

PHENOTYPICAL VARIABILITY OF FUNCTIONAL GROUPS OF PLANTS IN AN URBAN RAINFOREST

ABSTRACT

The functional characteristics of plants can be used to understand the changes of vegetation under different environmental pressures, since during the process of succession, the species deal with variations of luminosity, an important resource for the regeneration and growth of plants in humid tropical forests. From the perspective that along the succession there is variation of light availability and that leaf characteristics such as specific leaf area, chlorophyll content and leaf dry matter content are more plastic in groups linked to the rapid acquisition of the resource at the beginning of the succession, it was tested the hypothesis that at the beginning of the succession, where there is greater availability of light, leaf characteristics would be more plastic for the acquisitive group. It was initially found that the geographic distances did not influence the values of the variability indices of the groups, which allows to infer that the distance between the areas does not interfere in the variability of the leaf characteristics. To answer the hypothesis that at the beginning of the succession, in which there is greater light availability, the leaf characteristics would be more plastic for the purchasing group than for the conservative ones, a simple linear regression analysis (ARLS) was performed in the indices of variability for the groups (acquisitive and conservative) and abiotic factor (light) in each area of occurrence. However, the hypothesis that at the beginning of the succession, where there is greater light availability, the characteristics of the leaf would be more plastic for the species was rejected for the species acquisitive, since all indices were reduced for the purchasing group. It is important to take into account that the variation of leaf characteristics as a function of the light availability in an urban tropical fragment is different from what occurs in the classic succession commonly reported, pointing out that possible disturbances caused by the surroundings are the main agents of the functional structure of the community.

Keywords: Leaf characteristics, Light, Atlantic Rainforest, Phenotypic plasticity.

1. INTRODUCTION

The evaluation of the functional characteristics of plants groups can be used to understand the changes of vegetation under different environmental pressures [1]. In forest environments, throughout the process of succession, the species deal with variations in the luminosity levels, an important resource for the regeneration and growth of plants in rainforests [2,3].

Plants respond to environmental variations through acclimatization (phenotypic plasticity) or adaptations (evolutionary response) [4]. Phenotypic plasticity is the ability to adjust the value

of a given characteristic from a single genotype, according to changes in the environment within the individual lifetime, while the adaptations result from selective pressure variations along the gradient, able to produce hereditary differences among species, through evolution process [5,6,7].

The study of functional characteristics of plants has increased in recent years [8], the reason for this growth is due to the fact that these characteristics have effects on growth, reproduction and plant survival [9]. In this respect, different authors have discussed in detail the relations between physiological and ecological aspects of those characteristics [10,11]. The most abundant species in environments with greater light availability are characterized by rapid growth, low wood density, leaves with a short life cycle, high values of specific leaf area, chlorophyll content and low dry matter content. The conservative ones have greater abundance in areas with less light availability and are characterized by higher heights, stems with denser wood, leaves with longer life, high investment in dry matter, low chlorophyll content and specific leaf area [12,13].

Although the most studies focus on interspecific variation [14,15], it is understood that knowing the intraspecific variation can help to better understand the formation of communities [16,17,18,19]. The knowing role of variation within the groups of acquisitive and conservative tree species can help to understand the processes that lead to the formation and the functioning of the communities [20,21,8].

Ideally, studying intraspecific variation throughout the succession would be the ideal condition, but hardly is found species present in all successional stages, so is chosen to study the variations of the characteristic values in groups of species with quite different functional strategies, the acquisitive and conservative. These strategies are widely recognized and confirmed by the literature, especially with regard to the change of abundance of their populations throughout the succession [13,22,23,24,25].

Assuming that there is variation in light availability throughout the succession, leaf characteristics such as specific leaf area, chlorophyll content and leaf dry matter content are more plastic in groups linked to the rapid acquisition of the resource at the beginning of the succession [26,27]. In this study, was studied four areas of tropical rainforest located in a basal area gradient as a successional gradient evaluated in [28]. We hypothesized that at the beginning of the succession, where there is greater availability of light, leaf characteristics would be more plastic for the acquisitive group. If this is true, greater plasticity is expected in leaf dry matter content, specific leaf area and chlorophyll content in the species of the acquisitive group in environments with greater light availability.

