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Does a Commercial Organic Fertilizer with Hydrogel or Biochar Guarantee the Quality of Eucalyptus Seedlings?

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Abstract

Our objective was to evaluate the effect of a commercial organic fertilizer and substrate conditioners on the production of *Eucalyptus benthamii* seedlings. Two experiments were conducted with different doses of organic fertilizer (0, 5, 10, 15, 20, and 25 kg m^{-3}) and levels of hydrogel (0 and 3 kg m^{-3}) and biochar (0 and 30%). In each experiment, plots were divided into two subplots, with one subplot receiving topdressing with mineral fertilizers. At the end of each experimental period, quality, root morphology, and physiological characteristics of the seedlings were assessed. When only the organic fertilizer was applied with substrate conditioners, seedlings exhibited limited growth, averaging 5.02 cm in height and 0.81 mm in stem diameter. Topdressing fertilization combined with higher organic fertilizer doses (20–25 kg m^{-3}) enhanced key traits, such as height (up to 24.15 cm) and stem diameter (up to 2.39 mm). Hydrogel and biochar often reduced seedling quality and root development. Some interactions between factors affected certain root variables, but physiological characteristics remained largely unaffected. Overall, even when combined with a substrate conditioner, the commercial organic fertilizer is insufficient to produce high-quality seedlings. Neither hydrogel nor biochar is recommended under our experimental conditions. However, the commercial organic fertilizer shows potential when used with mineral fertilizers and further should be conducted to explore this possibility.

Keywords: *Eucalyptus benthamii*; pelletized; substrate conditioners; forest nursery

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1. Introduction

Recently, the planted forest area in Brazil reached 10.2 million hectares. Of this total, approximately 76% corresponds to Eucalyptus plantations, distributed across all regions of the country [1]. In tropical climate areas, high-yield species and hybrids stand out, such as *E. grandis* and *E. urograndis*, respectively. On the other hand, in subtropical regions, it is

essential to use species that combine high productivity with resistance to cold and frost. In this context, *E. benthamii* is a considerably relevant species [2].

In terms of productivity, *E. benthamii* can reach values above 400 m^3 ha^{-1}, showing superior performance compared to other species, such as *E. dunnii* and *E. grandis*, depending on the cultivation conditions [3]. From an economic perspective, the species stands out for the versatility of its wood, which can be used for sawn timber, panels, and energy generation [4–6].

Although *E. benthamii* is consolidated in the forestry sector, strategies are still needed to increase its productivity and adaptability. The production of quality seedlings depends on different factors, among them the substrate and fertilization. In this context, management practices that combine production efficiency with the rational use of inputs, such as fertilizers and substrate conditioners, represent sustainable alternatives to improve seedling quality and reduce environmental impacts.

The appropriate choice of substrate plays a central role, since it directly influences seedling growth and final quality. However, substrates available on the market alone do not fully meet the nutritional demands of plants, making the use of fertilizers indispensable. Controlled-release fertilizers are widely employed in *Eucalyptus* seedlings, as they promote the gradual release of nutrients [7], reducing application costs and environmental risks, such as leaching [8]. Nevertheless, they still have a higher cost compared to other fertilizers.

Thus, a promising alternative is the use of organic fertilizers. These fertilizers can be obtained from plant, animal, industrial, or domestic residues, supplying essential nutrients to plants and showing potential for improvement when associated with other components [9]. In addition to nutritional supply, these inputs favor the sustainability of production systems by promoting waste reuse and reducing dependence on mineral fertilizers [10]. In the soil, they contribute to aggregate formation, increase water retention capacity and cation exchange, and stimulate microbial communities [11,12]. When incorporated into substrates for seedling production, they can improve biometric and biochemical characteristics of plants [13].

In general, organic fertilizers have shown potential for the production of seedlings of native and exotic forest species. However, despite this potential, organic fertilizers alone are usually not sufficient to meet seedling nutritional demand. Nevertheless, when used in combination with other fertilizers or products, this limitation can be overcome [14–16]. Among the most relevant conditioners for seedling production are hydrogel and biochar.

Hydrogel is a hydrophilic polymer capable of retaining several times its weight in water [17]. When incorporated into the substrate, it increases water availability and storage for seedlings, potentially reducing mineral fertilizer requirements by up to 50% [18], as well as improving physical and chemical properties of the substrate and enhancing morphological and physiological plant traits [18,19].

Biochar, in turn, is a carbon-rich material obtained through the thermal conversion of biomass under limited oxygen conditions [20] and can be produced from forest by-products [21]. When added to the soil, this product can contribute to physical characteristics, such as greater water availability [22], biological aspects, such as richness and diversity of microorganisms [23], and chemical characteristics, due to nutrient availability, such as phosphorus [24]. In substrates for seedling production of forest species, biochar can also positively influence plant growth and physiology [21,25].

Considering the relevance of the *Eucalyptus* genus and the need to optimize the use of fertilizers and substrate conditioners in the production of forest species seedlings, our objective was to evaluate the effects of a commercial organic fertilizer, in combination with hydrogel or biochar, on the characteristics of *E. benthamii* seedlings. In this way, we sought to answer the following question: when associated with a substrate conditioner, is a

commercial organic fertilizer sufficient to ensure the quality of *E. benthamii* seedlings? Our hypotheses were: (1) when associated with a substrate conditioner, the organic fertilizer is sufficient to ensure the quality of *E. benthamii* seedlings; and (2) Hydrogel or biochar can be effectively combined with the commercial organic fertilizer to produce high-quality *E. benthamii* seedlings.

2. Materials and Methods

2.1. The Experiments

Two experiments were carried out at the Forest Nursery of the University of Santa Catarina State (UDESC), Lages, Santa Catarina, between November 2023 and May 2024. According to the Köppen climate classification, the region's climate is humid subtropical (Cfb), with average temperatures during the warmest season being below 22 °C [26].

