Variations in light intensity can lead to important anatomical and morphophysiological changes in plants. Aiming to increase knowledge about the Amazonian tree species, this study examines the influence of shade on the cambial activity and development of *Parkia gigantocarpa* Ducke and *Schizolobium parahyba* var. *amazonicum* (Huber ex Ducke) Barneby seedlings. Seedlings of the two species were grown in a nursery under four shade intensities (treatments): full sun, low, moderate, and high shade (resp., 0%, 23%, 67%, and 73% of shade, or 2000, 1540, 660, and 540 \( \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \)) obtained with polyethylene screens. We measured plant height, stem diameter, biomass production, stomatal conductance (\( g_s \)), transpiration (\( E \)), photosynthesis (\( A \)), and cambial activity (CA) (xylem, cambium, and phloem). Also, we calculated the Dickson Quality Index (DQI). The highest values of biomass production, \( g_s \), \( E \), \( A \), and DQI, were found under full sun, in *P. gigantocarpa*, and under low shade intensity in *S. parahyba*. In both species high shade intensity reduced CA. We concluded that the CA and the physiological and morphological attributes work together, explaining the radial growth and increasing seedlings quality, which optimized efficient seedling production under full sun, in *P. gigantocarpa*, and under low shade intensity in *S. parahyba*.

1. Introduction

Although environmental factors do not operate alone in plants, light is particularly important as it is the direct source of energy for photosynthesis, influencing plant growth [1, 2]. However, all plants possess some degree of plasticity to more efficiently use solar radiation [3, 4]. This is commonly observed in studies of forest tree species under restricted light conditions [5–7]. According to these studies, stem diameter is commonly measured, because its development is essential for growth, ensuring plant survival in the field under environmental stress and is also important for wood formation [8, 9].

Variation in stem diameter in response to light is a form of environmental adaptation that probably occurs because of cambial activity, which is stimulated by carbohydrates produced by photosynthesis, and hormonal activity [10, 11]. According to Dénardin et al. [12], increased cambial activity amounts to produce xylem, thus increasing radial growth (i.e., stem diameter), resulting in wood formation. Cambial activity can be influenced by environmental factors such as light, and by intrinsic factors to the plant, such as genetics and physiology [10, 13]. Since cambial activity is affected by light, it could be suggested that seedling quality would also be affected by this environmental factor.

Thus, in order to expand the knowledge on the anatomical and morphophysiological responses of Amazonian Leguminosae tree species to shade, aiming to devise a more efficient seedling production strategy, this study intends to examine the influence of shade intensity on cambial activity and on morphological and physiological characteristics of *Parkia gigantocarpa* Ducke and *Schizolobium parahyba* var. *amazonicum* (Huber ex Ducke) Barneby seedlings grown in a nursery.
2. Materials and Methods

2.1. Plant Material. The experiment was conducted in a semiconrolled environment, in the nursery seedling production unit of the Plant Ecophysiology Department of the Brazilian Agricultural Research Corporation (Embrapa) (01° 28’ 40” S, 48° 26’ 59” W) in Belém, PA, Brazil. The mean air temperature and relative humidity, measured in a agrometeorological station close to the experimental unit, during the experimental period, were 27°C and 81%.

Seeds of Parkia gigantocarpa and Schizolobium parahyba var. amazonicum used in the study were collected from trees grown in a reforested area, close to the city of Paragominas, PA (02° 59’ 45” S, 47° 21’ 10” W), in the eastern Brazilian Amazon.

Prior to sowing, seeds of both species were subjected to mechanical scarification with emery to break dormancy. Six days after germination, seeds were sown in plastic trays filled with a substrate composed of sand and sawdust (1:1). Seedlings were transplanted to black polyethylene bags (15×25 cm) with substrate made from a mixture of surface forest soil and sheep manure (3:2). Seedlings were acclimated for 14 days under 75% shade intensity. Fifteen days after germination, both species were fertilized with 3 g of NPK (10-28-20) per plant.