2. MATERIAL AND METHODS

2.1 Study area

The research was carried out in the Dois Irmãos State Park (PEDI), located northwest of the municipality of Recife-PE, between coordinates 7° 57' 21" and 8° 00' 54" S; 34° 55' 53" and 34° 58' 38" W. In the area predominates Ombrophilous Dense Lowland vegetation [29], with geological formation Barreiras and soils of the podzolic type, with subordinate latosols, usually sandy-clayey, ranging from deep to very deep, and the soil acidity varies from medium to high [30]. The local climate is As' type (tropical humid or tropical coastal), with average monthly temperatures above 23 °C, average annual rainfall of 2460 mm and rainy season in the autumn-winter period [31].

2.2 Assembly of plots, inclusion criterion and floristic list

73 In the PEDI area, a module of the Biodiversity Research Program (PPBio), Mata Atlântica
74 Network, is installed using the RAPELD method: consisting of a combination of Rapid
75 Inventory (RAP) and ecological long-term research (PELD) [32]. The method consists in the
76 opening of two straight trails of 5000 m of extension, parallel with distance of 1000 m to each
77 other, along which sampling plots are installed according to standard protocol [32].

78 From the two trails installed by the PPBio researchers, was selected one, in which was
79 analyzed four plots (250 × 40 m) each, distancing 1000 m from each other, totaling four
80 areas. Was assume that these four areas represent different successional stages depending
81 on the variation of the basal area [28]. Thus, was hypothesized that there is variation in light
82 availability throughout the sequence.

83 For each plot, a 250 m corridor was installed, following the ground level curve, according to
84 the protocol defined by [33]. Within each hectare 20 plots of 10 × 20 m without overlap were
85 selected, where botanical samples were collected from all plants with stem diameter at
86 breast height (DBH) ≥ 5 cm. Only the functional characteristics of the species present with
87 five or more individuals in the four areas were collected.

88 All botanical material was identified, following the classification system [34] and deposited in
89 the Vasconcelos Sobrinho Herbarium (HVS) at Rural Federal University of Pernambuco
90 (UFRPE).

91 **2.3 Light data collection**

92 The total radiation (luminosity) was obtained in each of the 80 plots of 10 × 20 m drawn (20
93 per area). Initially hemispheric photos were taken in the center of each plot with a Nikon D50
94 camera with a hemispherical lens (Nikon DX 18-105 mm adapted fisheye 67-58 mm) on a
95 tripod adjustable to one meter above the ground, horizontally leveled, positioned with the
96 upper part aligned with magnetic north. The photographs were taken between August and
97 December 2015, between 8:30 and 11:00 hours [35]. The image processing was done with
98 the GLA software (Gap Light Analyzer) version 2.0 [36], in order to obtain the total radiation
99 that crosses the canopy (luminosity).

100

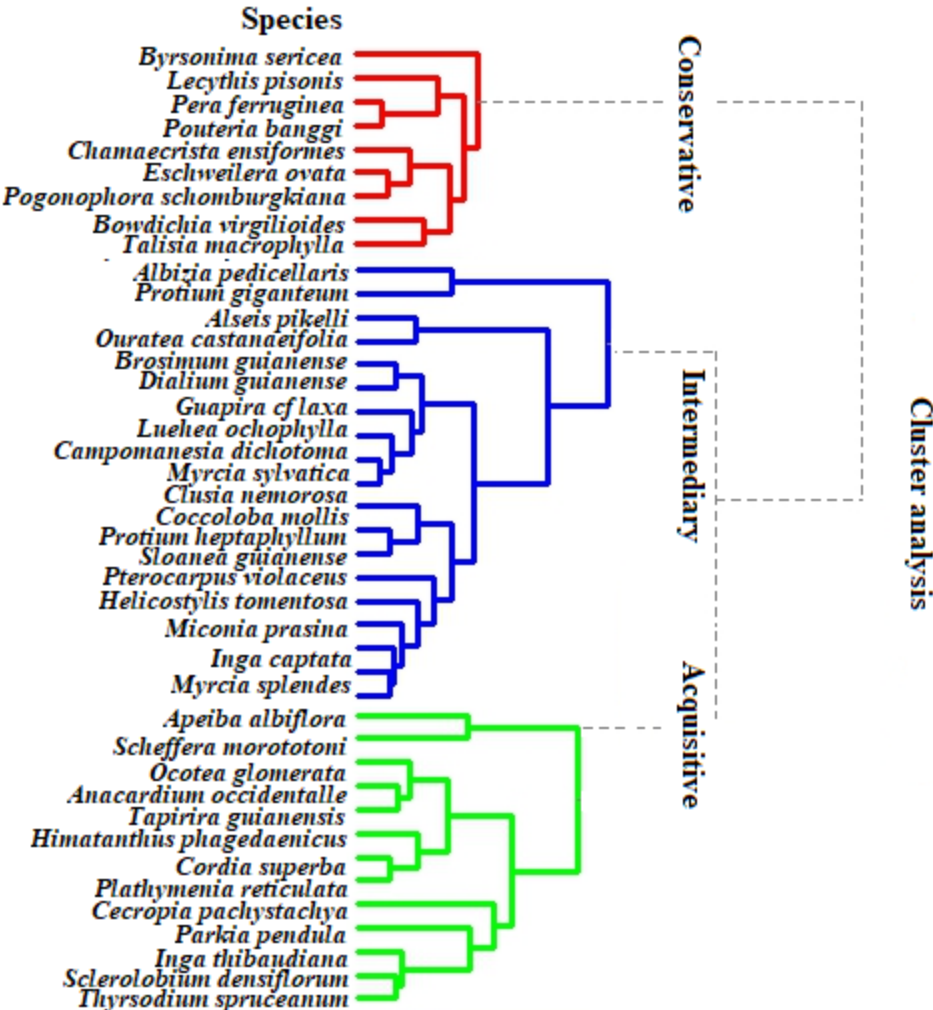
101 **2.4 Identification of functional groups**