Both experiments were set up using a completely randomized design, applying a commercial organic fertilizer (a pelleted organic fertilizer produced from poultry litter, wood shavings, other agro-industrial residues and 0.2% of amylaceous), hydrogel (Hydroplan-EB, Vinhedo, Brazil), and biochar (obtained from the ash of forest biomass combustion in an industrial boiler and produced at 800 °C) (Table 1). In the first experiment (Experiment 1), we sowed seeds of *E. benthamii* (from one to up to three seeds, which were obtained from a seed production area of a local company) directly into 55 cm^3 tubes (manufactured by Dacko, Erval Grande, Brazil) filled with commercial substrate (also manufactured by Dacko, composed of Peat, vermiculite, agro-industrial organic residue and limestone. pH = 5.5 and electrical conductivity = 0.4 dS m^{-1}, density = 130 kg m^{-3} and water holding capacity = 300%). After germination, the excess of seedlings (when more seeds were implanted) were removed to maintain only one seedling per tube at 30 days. The treatments consisted of the combination of different doses of the commercial organic fertilizer (0, 5, 10, 15, 20, and 25 kg m^{-3}) and different levels of hydrogel (0 and 3 kg m^{-3}), totaling 12 treatments, with 4 replications and 25 seedlings per replication. The doses of the commercial organic fertilizer were defined arbitrarily due to the lack of previous studies with this specific fertilizer, while the hydrogel levels were determined based on available literature [27].

Table 1. Characteristics of the pelleted organic fertilizer and biochar used in the experiments.

Product	Total Organic Carbon	Total Nitrogen	pH	Cation Exchange Capacity	C/N Ratio
	%		-	mmolc kg^{-1}	-
Organic fertilizer	15.0	0.5	7.0	75	20
Biochar [1]	12.8	0.1	7.9	-	-

[1] Data were provided by [28], where further details can be found upon request.

In the second experiment (Experiment 2), we transplanted the excess seedlings from Experiment 1 that were up to 5 cm in height into 55 cm^3 tubes filled with commercial substrate (the tubes and the substrate were from the same manufacturer as those used in Experiment 1). The transplanted seedlings remained in a mini-tunnel system with average temperature and humidity of 25.2 °C (±6.3) and 96.9% (±5.1), respectively, for 30 days after transplantation. The treatments were the combination of doses of the same pelleted organic fertilizer (0, 5, 10, 15, 20, and 25 kg m^{-3}) and different levels of biochar (0 and 30% of the substrate volume), totaling 12 treatments, with 4 replicates and 25 seedlings per replicate. The biochar levels were determined based on previous experiments (unpublished data). The treatments of the experiments are summarized in Table 2.

Table 2. Description of the treatments in Experiment 1 and Experiment 2.

Treatments	Experiment 1		Experiment 2	
	Organic Fertilizer Doses	Hydrogel Levels	Organic Fertilizer Doses	Biochar Levels
	kg m^{-3}	kg m^{-3}	kg m^{-3}	%
1	0	0	0	0
2	5		5	
3	10		10	
4	15		15	
5	20		20	
6	25		25	
7	0	3	0	30
8	5		5	
9	10		10	
10	15		15	
11	20		20	
12	25		25	

Experiment 1 and Experiment 2 were conducted for 150 and 140 days, respectively. During this period, the vegetal materials were initially kept in a shade house, with an average temperature of 20.6 °C (±7.2). At 75 days (Experiment 1) and 45 days (Experiment 2), 12 seedlings from each plot received topdressing fertilization with Potassium Chloride (60% K_2O) and Monoammonium Phosphate (58% P_2O_5 and 12% N) every 15 days [29], totaling four fertilizations for each experiment. Plants without and with topdressing fertilization were considered as subplots. At 135 and 115 days, respectively, for Experiment 1 and Experiment 2, the seedlings were transferred to the hardening area under full weather conditions, remaining there until the end of the experimental period. Throughout this period, we irrigated and monitored the occurrence of pests, diseases, and spontaneous plants. Furthermore, we emphasize that the differing periods for interventions and evaluations were driven by operational constraints, based on the resources available at the time.

2.2. Evaluated Characteristics

At the end of the experimental periods (150 and 140 days, respectively), we evaluated the quality, root morphology and physiological characteristics of the seedlings.

Regarding the quality characteristics, the height (*H*—cm) and stem diameter (*SD*—mm) of all seedlings were measured. Subsequently, the aerial part and root of two representative seedlings (with the values closest to the average subplot diameter) per subplot were separated. These components were dried in a forced-air oven at 65 °C for 72 h and then weighed (g) to obtain the shoot dry mass (*SDM*) and root dry mass (*RDM*), respectively. Based on these data, the total dry mass (*TDM*—g), the height/stem diameter ratio (*H*/*SD*), and shoot dry mass/root dry mass ratio (*SDM*/*RDM*) were calculated. Finally, the Dickson Quality Index (*DQI*) [30] was calculated, using the formula:

$$DQI = \frac{TDM}{\left(\frac{H}{SD}\right) + \left(\frac{SDM}{RD}\right)}$$

Regarding the root morphology characteristics, in each subplot, the root of one representative seedling (with the value closest to the average subplot diameter) was separated,

washed, and stored in a solution of water and 70% alcohol (1:1). Subsequently, this material was transferred to acrylic trays and analyzed with an EPSON 1200XL scanner (Epson do Brasil, Barueri, Brazil), using the WinRHIZO® software (version 2021a), to obtain the average diameter (mm), length (cm), surface area (cm^2), volume (cm^3), number of tips, forks, and crossings of the roots.

For the physiological characteristics, an Infrared Gas Analyzer—IRGA (model Li-6400xt, manufactured by LI-COR, LincoIn, NE, United States) was used. Thus, readings were taken on one representative seedling (with the value closest to the average subplot diameter) per subplot for the assimilation rate—*A* (μmol CO_2 m^{-2} s^{-1}), stomatal conductance—*gs* (mol H_2O m^{-2} s^{-1}), intercellular CO_2 concentration—*Ci* (μmol CO_2 mol^{-1}), transpiration rate—*E* (mmol H_2O m^{-2} s^{-1}), and the ratio between intercellular and atmospheric CO_2 concentrations—*Ci*/*Ca* (μmol CO_2). Subsequently, water use efficiency—*A*/*E* (μmol CO_2 m^{-2} s^{-1}/ mmol H_2O m^{-2} s^{-1}) and carboxylation efficiency—*A*/*Ci* (μmol CO_2 m^{-2} s^{-1}/ μmol CO_2 mol^{-1}) were calculated.

2.3. Statistical Analyses

To evaluate the effect of topdressing fertilization on the seedlings, for each characteristic, the averages of the subplot pairs were compared. For this, first, the Shapiro–Wilk test was performed, and then the paired t-test was conducted. The effect size chosen for this analysis was Glass's Δ. This effect size and its corresponding confidence interval were calculated [31,32].