After acclimation (20 days after germination), seedlings were distributed in the following light environment (treatments): full sun, low, moderate, and high shade intensities (resp., 0%, 23%, 67%, and 73% of shade, or 2000, 1540, 660, and 540 μmol m⁻² s⁻¹, at canopy height, measured at 11 am, local time, on a cloudless day) obtained with polyethylene screens. *P. gigantocarpa* and *S. parahyba* received daily irrigation and remained for 60 days under these light environments.

2.2. Morphological Parameters. Stem diameter (*D*) was measured on two opposite points at the same height with a digital caliper to an accuracy of 0.01 mm. The mean *D* was the average of these two points. Seedling height (*H*) was measured with a ruler, from the level of the soil substrate to the apical bud. Both *D* and *H* were measured when plants were for 15 and 60 days under the treatments. Based on data collected at the second time of collection, we calculated the ratio *H/D*.

At the end of the experiment (60 days under the treatments), plants were cut and fractionated into leaves, stems, and roots. Plant dry mass was obtained by drying the plant material in an oven at 60°C until constant mass. Total dry mass (*TDM*) was calculated by adding leaf dry mass (*LDM*), stem dry mass (*SDM*), and root dry mass (*RDM*). Aerial dry mass (*ADM*) was obtained by adding *LDM* and *SDM*. All of the morphological parameters were calculated according to Hunt [14].

Dickson Quality Index (DQI = [TDM (g)/[(H (cm)/D (mm)) + (ADM (g)/RDM (g))]]) was calculated according to Dickson et al. [15].

2.3. Physiological Parameters. Stomatal conductance (*g₅*), transpiration (*E*), and net photosynthesis (*A*) were measured at the end of the experiment (60 days under the treatments), between 9:00 and 10:00 am, in fully developed and expanded leaves, with an infrared gas analyzer (IRGA) (model LCpro, ADC Bioscientific Ltd., UK) under natural CO₂ conditions and photosynthetic active radiation of 500 μmol m⁻² s⁻¹ (obtained by an artificial light source coupled to the IRGA chamber). This light level was chosen to mimic the lowest light level used in the experiment. All gas exchange parameters were calculated on a leaf area basis.

2.4. Anatomical Parameters. To analyze cambial activity in the stem, plants were sampled on two occasions: 15 days and 60 days after the imposition of the treatments. After the stems have been collected, fragments located between the first node and the substrate level were selected and preserved in ethyl alcohol 70% [16].

For the preparation of the slide collection, sections were cleared by immersion in a sodium hypochlorite (NaClO) solution and then washed with distilled water. Subsequently, they were stained with safranin (1%) and Astra blue (1%) [17]. Semipermanent slides and freehand sections of the stem cross sections were prepared with glycerin. In sectioned samples structures derived from the cambial activity that is the vascular system formed by xylem, cambium, and phloem were measured, with the aid of an objective and eyepiece 10x micrometer microscope Zeiss Axioslab. Photographs were obtained from a 3.0 M pixel camera (Moticam).

2.5. Experimental Design and Statistical Analysis. The experimental design for each species was completely randomized with four treatments and five replicates for *H/D*, biomass production, *A*, *g₅*, *E*, DQI. Completely randomized in a 4 × 2 factorial arrangement (treatment × evaluation period), with five replicates for *H*, *D*, and CA. Data were subjected to analysis of variance and, when appropriate, the means were compared by Tukey test at 5%. Correlation analysis between CA, D, SDM, TDM, DQI, *A*, *g₅*, and *E* was calculated by parametric (Pearson correlation) or nonparametric (Spearman correlation) statistics, according to the nature of the data. All statistical calculations were performed by the software STATISTICA 7.