102 Considering that there is greater leaf plasticity in groups of species linked to the fast use of
103 resource in environments with greater light availability, was chosen to test species with
104 acquisitive and conservative strategies in the four areas, since these strategies are more
105 easily identified. For this, was studied 10 functional characteristics (leaf, stem and root) of
106 the 41 species evaluated in [28] as follows: 1) was performed a hierarchical clustering
107 analysis based on the abundance of the 10 functional characteristics, based on the Gower
108 dissimilarity matrix [37]. There was no phylogenetic signal for functional characteristics
109 throughout the succession according to [28]. A nonparametric multivariate analysis of
110 variance (PERMANOVA) was then performed to verify the optimal number of groups. The
111 choice of the best number of groups was one in which the increase in the amount of
112 variance was higher than 15% [38]. It is important to note that average values of all 10
113 characteristics (leaf, stem and root) were used in all four areas to identify the formation of
114 both groups (acquisitive and conservative).

115 Plants with high chlorophyll content, higher specific leaf area, leaf area, low dry matter
116 content [39], less dense stem and root woods, higher amount of saturated water and lower
117 contents of dry matter [40,41,42], are related to the acquisition group resource and dominate
118 in areas at the beginning of the succession, while plants that present low content of

119 chlorophyll, specific leaf area, leaf area, higher dry matter content, denser stem and root
120 woods, less saturated stem and root water and higher dry matter contents of stem and root
121 [12] predominate in environments related to conservative use. The hierarchical cluster
122 analysis and PERMANOVA were performed with the "ggplot2", "ggdendro", "vegan" and
123 "cluster" packages in R [43]. As results, 13 species were identified and nine were
124 conservative.

125 **Fig 1. Hierarchical cluster analysis performed by the Ward method for the 41 species**
126 **revealed that the optimal number of groups of strategies was three ($k = 3$), with $R^2 =$**
127 **0.54, studied in the four areas of an urban forest fragment.**



128

129 Source: Leite MJH (2017)

130 It is known that studying phenotypic plasticity throughout the succession would be an ideal
131 condition, but hardly is found species in all successional stages, so was chosen to study the
132 variations of the characteristic values in groups of species with very different functional
133 strategies (acquisitive and conservative). For this was used the standard deviation because

134 it is considered as a measure of dispersion around the population mean of a random
135 variable and for indicating the degree of variation of a set of elements. Based on the
136 characteristic values (TMSF, AFE and Cc_mass) was calculated the standard deviation of
137 each group of species present in each area. Was considered only the species that presented
138 standard deviation of 0.1. While species that exhibited values below or above 0.1 was not
139 used to avoid outliers in the results. The literature reports that the standard deviation is
140 considered an important characteristic of the normal distribution, since species with a
141 deviation of 0.1 their characteristics tend to be closer to the mean (Table 1).