For the analysis of the effect of factors (organic fertilizer doses and hydrogel or biochar levels) on the seedlings with topdressing, the assumptions of normality of residuals and homogeneity of variances were verified, respectively, with the Shapiro–Wilk and Bartlett tests. When at least one of these assumptions was not met, the data were transformed using common transformations (x^2, $x^{0.5}$, log(x), $x^{-0.5}$, or x^{-1}). Once the assumptions were met, a two-way Analysis of Variance (ANOVA) was performed, and when an interaction or isolated effect of one or more factors was observed, the Scott-Knott test (organic fertilizer doses) and/or t-test for independent samples (hydrogel or biochar levels) was conducted. The effect size chosen for the two-way ANOVA was the partial omega squared (Ω^2 *p*). This effect size and its confidence interval were calculated [33].

For all these tests, analyses, and calculations, the significance level adopted was 5% ($\alpha = 0.05$), and all these procedures, in addition to the creation of the graphs, were performed in RStudio version 4.3.2—"Eye Holes" [34]. Figure 1 illustrates the statistical procedure.

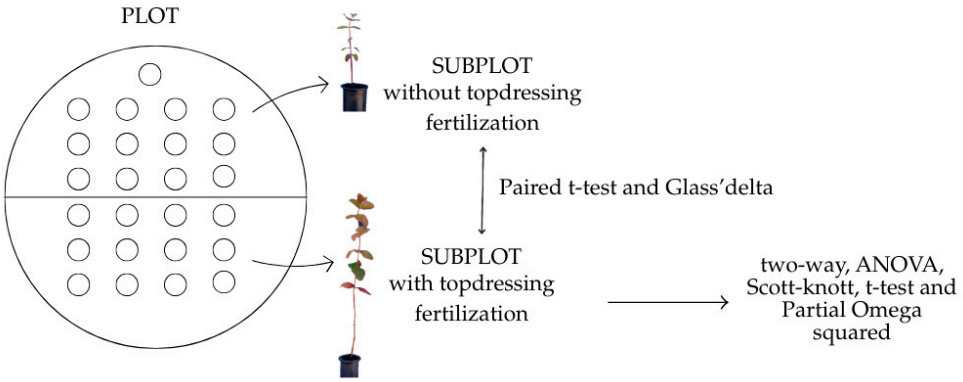

Figure 1. Flowchart of the statistical procedure for the analysis of Experiment 1 and Experiment 2.

3. Results

3.1. Experiment 1

3.1.1. Quality Characteristics

In general, there was a significant difference ($p < 0.05$) between the averages without and with topdressing fertilization for the seedling quality variables of *E. benthamii*. On average, the Glass's Δ value for these variables was 17.903 (Supplementary Table S1), and the values of the main quality characteristics (without topdressing fertilization) were: 5.48 cm, 1.06 mm, 0.10 g, and 0.01, for height, stem diameter, total dry mass, and Dickson's Quality Index, respectively.

There was no significant interaction ($p > 0.05$) between factors for the seedling quality traits of *E. benthamii* with topdressing fertilization. However, there was an isolated effect of the factors for stem diameter; an isolated effect of organic fertilizer doses for the height/stem diameter ratio; and an isolated effect of hydrogel levels for height, total dry mass, Dickson's Quality Index, aerial part dry matter, and root dry matter. On average, the Ω^2 p value was 0.086, 0.188, and 0.024 for organic fertilizer doses, hydrogel levels, and the interaction between these factors, respectively (Supplementary Table S2).

In general, the values of the seedling quality traits of *E. benthamii* were better in the treatments without hydrogel. Regarding the doses of organic fertilizer, no difference was found between doses for stem diameter. On the other hand, for the height/stem diameter ratio, the values were higher starting from the dose of 20 kg m^{-3} (Figure 2 and Supplementary Figure S1). Without hydrogel (with topdressing fertilization), the average values of height, stem diameter, total dry mass, and Dickson Quality Index were 21.02 cm, 2.27 mm, 0.74 g, and 0.06, respectively.