3. Results and Discussion

3.1. Plant Growth. For both species, there was no evaluation period × treatment interaction for plant height and stem diameter. However, when each evaluation period was considered individually, significant differences among treatments were found for these variables (Figures 1(a), 1(b), 2(a), and 2(b)). For the first evaluation period, the highest values of plant height were measured in full sun plants of both species. In the second evaluation period, both species maintained the greater plant heights under full sun, but in *P. gigantocarpa* this greater plant height was also shared by plants grown under low and high shade intensity, while in *S. parahyba*, plants were also higher under moderate and high shade intensities.

Plant height growth of *P. gigantocarpa* and *S. parahyba* did not follow the expected response pattern. This expected
response is a higher stem elongation under low light intensity, producing a greater plant height [18]. Studies conducted with forest species indicate that this strategy is an adaptive mechanism to low light environments [5, 6, 19].

In general, forest seedlings achieve their critical point of field adaptation (i.e., improved chances of survival in the field) when they reach around 20 cm in height and 3 mm in stem diameter [20]. In our study, both species have accelerated growth, reaching a “state of steadiness” in their ability to modify morphophysiological patterns, in response to the experimental shade intensities, on average, 60 days under these light environments. Therefore, the absence of a higher stem elongation in low light plants from both species, found in our study, could indicate that seedling of these species may have differential adaptive mechanisms to cope with low light environments. Similar results were found in Caesalpinia ferrea Mart. ex Tul. [21] and in Talisia subalbens (Mart.) Radlk. seedlings [22].

For the first evaluation period, plant diameter did not vary among treatments in P. gigantocarpa; however, in
**Figure 2:** *S. parahyba* responses to shade. (a) Height (*H*), (b) diameter (*D*), (c) ratio *H/D*, (d) Dickson Quality Index (*DQI*), (e) stomatal conductance (*g*s), (f) transpiration (*E*), (g) net photosynthesis (*A*), and (h) cambial activity. Mean ± SE (*n* = 5). Columns with different letters, within the same evaluation period, are significantly different (*P* < 0.05, Tukey test).

*S. parahyba* stem diameter was larger for full sun and low shade intensity plants. In the second evaluation period, stem diameter in *P. gigantocarpa* was lower under high shade intensity (Figure 1(b)); this is a common response in forest species [19, 23, 24]. However, some forest species do not vary stem diameter in response to light availability [5, 25, 26], corroborating the lack of response found in *S. parahyba* (Figure 2(b)).

The ratio between height and diameter (*H/D*) is a parameter used to determine seedling quality; this ratio is an indication of reserve accumulation and seedling survival in the field [15, 23, 25]. Lower *H/D* values indicate a balanced growth in stem diameter and height, increasing survival ability. Conversely, seedlings with high *H/D* values show disproportionate growth, reducing the chances of survival in the field, since etiolated plants are more susceptible to tipping. The *H/D* values of *P. gigantocarpa* indicated that an adequate growth balance was achieved under lower shade intensity (Figure 1(c)), while in *S. parahyba* this balance was achieved in plants grown under low shade intensity (Figure 2(c)).

### 3.2. Biomass Production

In *P. gigantocarpa* leaf dry mass and stem dry mass were lower under high shade intensity. Stem dry mass was also lower in low shade intensity plants.
Table 1: Biomass production of *P. gigantocarpa* and *S. parahyba* under different shade intensities. Leaf dry mass (LDM), stem dry mass (SDM), root dry mass (RDM), total dry mass (TDM), aerial dry mass (ADM), and the ratio between ADM and RDM.