UNDER PEER REVIEW

1 **Table 1. Standard deviation of the functional characteristics of the acquisitive and conservative species in the four areas of a fragment of urban**
2 **forest.**

ACQUISITIVE SPECIES				CONSERVATIVE SPECIES					
		STANDARD DEVIATION					STANDARD DEVIATION		
Areas	Species	AFE	TMSF	Cc. mass	Areas	Species	AFE	TMSF	Cc. mass
A1>AB	<i>Inga thibaudiana</i> DC.	0.1359	0.0606	0.1088	A1>AB	<i>Bowdichia virgilioides</i> Kunth	0.1282	0.0660	0.0573
	<i>Ocotea glomerata</i> (Nees) Mez	0.0780	0.0416	0.0474		<i>Eschweilera ovata</i> (Cambess.) Mart. ex Miers	0.1751	0.1024	0.1770
	<i>Parkia pendula</i> (Willd.) Benth	0.1867	0.1451	0.1887		<i>Pogonophora schomburgkiana</i> Miers ex Benth.	0,1870	0.1477	0.1885
	<i>Schefflera morototoni</i> (Aubl.) Maguire, Steyerl. & Frodin	0.1224	0.0679	0.1202		<i>Pouteria banggi</i> (Rusby) T.D. Penn.	0.1476		0.1549
	<i>Sclerolobium densiflorum</i> (Benth.)	0.1706	0.1746	0.1032		<i>Talisia macrophylla</i> (Mart.) Radlk.	0.3925	0.1568	0.2416
	<i>Thyrsodium spruceanum</i> Benth.	0.0475	0.6173	0.7788					
A2ABI	<i>Anacardium occidentale</i> L.	0.1596	0.1970	0.1469	A2ABI	<i>Bowdichia virgilioides</i> Kunth	0.8704	0.4118	0.0210
	<i>Cecropia pachystachya</i> Trécul	0.1278	0.1887	0.1293		<i>Byrsonima sericea</i> DC.	0.1470	0.1853	0.1459
	<i>Cordia superba</i> Cham.	0.1807	0.1819	0.1324		<i>Eschweilera ovata</i> (Cambess.) Mart. ex Miers	0.1232	0.1245	0.1357
	<i>Himatanthus phagedaenicus</i> (Mart.) Woodson	0.1460	0.1200	0.1294		<i>Lecythis Pisonis</i> Cambess.	0.3129	0.0791	0.0998
	<i>Plathymenia reticulata</i> Benth.	0.1244	0.1023	0.1802	<i>Pera ferruginea</i> (Schott) Müll. Arg	0.1360	0.1054	0.1126	
	<i>Schefflera morototoni</i> (Aubl.) Maguire, Steyerl. & Frodin	0.1063	0.1804	0.1304	<i>Pogonophora schomburgkiana</i> Miers ex Benth.	0.1155	0.1047	0.1797	
	<i>Tapirira guianensis</i> Aubl.	0.1038	0.1390	0.1397	A3ABI	<i>Bowdichia virgilioides</i> Kunth	0.7810	0.0705	0.0219
	<i>Thyrsodium spruceanum</i> Benth.	0.1170	0.0181	0.0397		<i>Byrsonima sericea</i> DC.	0.1245	0.1031	0.1264
						<i>Chamaecrista ensiformes</i> (Vell.) H.S.Irwin & Barneby	0.1262	0.1026	0.1302
	A3ABI	<i>Apeiba albiflora</i> Ducke	0.1380	0.0660	0.1214	A4<AB	<i>Eschweilera ovata</i> (Cambess.) Mart. ex Miers	0.1349	0.1208
<i>Cecropia pachystachya</i> Trécul		0.1409	0.1377	0.1296	<i>Lecythis Pisonis</i> Cambess.		0.8827	0.5922	0.3496
<i>Ocotea glomerata</i> (Nees) Mez		0.0519	0.0344	0.0422	<i>Pera ferruginea</i> (Schott) Müll. Arg		0.1406	0.1306	0.1876
<i>Plathymenia reticulata</i> Benth.		0.3646	0.1417	0.1494	<i>Pogonophora schomburgkiana</i> Miers ex Benth.		0.1761	0.1348	0.1312
					<i>Chamaecrista ensiformes</i> (Vell.) H.S.Irwin & Barneby			0,1977	0.1713
				<i>Pera ferruginea</i> (Schott) Müll. Arg	0.1551				
				<i>Pogonophora schomburgkiana</i> Miers ex Benth.	0.1368		0.0503	0.1296	
					0.1285		0.1345	0.1502	

3 *DP_AFE* – Standard deviation of the leaf area, *DP_TMSF* - Standard deviation of leaf dry matter, *DP_Cc_mass* - Standard deviation of chlorophyll content, *A1>AB* (area with
4 greater basal area), *A2ABI* (basal intermediate area), *A3<AB* basal area) and *A4<AB* (area with the lowest basal area).