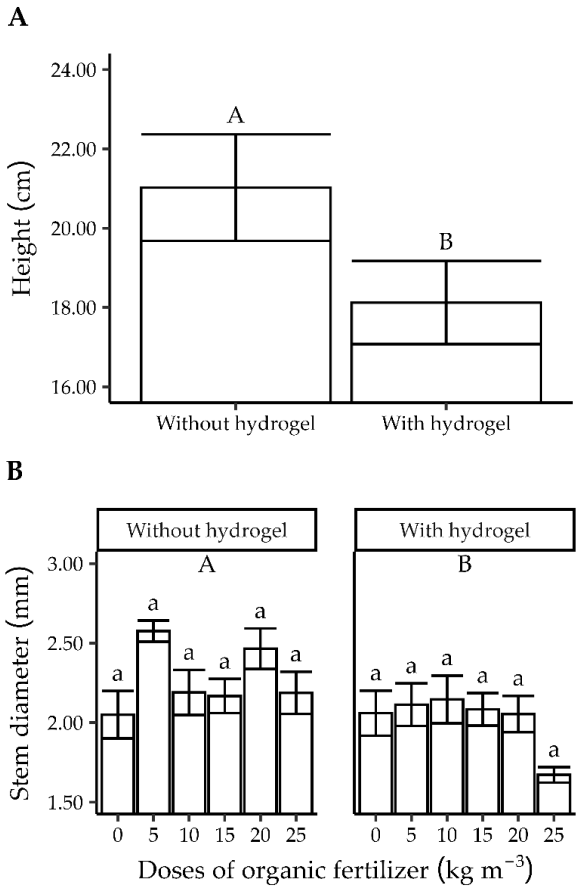

Figure 2. *Cont.*

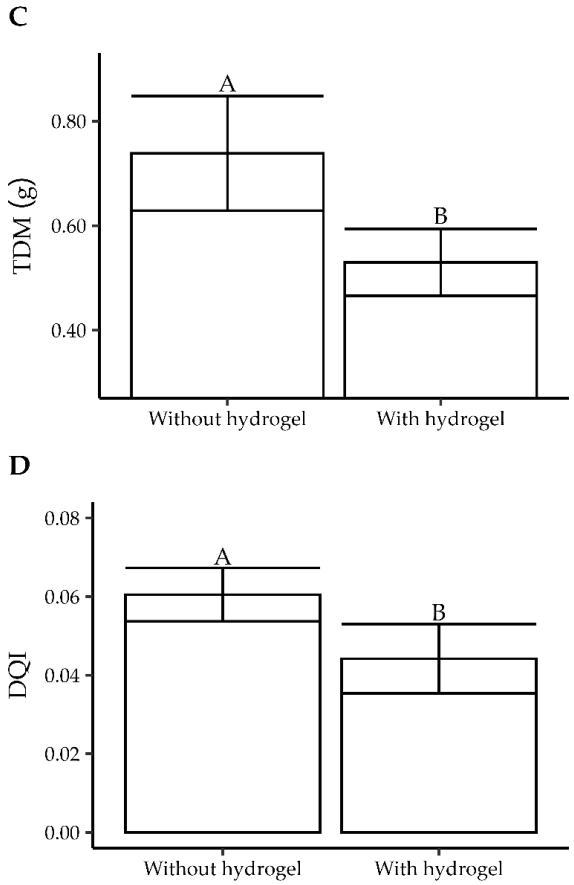

Figure 2. Height (**A**), stem diameter (**B**), total dry mass (**C**), and Dickson's Quality Index (**D**) of *Eucalyptus benthamii* seedlings produced with organic fertilizer doses, hydrogel levels, and topdressing fertilization. TDM: total dry mass; DQI: Dickson Quality Index. Averages ± standard error followed by the same lowercase letter (organic fertilizer doses) and uppercase letter (hydrogel levels) do not differ from each other, respectively, according to the Scott-Knott test and *t*-test ($\alpha = 0.05$).

3.1.2. Root Morphological Characteristics

Commonly, there was a significant difference ($p < 0.05$) between the averages with and without topdressing fertilization for the seedling morphology variables of *E. benthamii* (Supplementary Table S1 and Figure S2). On average, the value of Glass's Δ for these variables was 10.979, and the value of the main root morphology traits (without topdressing fertilization) was 0.58 mm, 111.21 cm, 19.58 cm^2, and 0.28 cm^3 for average diameter, length, surface area, and root volume, respectively.

There was an interaction between the factors ($p < 0.05$) for all root morphological variables of *E. benthamii* seedlings with topdressing fertilization, except for average diameter. On average, the Ω^2 p value was 0.123, 0.003, and 0.256 for the doses of organic fertilizer, hydrogel levels, and the interaction between these factors, respectively (Supplementary Table S2).

In general, at the dose of 0.0 kg m^{-3} of organic fertilizer, the plants without hydrogel showed higher values for the root morphological variables. Additionally, when considering the treatments without hydrogel, the use of organic fertilizer led to a reduction in the values of these variables, although they did not differ from the treatments with hydrogel (Table 3). On average, the values of average diameter, length, surface area, and root volume without organic fertilizer and hydrogel (with topdressing fertilization) were, respectively, 0.66 mm, 412.37 cm, 91.03 cm^2, and 1.61 cm^3.

Table 3. Root morphology and physiological characteristics of *Eucalyptus benthamii* seedlings with topdressing fertilization based on doses of organic fertilizer and hydrogel levels at 150 days.

Variable	Hydrogel Levels	Doses of Organic Fertilizer (kg m^{-3})					
		0	**5**	**10**	**15**	**20**	**25**
Average diameter (mm)	Without hydrogel	0.70 ns	0.65	0.66	0.62	0.75	0.69
	With hydrogel	0.64	0.67	0.60	0.65	0.67	0.62
Length (cm)	Without hydrogel	412.37 Aa	301.30 Ab	235.81 Ab	239.86 Ab	261.74 Ab	249.85 Ab
	With hydrogel	220.97 Bb	354.46 Aa	310.71 Aa	230.34 Ab	257.89 Ab	300.18 Aa
Surface area (cm^2)	Without hydrogel	91.03 Aa	60.44 Ab	48.53 Ab	46.89 Ab	62.05 Ab	53.31 Ab
	With hydrogel	44.20 Ba	74.40 Aa	58.49 Aa	47.08 Aa	53.59 Aa	59.14 Aa
Volume (cm^3)	Without hydrogel	1.61 Aa	0.98 Ab	0.80 Ab	0.74 Ab	1.18 Ab	0.95 Ab
	With hydrogel	0.71 Ba	1.25 Aa	0.88 Aa	0.77 Aa	0.89 Aa	0.95 Aa
Tips	Without hydrogel	1037.75 Aa	850.75 Aa	609.25 Bb	682.50 Ab	634.00 Ab	648.75 Ab
	With hydrogel	544.75 Bb	1087.50 Aa	928.50 Aa	600.25 Ab	705.50 Ab	812.00 Aa
Crossings	Without hydrogel	1509.00 Aa	819.75 Ab	585.50 Ab	572.75 Ab	818.50 Ab	620.50 Ab
	With hydrogel	537.75 Ba	1089.50 Aa	906.00 Aa	599.50 Aa	679.75 Aa	909.75 Aa
Crossings	Without hydrogel	666.00 Aa	257.50 Ab	178.00 Ab	157.75 Ab	286.50 Ab	192.25 Ab
	With hydrogel	143.50 Ba	370.50 Aa	287.00 Aa	159.50 Aa	216.50 Aa	328.25 Aa
A	Without hydrogel	3.63 ns	4.19	2.68	3.84	3.70	4.10
	With hydrogel	2.67	3.20	3.71	3.81	5.34	4.11
gs	Without hydrogel	0.21 ns	0.15	0.34	0.23	0.19	0.18
	With hydrogel	0.21	0.24	0.32	0.20	0.30	0.18
E	Without hydrogel	3.22 ns	2.67	3.42	3.52	2.95	3.13
	With hydrogel	3.33	3.30	3.87	3.22	3.78	2.85
Ci	Without hydrogel	243.28 ns	224.07	263.34	248.75	233.23	237.18
	With hydrogel	256.69	251.77	250.18	240.10	237.14	224.76
Ci/*Ca*	Without hydrogel	0.86 ns	0.80	0.92	0.88	0.83	0.84
	With hydrogel	0.88	0.88	0.89	0.85	0.85	0.80
A/*Ci*	Without hydrogel	0.02 ns	0.02	0.01	0.02	0.02	0.02
	With hydrogel	0.01	0.01	0.02	0.02	0.02	0.02
A/*E*	Without hydrogel	1.16 ns	1.59	0.79	1.05	1.37	1.30
	With hydrogel	0.90	0.99	1.03	1.26	1.46	1.60

Averages followed by the same uppercase letter (hydrogel levels) and lowercase letter (organic fertilizer dose) do not differ from each other by the Scott-Knott test and independent *t*-test, respectively; ns: not significant for the isolated effect or interaction between the factors ($\alpha = 0.05$). *A*: assimilation rate (μmol CO_2 m^{-2} s^{-1}); *gs*: stomatal conductance (mol H_2O m^{-2} s^{-1}); *E*: transpiration rate (mmol H_2O m^{-2} s^{-1}); *Ci*: intercellular CO_2 concentration (μmol CO_2 mol^{-1}); *Ci*/*Ca*: ratio between intercellular and atmospheric CO_2 concentration (μmol CO_2); *A*/*E*: water use efficiency (μmol CO_2 m^{-2} s^{-1}/mmol H_2O m^{-2} s^{-1}); *A*/*Ci*: carboxylation efficiency (μmol CO_2 m^{-2} s^{-1}/μmol CO_2 mol^{-1}).