<table>
<thead>
<tr>
<th>Species</th>
<th>Shade intensity</th>
<th>LDM (g)</th>
<th>SDM (g)</th>
<th>RDM (g)</th>
<th>TDM (g)</th>
<th>ADM/RDM</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. gigantocarpa</em></td>
<td>Full sun</td>
<td>1.33 (±0.17)*</td>
<td>1.28 (±0.21)*</td>
<td>0.50 (±0.12)*</td>
<td>3.11 (±0.44)*</td>
<td>5.22 (±0.76)</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>1.01 (±0.04)ab</td>
<td>0.71 (±0.06)b</td>
<td>0.31 (±0.02)b</td>
<td>2.03 (±0.12)b</td>
<td>5.55 (±0.15)</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>1.35 (±0.04)*</td>
<td>1.05 (±0.01)ab</td>
<td>0.39 (±0.02)b</td>
<td>2.79 (±0.04)ab</td>
<td>6.15 (±0.53)</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>0.80 (±0.01)b</td>
<td>0.66 (±0.03)b</td>
<td>0.30 (±0.03)b</td>
<td>1.76 (±0.03)b</td>
<td>4.87 (±0.61)</td>
</tr>
<tr>
<td><em>S. parahyba</em></td>
<td>Full sun</td>
<td>1.83 (±0.01)bd</td>
<td>3.11 (±0.19)b</td>
<td>4.12 (±0.39)*</td>
<td>9.06 (±0.19)*</td>
<td>1.20 (±0.17)</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>3.99 (±0.25)cd</td>
<td>6.30 (±0.70)c</td>
<td>4.40 (±0.03)a</td>
<td>14.69 (±0.82)c</td>
<td>2.27 (±0.23)</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>3.31 (±0.04)bd</td>
<td>4.78 (±0.23)bd</td>
<td>4.42 (±0.92)a</td>
<td>12.51 (±0.98)ab</td>
<td>1.83 (±0.38)</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>2.63 (±0.03)bd</td>
<td>3.86 (±0.16)bd</td>
<td>2.86 (±0.43)bd</td>
<td>9.35 (±0.62)b</td>
<td>2.27 (±0.33)</td>
</tr>
</tbody>
</table>

* Values are mean ± SE (n = 5). Means followed by the same letter in each column are not significantly different (P < 0.05, Tukey test).

(Table 1). In *S. parahyba* leaf dry mass was higher under the moderate shade intensity and the stem dry mass was higher under the low and moderate shade intensity. In both species root dry mass tended to decrease under high shade and the ratio ADM/RDM was not affected by treatments. Total biomass (TDM) was higher under full sun in *P. gigantocarpa* and under low shade intensity in *S. parahyba* (Table 1). It could be inferred that under the above conditions these species were more efficient in the use of light.

Under higher shade intensity, the supplied light energy was apparently inadequate for both species needs, reducing the total biomass (TDM). This indicates that these species have a highlight compensation point; that is, carbon consumed by respiration is greater than the one produced by photosynthesis, leading to a reduction in total biomass production [1, 2]. Similar results are also reported for seedlings of the forest tree species *Caesalpinia ferrea* Mart. ex Tul. [21], *Talisia subalbens* (Mart.) Radlk. [22], and *Simarouba amara* Aubl. [27].

The total biomass of *S. parahyba* was also reduced under full sun; this was probably caused by increased leaf temperature, which decreased stomatal conductance, before affecting photosynthesis. In addition, because under full sun roots were the preferred drain, as opposed to stem and leaves (Table 1), total biomass was lower under this light environment.

This behavior of tending to increase root biomass under higher light intensities, in both species, possibly was an acclimation strategy to increase water uptake in response to increased transpiration and thus to soil drying [28]. In both species the variation of light did not affect the ratio between aerial dry mass and root dry mass (Table 1), suggesting a certain balance in the biomass distribution of these species under this abiotic stress. Similar behavior was also found in studies with forest tree seedlings [5, 7, 22].

### 3.3. Seedling Quality

Dickson Quality Index in *P. gigantocarpa* ranged from 0.19, in high shade intensity, to 0.31, in full sun (Figure 1(d)). In *S. parahyba* this index ranged from 1.05, in high shade intensity plants, to 1.81, in low shade intensity plants (Figure 2(d)).