2.5 Functional characteristics

From the 10 characteristics studied in [28], only three foliar characteristics were studied because they are considered very plastic: specific leaf area, chlorophyll content and leaf dry matter content [26,27,44] in the 22 species selected in the two groups, nine conservative and thirteen acquisitive. The data collection occurred in five individuals per species. From each individual, 10 mature leaves were collected at the intermediate height of the crown (exposed to the sun), without evident symptoms of pathogen or herbivore attack [39]. For the determination of the leaf area (FA), the "Image-Tool" program was used [45]. The specific leaf area (AFE) was the ratio between leaf area and dry weight (Table 2).

The chlorophyll content in the leaves was measured with the aid of a SPAD chlorophyll meter (Minolta SPAD 502 D Spectrum Technologies Inc., Plainfield, IL, USA). The content of chlorophyll by mass was determined by the following formula: (Cmassa; Chlorophyll content* (AFE / 10000 [46])). After rehydration, the leaves were weighed in an analytical scale to obtain the saturated weight of water. They were then scanned for leaf area measurement using the computer program "Image-Tool" [45].

Table 2. List of functional characteristics analyzed in an urban Rainforest fragment, adapted from [47].

Functional Feature	Description	Functional Relationship
AFE	Specific leaf area (AF / PS) Dry matter content of leaf (PUF-PSF)	Photosynthetic rate, leaf longevity, relative growth rate
TMSF	Chlorophyll Concentration	Resistance to physical hazards (herbivory)
Cc_mass	(Cmassa, chlorophyll content * (AFE / 10000))	Photosynthetic process, acting in the conversion of light energy into chemical energy

AFE - specific leaf area ($\text{cm}^2.\text{mg}^{-1}$); CC_mass - concentration of chlorophyll (micromol.g^{-1}); TMSF - leaf dry matter content (mg.g^{-1}).

2.6 Phenotypic Plasticity

Was calculated the phenotypic plasticity index proposed by [26] for three leaf characteristics (AFE, Cc_mass and TMSF) of the 13 species of the group of the acquisitive and nine conservative species, in each of the four areas. This index can vary from zero to one, with IP 1 inferring high plasticity. In order to calculate the IP, the following formula was used: $\text{IP} = \frac{\text{maximum average value} - \text{minimum average value}}{\text{maximum average value}}$ of each characteristic for each group of acquisitive and conservative species in each area.

2.7 Data Analysis

In order to verify if the phenotypic plasticity indices of the two groups of species were influenced by the geographic distances, was used the Mantel Partial test in each of the 80 plots drawn (20 per area).

The Mantel Partial test and simple regression analysis were performed using the nortest, vegan and APE packages in the R environment version 3.0.2 [43].

To test the hypothesis that at the beginning of the succession, where there is greater light availability, leaf characteristics would be more plastic for the acquisitive group, a simple linear regression analysis (ARLS) was performed, on the plasticity indices of the groups (acquisitive and conservative, response variables) and abiotic factor (light) in each area.

3. RESULTS

According to the Partial Mantel test, the geographic distances did not influence the values of the plasticity indices of the groups ($r = -0.2977$; $p = .001$). This result allows to infer that the distance between the areas does not interfere in the plasticity of the foliar characteristics.

To test the hypothesis that at the beginning of the succession, where there is greater light availability, leaf characteristics would be more plastic for the acquisitive group, was performed a simple linear regression analysis between the light percentages and the plasticity indices of the two groups of species with and conservative strategies (Fig. 2).