3.1.3. Physiological Characteristics

In general, there was no significant difference ($p > 0.05$) between the averages with and without topdressing fertilization for the physiological variables of *E. benthamii* seedlings. On average, the value of Glass's Δ for assimilation rate, carboxylation efficiency, and water use efficiency was 9.036, and −2.313 for the other variables (Supplementary Table S1). On average, the value of the main physiological variables (without topdressing fertilization) was 1.11 μmol CO_2 m^{-2} s^{-1} 0.34 mol H_2O m^{-2} s^{-1}, and 4.80 mmol H_2O m^{-2} s^{-1}, respectively, for assimilation rate, stomatal conductance, and transpiration rate.

Furthermore, there was also no interaction or isolated effect of the factors ($p > 0.05$) for the physiological variables of *E. benthamii* seedlings with topdressing fertilization (Table 3). On average, the value of Ω^2 *p* was −0.015, −0.017, and −0.043 for the doses of

organic fertilizer, hydrogel levels, and the interaction between these factors, respectively (Supplementary Table S2). On average, the value of the main physiological variables (with topdressing fertilization) was 3.74 μmol CO_2 m^{-2} s^{-1}, 0.23 mol H_2O m^{-2} s^{-1}, and 3.27 mmol H_2O m^{-2} s^{-1}, respectively, for assimilation rate, stomatal conductance, and transpiration rate.

3.2. Experiment 2

3.2.1. Quality Characteristics

In general, there was a significant difference ($p < 0.05$) between the averages with and without topdressing fertilization for the seedling quality variables of *E. benthamii*. On average, the Glass's Δ value for these variables was 28.543 (Supplementary Table S3). On average, the value of the main quality characteristics (without topdressing fertilization) was 4.60 cm, 0.88 mm, 0.07 g, and 0.01, in order, for height, collar diameter, total dry matter, and Dickson Quality Index.

There was an isolated effect ($p < 0.05$) of at least one factor for all the quality variables of *E. benthamii* seedlings, except for the aerial part/root dry mass ratio. On average, the value of Ω^2 p was 0.272, 0.181, and 0.051 for organic fertilizer doses, biochar levels, and the interaction between these factors, respectively (Supplementary Table S4).

In general, the values for the quality characteristics of *E. benthamii* seedlings were better in the treatments without biochar. Regarding the doses of organic fertilizer, the values were also higher from the dose of 20 kg m^{-3} onwards (Figure 3 and Supplementary Figure S3). Without biochar and, in general, 20 to 25 g dm^{-3} of organic fertilizer (with topdressing fertilization), the average values for height, diameter, total dry matter, and Dickson Quality Index were 23.46 cm, 2.31 mm, 0.84 g, and 0.07, in that order.