Based on the ideal minimum DQI value of 0.20, suggested by Hunt [14] (1990), it is reasonable to infer that when grown under full sun, seedlings of *P. gigantocarpa* would have a better quality for reforestation (Figure 1(d)). For *S. parahyba*, although the DQI values for all treatments were always above the minimum ideal value suggested by Hunt [14], these values were higher under low shade intensity, which could be considered as the adequate light environment for the production of seedlings of this species (Figure 2(d)). Similarly, Melo et al. [29] and Aguiar et al. [23], studying *Enterolobium contortisiliquum* (Vell.) and *Caesalpinia echinata* Lam under different light intensities, considered higher DQI values (greater than 0.20), as the seedlings with the best quality for reforestation purposes.

### 3.4. Physiological Parameters

One of the first responses of plants to environmental stress is a reduction in stomatal conductance [30]. Therefore, it can be inferred that because the highest stomatal conductance values for *P. gigantocarpa* were found under full sun and for *S. parahyba* were measured under low shade intensity, these light environments were adequate for these species, allowing a greater stomata aperture and increased transpiration. Based on the gas exchange behavior of the studied species, it can be concluded that these tropical forest tree species require high light intensities for maximum photosynthesis rates (Figures 1(e)–1(g) and 2(e)–2(g)). Accordingly, *Cupania vernalis* Camb. [31] and *Dipteryx alata* Vog. [32] show higher gas exchange values when grown at higher light intensity.

### 3.5. Anatomy Description

In *P. gigantocarpa*, stem cross section made on the first evaluation period (Figures 3(a)–3(d) and 3(i)) revealed trichomes, epidermis with polygonal cells, principle of lenticels; cortex with angular collenchyma without intercellular spaces, followed by layers of isodiametric parenchyma cells with intercellular spaces (Figures 3(i) and 3(j)). Sequentially, we observed sclerenchyma bands adjacent to the vascular system. This vascular system showed the greatest development in seedlings grown under full sun, resulting from the greater cambial activity (ca. 500 μm). In the second evaluation period (Figures 3(e)–3(h) and 3(j)), we observed the periderm, isodiametric parenchyma cells with smaller intercellular spaces, sclerenchyma cap, and increased vascular system, consisting of phloem, cambium, xylem, and medulla. This vascular system, measured in the second evaluation period, was higher in seedlings under lower shade.
intensity, resulting from the greater cambial activity (ca. 800 μm) (Figure 1(h)).

In *S. parahyba*, stem cross section revealed absence of trichomes. In the first evaluation period, we found that the cortex is composed of isodiametric parenchyma cells without intercellular spaces, sclerenchymatic cap interrupted, followed by phloem, cambium, xylem, and medulla (Figures 4(a)–4(d) and 4(i)). The highest values of cambial activity were close to 800 μm, which were measured in plants grown under full sun and at low shade intensity. In the second evaluation period (Figures 4(e)–4(h) and 4(j)), we observed that the stem was composed of periderm, lenticels, smaller

Figure 3: *P. gigantocarpa* cross sections stem. (a–d, i) Plants 15 days under different shade intensities. (e–h, j) Plants 60 days under different shade intensities. (a, e) Seedlings in full sun, (b, f) low, (c, g) moderate, and (d, h) high shade intensity. (i, j) Epidermis and cortex. Cambial activity (CA), xylem (x), cambium (c), and phloem (p).
area layers of parenchyma cells, and well-developed vascular system, resulting from the high cambial activity (ca. 2500 \( \mu \text{m} \)), tending to be higher in low shade intensity plants (Figure 2(h)). In the second evaluation period, cambial activity was lower under high shade intensity in \textit{P. gigantocarpa} and tended to decrease in \textit{S. parahyba}. On both evaluation periods and on both species, the cambium was already well developed, with the complete ring cambium in full activity.