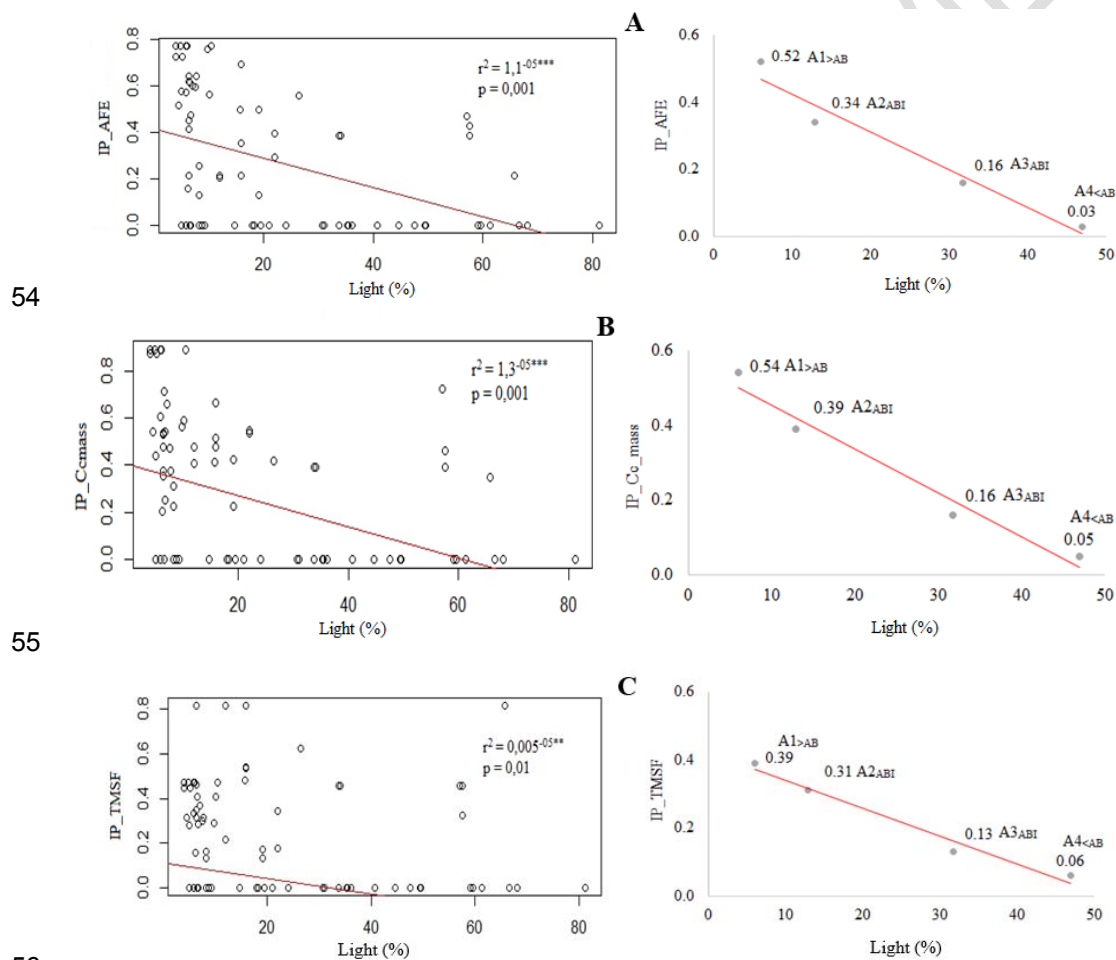


Fig 2. Simple regression analysis between light percentages and phenotypic plasticity indices of the group with acquisition strategy in the four areas of a fragment of urban Rainforest. A, B and C (purchasing group). IP_AFE (specific leaf area index), IP_Cc_mass (plasticity index of chlorophyll content), IP_TMSF (leaf dry matter content plasticity index).

62 *IP_AFE* - *IP* of specific leaf area (acquisitive group, A), *IP_Cc_mass* - *IP* of chlorophyll content by
63 mass (acquisitive group, B), *IP_TMSF* - *IP* of leaf dry matter content (acquisitive group, C), *IP_*
64 *Cc_mass* - *IP* of chlorophyll content (conservative group, D). (Area with the lowest basal area), A2ABI
65 (intermediate basal area), A3 <AB (intermediate basal area) and A4 <AB (area with the lowest basal
66 area). *F* values were obtained with ANOVA (* = $P < .05$; ** = $P < .01$; *** = $P < .001$).
67

68 The results of this analysis revealed that within the acquisitive species, as the light
69 percentages increased throughout the succession, all leaf characteristics (*IP_AFE*,
70 *IP_Cc_mass* and *IP_TMSF*) presented lower plasticity (Fig. 2). In relation to the conservative
71 group, was observed no relation with the abiotic light factor in the succession.