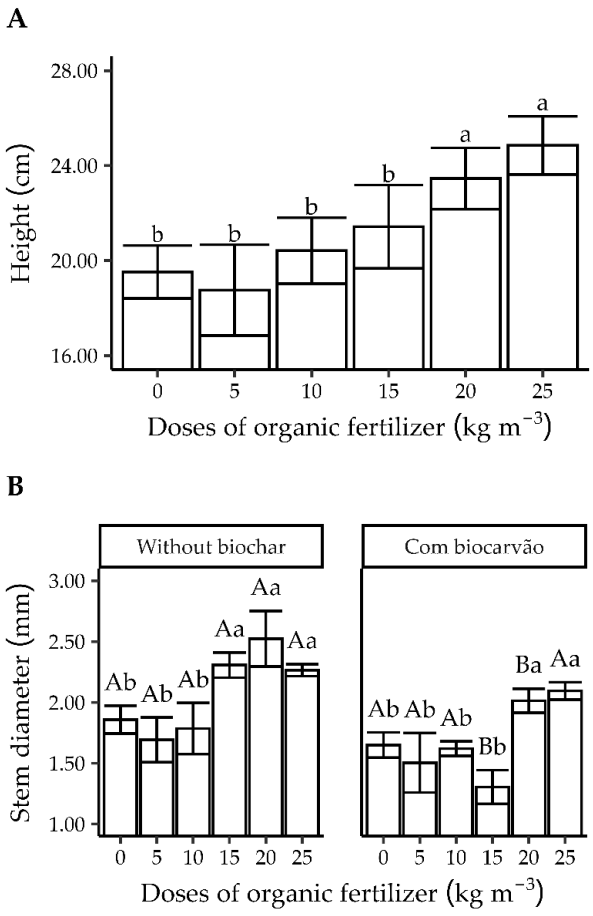

Figure 3. *Cont.*

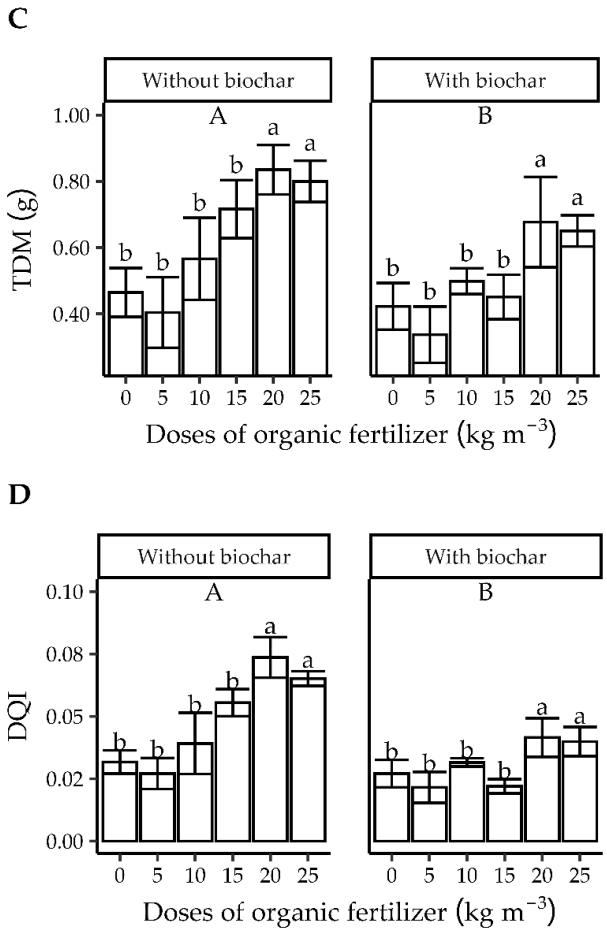

Figure 3. Height (**A**), stem diameter (**B**), total dry mass (**C**), and Dickson Quality Index (**D**) of *Eucalyptus benthamii* seedlings produced with organic fertilizer doses, biochar levels, and topdressing fertilization. TDM: total dry mass; DQI: Dickson Quality Index. Averages ± standard error followed by the same lowercase letter (organic fertilizer doses) and uppercase letter (biochar levels) do not differ between each other, respectively, by the Scott-Knott test and *t*-test ($\alpha = 0.05$).

3.2.2. Root Morphological Characteristics

Frequently, there was a significant difference ($p < 0.05$) between the means without and with cover fertilization for the morphological root variables of *E. benthamii* seedlings (Supplementary Table S3 and Figure S2) On average, the Glass Δ value for these variables was 13.152, and the values of the main root morphology traits (without cover fertilization) were 0.52 mm, 131.38 cm, 20.81 cm^2, and 0.26 cm^3 for mean diameter, length, surface area, and volume, respectively.

According to ANOVA, there was either an interaction or an isolated effect of the organic fertilizer doses ($p < 0.05$) for all analyzed variables, except for mean diameter. On average, the Ω^2 p values were 0.217, 0.005, and 0.164 for organic fertilizer doses, biochar levels, and the interaction between these factors, respectively (Supplementary Table S4).

In general, at the dose of 0.0 g dm^{-3} of organic fertilizer, plants with biochar showed higher values for root morphological variables. Furthermore, when considering these treatments with biochar, the doses of 0.0 and 10.0 g dm^{-3} were not different from each other but were higher than the other doses of organic fertilizer (Table 4). On average, the values of the main root morphology traits with 0.0 and 10.0 g dm^{-3} of organic fertilizer and with biochar (with topdressing fertilization) were 404.61 cm, 81.82 cm^2, and 1.32 cm^3 for length, surface area, and volume, respectively. The mean diameter was 0.66 mm on average.

Table 4. Root morphology and physiological characteristics of *Eucalyptus benthamii* seedlings with topdressing fertilization based on organic fertilizer doses and biochar levels at 140 days.

Variable	Biochar Levels	Doses of Organic Fertilizer (kg m^{-3})					
		0	**5**	**10**	**15**	**20**	**25**
Average diameter (mm)	Without biochar	0.60 ns	0.67	0.70	0.68	0.65	0.66
	With biochar	0.65	0.63	0.65	0.62	0.68	0.70
Length (cm)	Without biochar	220.09 Ba	320.38 Aa	304.49 Ba	259.79 Aa	247.18 Aa	264.61 Aa
	With biochar	383.32 Aa	271.52 Ab	425.89 Aa	240.03 Ab	204.78 Ab	266.45 Ab
Surface area (cm^2)	Without biochar	41.78 Ba	68.93 Aa	65.98 Aa	55.50 Aa	49.96 Aa	54.82 Aa
	With biochar	77.35 Aa	53.31 Ab	86.28 Aa	45.98 Ab	43.08 Ab	55.13 Ab
Volume (cm^3)	Without biochar	0.64 Ba	1.19 Aa	1.15 Aa	0.95 Aa	0.81 Aa	0.90 Aa
	With biochar	1.24 Aa	0.83 Ab	1.39 Aa	0.71 Ab	0.72 Ab	0.95 Ab
Tips	Without biochar	734.25	1118.25	1330.50	621.50	774.00	723.50
	With biochar	1008.50	898.75	1469.25	685.00	703.50	1093.50
Crossings	Without biochar	514.75 Bb	1018.00 Aa	837.75 Ba	547.00 Ab	562.25 Ab	611.00 Ab
	With biochar	1271.25 Aa	606.50 Bb	1307.75 Aa	514.50 Ab	475.25 Ab	616.00 Ab
Crossings	Without biochar	146.00 Bb	376.25 Aa	257.25 Ba	149.25 Ab	163.75 Ab	173.50 Ab
	With biochar	480.50 Aa	198.00 Bb	492.25 Aa	152.25 Ab	145.75 Ab	203.00 Ab
A	Without biochar	8.90 ns	7.27	9.71	7.35	8.59	6.44
	With biochar	6.94	5.48	7.27	6.92	7.80	8.59
gs	Without biochar	0.50 * A	0.41	0.60	0.39	0.56	0.32
	With biochar	0.34 B	0.27	0.32	0.30	0.44	0.42
E	Without biochar	3.91 ns	3.59	3.99	3.57	3.96	3.43
	With biochar	3.57	3.08	3.55	3.01	3.93	3.29
Ci	Without biochar	252.53 ns	254.13	254.95	250.76	258.12	249.56
	With biochar	248.62	254.65	242.05	236.56	252.86	239.28
Ci/*Ca*	Without biochar	0.88 ns	0.88	0.89	0.87	0.90	0.86
	With biochar	0.86	0.87	0.84	0.81	0.88	0.83
A/*Ci*	Without biochar	0.04 ns	0.03	0.04	0.03	0.03	0.03
	With biochar	0.03	0.02	0.03	0.03	0.03	0.04
A/*E*	Without biochar	2.32 ns	2.04	2.45	2.06	2.14	1.87
	With biochar	1.95	1.76	2.05	2.38	1.99	2.59

Averages followed by the same uppercase letter (biochar levels) and lowercase letter (organic fertilizer dose) do not differ from each other by the Scott-Knott test and independent *t*-test, respectively; ns: not significant for interaction or the isolated effect of the factors; isolated effect of biochar levels; *: isolated effect of biochar levels ($\alpha = 0.05$). *A*: assimilation rate (μmol CO_2 m^{-2} s^{-1}); *gs*: stomatal conductance (mol H_2O m^{-2} s^{-1}); *E*: transpiration rate (mmol H_2O m^{-2} s^{-1}); *Ci*: intercellular CO_2 concentration (μmol CO_2 mol^{-1}); *Ci*/*Ca*: ratio between intercellular and atmospheric CO_2 concentration (μmol CO_2); *A*/*E*: water use efficiency (μmol CO_2 m^{-2} s^{-1}/mmol H_2O m^{-2} s^{-1}); *A*/*Ci*: carboxylation efficiency (μmol CO_2 m^{-2} s^{-1}/ μmol CO_2 mol^{-1}).

