrossRef](#)] [[PubMed](#)]
73. Newbold, T.; Hudson, L.N.; Hill, S.L.L.; Contu, S.; Lysenko, I.; Senior, R.A.; Börger, L.; Bennett, D.J.; Choimes, A.; Collen, B.; et al. Global effects of land use on local terrestrial biodiversity. *Nature* **2015**, *520*, 45–50. [[CrossRef](#)] [[PubMed](#)]

74. Fletcher, R.J.; Didham, R.K.; Banks-Leite, C.; Barlow, J.; Ewers, R.M.; Rosindell, J.; Holt, R.D.; Gonzalez, A.; Pardini, R.; Damschen, E.I.; et al. Is habitat fragmentation good for biodiversity? *Biol. Conserv.* **2018**, *226*, 9–15. [[CrossRef](#)]
75. Fahrig, L. Ecological Responses to Habitat Fragmentation Per Se. *Annu. Rev. Ecol. Evol. Syst.* **2017**, *48*, 1–23. [[CrossRef](#)]
76. Rodríguez, C.; Leoni, E.; Lezama, F.; Altesor, A. Temporal trends in species composition and plant traits in natural grasslands of Uruguay. *J. Veg. Sci.* **2003**, *14*, 433–440. [[CrossRef](#)]
77. Van Auken, O.W. Shrub invasions of north american semiarid grasslands. *Annu. Rev. Ecol. Syst.* **2000**, *31*, 197–215. [[CrossRef](#)]
78. Guido, A.; Salengue, E.; Dresseno, A. Effect of shrub encroachment on vegetation communities in Brazilian forest-grassland mosaics. *Perspect. Ecol. Conserv.* **2017**, *15*, 52–55. [[CrossRef](#)]
79. Connell, J.H. Diversity in tropical rain forests and coral reefs. *Science* **1978**, *199*, 1302–1310. [[CrossRef](#)]
80. Milchunas, D.G.; Sala, O.E.; Lauenroth, W.K. A Generalized model of the effects of grazing by large herbivores on grassland community structure. *Am. Nat.* **1988**, *132*, 87–106. [[CrossRef](#)]
81. Yuan, Z.Y.; Jiao, F.; Li, Y.H.; Kallenbach, R.L. Anthropogenic disturbances are key to maintaining the biodiversity of grasslands. *Sci. Rep.* **2016**, *6*, 1–8. [[CrossRef](#)]
82. Costa, F.V.; Blüthgen, N.; Viana-Junior, A.B.; Guerra, T.J.; Di Spirito, L.; Neves, F.S. Resilience to fire and climate seasonality drive the temporal dynamics of ant-plant interactions in a fire-prone ecosystem. *Ecol. Indic.* **2018**, *93*, 247–255. [[CrossRef](#)]
83. Blüthgen, N.; Fründ, J.; Vazquez, D.P.; Menzel, F. What do interaction network metrics tell us about specialization and biological traits? *Ecology* **2008**, *89*, 3387–3399. [[CrossRef](#)] [[PubMed](#)]
84. Jordano, P.; Bascompte, J.; Olesen, J.M. Invariant properties in coevolutionary networks of plant-animal interactions. *Ecol. Lett.* **2002**, *6*, 69–81. [[CrossRef](#)]
85. Vázquez, D.P.; Aizen, M.A. Community-wide patterns of specialization in plant–pollinator interactions revealed by null models. In *Plant–Pollinator Interactions: From Specialization to Generalization*; Waser, N.M., Ollerton, J., Eds.; University of Chicago Press: Chicago, IL, USA, 2006; pp. 200–219.
86. Montoya, J.M.; Pimm, S.L.; Sole, R. V Ecological networks and their fragility. *Nature* **2006**, *442*, 259–264. [[CrossRef](#)] [[PubMed](#)]
87. Schleuning, M.; Fründ, J.; Klein, A.M.; Abrahamczyk, S.; Alarcón, R.; Albrecht, M.; Andersson, G.K.S.; Bazarrian, S.; Böhning-Gaese, K.; Bommarco, R.; et al. Specialization of mutualistic interaction networks decreases toward tropical latitudes. *Curr. Biol.* **2012**, *22*, 1925–1931. [[CrossRef](#)] [[PubMed](#)]
88. Fidelis, A.; Appezzato-da-Glória, B.; Pillar, V.D.; Pfadenhauer, J. Does disturbance affect bud bank size and belowground structures diversity in Brazilian subtropical grasslands? *Flora Morphol. Distrib. Funct. Ecol. Plants* **2014**, *209*, 110–116. [[CrossRef](#)]
89. Giannini, T.C.; Garibaldi, L.A.; Acosta, A.L.; Silva, J.S.; Maia, K.P.; Saraiva, A.M.; Guimarães, P.R.; Kleinert, A.M.P. Native and non-native supergeneralist bee species have different effects on plant-bee networks. *PLoS ONE* **2015**, *10*, e0137198. [[CrossRef](#)]
90. Kerr, W.E. The history of the introduction of african bees in Brazil. *South African Bee, J.* **1967**, *39*, 33–35.
91. Guzman-Novoa, E.; Page, R.E.J. The impact of africanized bees on mexican beekeeping. *Am. Bee J.* **1994**.
92. Bascompte, J.; Jordano, P.; Olesen, J.M. Asymmetric coevolutionary networks facilitate biodiversity maintenance. *Science* **2006**, *312*, 431–433. [[CrossRef](#)]
93. Freitas, L.; Bolmgren, K. Synchrony is more than overlap: Measuring phenological synchronization considering time length and intensity. *Rev. Bras. Bot.* **2008**, *31*, 721–724. [[CrossRef](#)]
94. Denslow, J.S. Disturbance-mediated coexistence of species. In *The Ecology of Natural Disturbance and Patch Dynamics*; Pickett, S.T.A., White, P.S., Eds.; Academic Press: San Diego, CA, USA, 1985.
95. Harrison, S.; Inouye, B.D.; Safford, H.D. Ecological heterogeneity in the effects of grazing and fire on grassland diversity. *Conserv. Biol.* **2003**, *17*, 837–845. [[CrossRef](#)]
96. Overbeck, G.E.; Müller, S.C.; Pillar, V.d.P.; Pfadenhauer, J. Floristic composition, environmental variation and species distribution patterns in burned grassland in southern Brazil. *Braz. J. Biol.* **2006**, *66*, 1073–1090. [[CrossRef](#)] [[PubMed](#)]
97. McNeely, J.A. Protected areas for the 21st century: Working to provide benefits to society. *Biodivers. Conserv.* **1994**, *3*, 390–405. [[CrossRef](#)]

98. Quinn, J.F.; Harrison, S.P. Effects of habitat fragmentation and isolation on species richness: Evidence from biogeographic patterns. *Oecologia* **1988**, *75*, 132–140. [[CrossRef](#)]
99. Tschardtke, T.; Steffan-Dewenter, I.; Kruess, A.; Thies, C. Contribution of small habitat fragments to conservation of insect communities of grassland–cropland landscapes. *Ecol. Appl.* **2002**, *12*, 354–363.



© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).