3.6. Cambial Activity. For both species, there was no evaluation period \( \times \) treatment interaction for CA. However, when each evaluation period was considered individually,
Table 2: Correlation coefficient (r) of the correlations between stem diameter (D), total dry mass (TDM), shoot dry weight (SDM), Dickson Quality Index (DQI), photosynthesis (A), stomatal conductance (gs), or transpiration (E) versus cambial activity of P. gigantocarpa and S. parahyba (n = 5).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>P. gigantocarpa</th>
<th>S. parahyba</th>
</tr>
</thead>
<tbody>
<tr>
<td>D</td>
<td>0.80*</td>
<td>0.94*</td>
</tr>
<tr>
<td>SDM</td>
<td>0.34</td>
<td>0.68</td>
</tr>
<tr>
<td>TDM</td>
<td>0.45</td>
<td>0.80</td>
</tr>
<tr>
<td>DQI</td>
<td>0.38</td>
<td>0.86</td>
</tr>
<tr>
<td>A</td>
<td>0.35</td>
<td>0.88</td>
</tr>
<tr>
<td>gs</td>
<td>0.21</td>
<td>0.75</td>
</tr>
<tr>
<td>E</td>
<td>0.27</td>
<td>0.83</td>
</tr>
</tbody>
</table>

*Results obtained by nonparametric correlations analysis.

significant differences among treatments were found for these variables. On both evaluation periods, the CA was reduced on high shade intensity plants of both species (Figures 1(h), 2(h), 3, and 4).

The higher vascular system development under increasing light observed in the studied species (Figures 1(h) and 2(h)) could be explained by the role of light in affecting cambial activity by interacting with hormones, such as auxin [13] and gibberellin [11, 13], stimulating photosynthesis and increasing the carbohydrate production that may be used to xylem and phloem buildup. Furthermore, environmental factors are known to affect the size and organization of the vascular elements [33, 34] in order to ensure adequate transport in conducting vessels, especially when the plant is subject to stress. Other studies also found the influence of environmental factors on the development and structure of the vascular system, which is derived from the cambial activity [10, 35–37].

3.7. Cambial Activity, Physiological Parameters, and Plant Growth. The positive correlation of the cambial activity with physiological (A, gS, and E), morphological (stem diameter, stem dry mass, and total dry mass), and anatomical (CA) parameters of P. gigantocarpa and S. parahyba (Table 2) suggested that the development of these forest seedlings is greatly affected by the cambium activity.

The higher CA and the consequent increase in xylem production observed for both species in the least shaded environments probably increased the pressure gradient in the phloem, as a result of the sugars loading and unloading [38, 39]. Thus, it could be inferred that in less shaded environments, increased cambial activity and, consequently, higher shoot development might have stimulated the photosynthetic rate. This happens because stem thickening increases the demand for photosynthates, because the stem is the preferred sink in the vegetative plant stage [40]. Finally, the higher cambial activity increased stem biomass and diameter, resulting in better seedlings quality. It is also important to note that environmental factors affect this mechanism. In the present study the highest shade intensity reduced cambial activity and decreased gas exchange and biomass production, resulting in reduced seedlings quality of both species, which would likely decrease their survival in the field.

4. Conclusions

We concluded that the cambial activity and the physiological and morphological attributes work together, explaining the radial growth and increasing seedlings quality (adequate Dickson Quality Index), which was optimized efficient seedling production under full sun, in P. gigantocarpa, and under low shade intensity in S. parahyba.

Abbreviations

A: Photosynthesis
gs: Stomatal conductance
E: Transpiration
CA: Cambial activity
DQI: Dickson Quality Index.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors’ Contribution

Marco Antônio Menezes Neto and Eniel David Cruz designed the experiments. Monyck Jeane dos Santos Lopes performed the experiments. Moacir Bernardino Dias-Filho analyzed the data. Monyck Jeane dos Santos Lopes and Moacir Bernardino Dias-Filho wrote the paper.

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