3.2.3. Physiological Characteristics

In general, there was a significant difference ($p > 0.05$) between the means without and with topdressing for the physiological variables of *E. benthamii* seedlings. On average, the Glass Δ value was −5.657 for intercellular CO_2 concentration and the ratio between intercellular and atmospheric CO_2 concentration, and 105.521 for the other variables (Supplementary Table S3). The values of the main physiological variables (without topdressing) were −0.07 μmol CO_2 m^{-2} s^{-1}, 0.02 mol H_2O m^{-2} s^{-1}, and 0.56 mmol H_2O m^{-2} s^{-1}, respectively, for assimilation rate, stomatal conductance, and transpiration rate.

There was no interaction or isolated effect of the factors ($p > 0.05$) for the physiological variables of *E. benthamii* seedlings with topdressing, except for stomatal conductance, which showed an isolated effect of biochar levels (Table 4). On average, the Ω^2 *p* values were

0.009, 0.043, and −0.026 for organic fertilizer doses, biochar levels, and the interaction between these factors, respectively (Supplementary Table S4). The values of the main physiological variables (with topdressing fertilization) were 7.61 μmol CO_2 m^{-2} s^{-1} and 3.57 mmol H_2O m^{-2} s^{-1} for assimilation rate and transpiration rate, respectively. For stomatal conductance, without biochar, the mean value was 0.46 mol H_2O m^{-2} s^{-1}.

4. Discussion

Our results did not confirm the first hypothesis that, when combined with a substrate conditioner, the commercial organic fertilizer would be sufficient to ensure the quality of *E. benthamii* seedlings. In fact, seedlings were only able to reach the minimum requirements recommended in the literature for certain characteristics when topdressing fertilization was applied, which showed an effect size that can be considered of enormous magnitude [35].

Although the benefits of organic fertilizers are widely reported in agricultural sciences [36], nutrient release from these sources may not fully meet the plants' demand, being limited by factors such as substrate temperature and moisture [37,38]. Moreover, the nutrient concentration in organic fertilizers is generally lower than in mineral fertilizers [39]. Altogether, these aspects may explain why the association of organic fertilizer with substrate conditioners was not sufficient in our experiments.

To better understand the influence of fertilization on seedling quality, it is important to consider the main quality indicators. Height and stem diameter are probably the two most widely used characteristics in the context of forest seedling production, as they are easy to measure. For the genus *Eucalyptus*, it is recommended that seedlings be sent to the field with minimum values of 20.00 cm and 2.00 mm [40], respectively. Dry mass, in turn, is a good indicator of seedling hardening [41] and, although no specific value is recommended, higher values are desirable [42]. Regarding the Dickson Quality Index (DQI), higher values indicate higher seedling quality, and for forest seedlings produced in small containers, a minimum of 0.20 can be considered adequate [43].

In both experiments, when topdressing fertilization was applied, the seedlings reached the reference values for the main quality indicators, with the exception of the Dickson Quality Index. This exception is likely related to the dry mass values, which are present in both the numerator and denominator of the index and therefore strongly influence its final value. In the literature, other authors performed additional fertilizations, along with a 30 30-day hardening period, and reported considerably higher dry mass values and, consequently, higher Dickson Quality Index values than those observed in the present study [44,45]. In our case, only four topdressing fertilizations were carried out and the seedlings were exposed to full sunlight for up to 20 days. Thus, E. benthamii seedlings may achieve higher quality than that observed here if subjected to greater fertilization inputs and longer hardening periods.

In addition to quality characteristics, root morphology characteristics are also important. The number of tips indicates the number of terminal root portions, while the number of forks represents lateral branching [46]. Crossings, in turn, represent distinct roots overlapping in a two-dimensional projection [47]. Together with the other root characteristics, these variables indicate the plant's ability to explore soil beyond the depletion zone, i.e., the capacity to capture water and nutrients and thereby sustain greater vegetative growth [48]. In this sense, higher values are desirable for all these characteristics. In the literature, for instance, the best treatments yielded values greater than 0.40 mm, 1500.00 cm, 200.00 cm^2, and 2.00 cm^3 for average root diameter, length, surface area, and volume, respectively, in seedlings of *E. dunnii*, *E. saligna*, and *E. urograndis* [49]. For *E. grandis* and *E. saligna*, values close to or exceeding 1.00 mm, 2000.00 cm, 200.00 cm^2, and 2.00 cm^3, in the same order, have also been reported [50]. Despite the similar climatic conditions and plant age,

these experiments used different genetic materials and larger containers (6.0 and 8.0 L, respectively), which most likely explains their superior results compared to ours.

Beyond morphology, physiological characteristics provide additional insights into seedling performance. The main variables that characterize photosynthesis under a given environmental condition are the assimilation rate and the transpiration rate, both of which are directly measurable through gas exchange [51]. These two variables are regulated by stomatal conductance [52], which is a measure of the rate of H_2O loss or CO_2 absorption by the leaf, and can be demonstrated by the mechanism of stomatal opening and closing [53].

Thus, higher values of assimilation rate are desired, and higher values of stomatal conductance and transpiration rate can accompany these values. However, the kinetics of stomatal response are considerably slower than the kinetics of photosynthetic response, resulting in a temporal mismatch between CO_2 assimilation and water loss [54]. In the literature, under the best conditions, values higher than those of the present article have often been observed for seedlings of 16 different genetic materials from the genera *Eucalyptus* and *Corymbia*, with an average of 12.30 μmol CO_2 m^{-2} s^{-1}, 0.33 mol H_2O m^{-2} s^{-1}, 4.15 mmol H_2O m^{-2} s^{-1} for assimilation rate, stomatal conductance, and transpiration rate, respectively [55]. These differences occurred due to the different genetic materials and cultivation conditions, such as fertilization, in addition to the age of the seedlings, which were older than those of the present study.