72 Was observed that the acquisitive group presented lower values of *IP_AFE* (0.03),
73 *IP_Cc_mass* (0.05) and *IP_TMSF* (0.06) in the initial phase of the succession (A4 <AB),
74 environment with higher incidence of light (46.97%). In the environment with less light
75 (6.09%, A1 <AB) this group was more plastic, with higher value of *IP_AFE* (0.52),
76 *IP_Cc_mass* (0.58) and *IP_TMSF* (0.31). Thus, was rejected the hypothesis that at the
77 beginning of the succession, where there is greater availability of light, leaf characteristics
78 would be more plastic for the acquisitive group (Fig. 2).
79

80 **4. DISCUSSION**

81
82 The hypothesis that at the beginning of the succession, where there is greater light
83 availability, leaf characteristics would be more plastic for the acquisitive species was
84 rejected. Since, as the light availability within the acquisitive group increased, the plasticity
85 indices of the characteristics such as *TMSF*, *AFE* and *Cc_mass* decreased. It is important to
86 emphasize that although these characteristics are highly plastic in more open environments,
87 their plasticity may have been reduced due to the constant perturbations in the area,
88 especially in the environment with a higher incidence of light (46.97%, A4 <AB). Lower *AFE*
89 and *Cc_mass* values were found in the more open area (A4 <AB), which expected higher
90 values. These results point to the hypothesis that because these characteristics are highly
91 plastic, especially in more open environments, the anthropic actions occurred in this area,
92 caused that these characteristics did not suffer increase of their values. Is worth to mention
93 that the species occurring in these environments present a short life cycle, colonize faster,
94 invest more in height and present high mortality, leading species of these environments to
95 be more susceptible to changes.

96 While the areas with lower incidences of light (A1 > AB, 6.09% and A2ABI, A3ABI 12.94%),
97 the values of those characteristics increased as they decreased light availability, contrary to
98 expectations. It is possible to hypothesize that this increase in plasticity in these areas has
99 occurred because species that grow in shaded environments experience several ontogenetic
100 changes in relation to low irradiance during the life cycle and therefore may demonstrate
101 greater plasticity in such characteristics.

102 According to [48,26], the plasticity of physiological characteristics are more plastic in open
103 environments, because they present rapid responses in the short term in relation to the
104 availability of the resource. However, there is evidence to suggest that the adjustments are
105 not necessarily related to the successional status of species [49,50].

106 For [51,52] phenotypic plasticity is more observed in seedlings, especially in the pioneer
107 ones, because they are more prone to acclimatization. On the other hand, [53] observed that
108 the leaf plasticity of pioneer species may be lower in shaded environments, because they
109 cannot survive long in this environment.

110 It is important to mention that, although the foliar characteristics are highly plastic in more
111 open environments, the plasticity can be reduced by the perturbations occurring in the area
112 where is found [54,55]. What could be proven with the results found in the present research
113 (lower IP_AFE, IP_Ccmass and IP_TMSF) in the environment with greater incidence of light.
114 The perturbations occurred in the area may have contributed to this reduction of plasticity
115 (Leite et al 2019), is important to note that species occurring in these environments present
116 a short life cycle, being more susceptible to changes in their values. For [56,57] both
117 conservative and acquisitive species can be plastic in characteristics important for its
118 functions. These authors also observe that groups of species adapted to high irradiation may
119 have greater plasticity in leaf characteristics related to photosynthesis, such as nitrogen
120 content and that shade tolerant species may present greater plasticity in specific leaf area
121 and chlorophyll content.

122 **4. CONCLUSIONS**

123
124 Different from what is expected, at the beginning of the succession, where there is greater
125 availability of light, the leaf characteristics would be less plastic for the acquisitive group, this
126 disturbances could change the classical path of succession in function of population
127 dynamics, especially in the area with greater light availability, which probably led to higher
128 plant mortality of the acquisition group, as a result, the variability of A4_{AB} leaf characteristics
129 decreased.

130
131 This research showed that the variation of leaf characteristics, as a function of the light
132 availability, in an urban Rainforest fragment is different from what occurs in the classic
133 succession commonly reported, pointing out that possible disturbances caused by the
134 surroundings are the main agents of the functional structure of the community.

135 **COMPETING INTERESTS**

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137
138 Authors have declared that no competing interests exist.

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