Therefore, our second hypothesis suggested that hydrogel or biochar could be effectively combined with the commercial organic fertilizer to produce high-quality *E. benthamii* seedlings, but our results did not confirm it. After all, considering both experiments, there was basically no significant interaction between these factors for the quality and physiological characteristics of the seedlings. In fact, in both experiments, the value of Ω^2 p for the interaction in the physiological variables was negative, which indicates the absence of an effect [33]. Moreover, although this interaction existed for most of the root morphology variables, it was not sufficiently beneficial for the plants to the point of justifying the use of these factors together. However, the isolated effects of organic fertilizer doses and substrate conditioner levels on seedlings with topdressing fertilization, in both experiments, bring us important perspectives.

Regarding the use of organic fertilizer, doses starting from 20 kg m^{-3} were able to provide significant effects with median (Ω^2 $p > 0.059$) and large (Ω^2 $p > 0.138$) magnitudes on the quality characteristics of the seedlings that received topdressing [56]. This indicates the potential of this organic fertilizer to be used in conjunction with mineral fertilizers. In the literature, several authors have pointed out the potential of combining these types of fertilizers [14–16]. Probably, the positive effect of the association between these fertilizers occurs for various reasons related to the improvement of the chemical and physical characteristics of the substrate. For example, there are reports of increased cation exchange capacity and water retention of the medium with the use of organic fertilizers [57,58], both characteristics inversely related to nutrient leaching, which may reduce the losses of nutrients provided by mineral fertilizers and, consequently, increase the efficiency of the use of these fertilizers. In fact, some studies have evaluated this combined use in field experiments with agricultural species and found the possibility of reducing the amount of mineral fertilizers used [59,60].

Regarding the use of conditioners in the substrate, these present significant effects of large magnitude (Ω^2 $p > 0.138$) on the quality characteristics of the seedlings [56]. The benefits of using hydrogel [18,61,62] and biochar [63–67] have been reported in the literature for forest seedlings. However, in the present study, the use of these conditioners negatively affected seedling characteristics, which does not support our second hypothesis that both substrate conditioners would be recommended for the production of Eucalyptus benthamii

seedlings. Negative effects of using hydrogel [68] or biochar [69] as substrate conditioners have also been recently reported.

Considering that the water demand of seedlings is closely related to local climatic conditions, such as temperature and humidity [70], it is possible that the divergence in the results is related to the different climatic characteristics of the experimental sites. For example, in Santa Maria, state of Rio Grande do Sul, where several studies with hydrogel were conducted [18,71], the average annual values for temperature (+3.3 °C) and humidity (−2.1%) differ from those observed in Lages, state of Santa Catarina [72], resulting in higher water demand. In this scenario, these results highlight the importance of understanding the ideal physical parameters for forest seedling production and emphasize that the use of substrate conditioners, such as hydrogel and biochar, must be linked to the proper adjustment of doses and irrigation regimes under different climatic conditions.

In the case of biochar, additional mechanisms may have contributed to the observed results. For instance, it can reduce available nitrogen [73] and contain potentially harmful elements or organic compounds, depending on feedstock and pyrolysis temperature [74]. If this is the case, our results suggest that the use of biochar for seedling production should consider not only the quantity but also the origin and production process of this material.

Finally, we emphasize that it is not our intention to suggest that eucalyptus seedlings should be produced with topdressing fertilization, as this entails operational costs and does not reflect the typical production context in the country, where controlled-release fertilizers are generally used. Therefore, topdressing fertilization was applied solely to (1) confirm that the lack of plant growth was due to nutritional limitations and (2) assess the potential of the commercial organic fertilizer to be used in combination with a mineral fertilizer. Both objectives were successfully achieved.

Furthermore, we note that we did not perform physical, chemical, or biological analyses of the substrate, and our study was limited to the nursery. Thus, we acknowledge the main limitations of our study. First, the effects of organic fertilizer application under field conditions remain unverified. Second, the mechanisms underlying the negative effects observed with substrate conditioners were not elucidated.

Notably, our work was the first to test this commercial organic fertilizer for seedling production. For future studies, we suggest evaluating higher doses of this fertilizer (e.g., up to 50 kg m^{-3}) in combination with controlled-release fertilizers, with assessments conducted both in the nursery and under field conditions. Furthermore, we reported appropriate effect size values for the statistical tests used, which is uncommon in the literature on this topic when correlation or regression analyses are not applied. We hope that this approach will encourage other researchers in agricultural sciences to go beyond the *p*-value. As this practice becomes more widespread, it will be possible to establish specific values for interpreting different effect sizes in the context of seedling production.

5. Conclusions

Even when combined with a substrate conditioner, the commercial organic fertilizer is insufficient to produce high-quality seedlings. Neither hydrogel nor biochar is recommended to be combined with this organic fertilizer under our experimental conditions.

However, the commercial organic fertilizer shows potential when used with mineral fertilizers, and further research should be conducted to explore this possibility.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f16091489/s1, Figure S1. Shoot dry matter—SDM (A), root dry matter—RDM (B), Shoot dry matter/Root dry matter—SDM/RDM (C), and Height/Steam Diameter ratio (D) of *Eucalyptus benthamii* seedlings with topdressing fertilization as a function of organic fertilizer doses and hydrogel levels at 150 days. Means ± standard error followed by the same

lowercase letter (organic fertilizer dose) and uppercase letter (hydrogel level) do not differ from each other, respectively, according to the Scott–Knott test and *t*-test ($\alpha = 0.05$). ns: not significant for interaction or isolated factor effect. Figure S2. Roots of Eucalyptus benthamii seedlings as a function of organic fertilizer rates and hydrogel levels, without and with topdressing fertilization, at 150 days. Figure S3. Shoot dry matter—SDM (A), root dry matter—RDM (B), Shoot dry matter/Root dry matter—SDM/RDM, and Height/Stem diameter ratio (H/SD) of Eucalyptus benthamii seedlings under topdressing fertilization as a function of organic fertilizer doses and biochar levels at 140 days. Means ± standard error followed by the same lowercase letter (organic fertilizer doses) and uppercase letter (biochar levels) do not differ from each other, respectively, according to the Scott–Knott test and *t*-test ($\alpha = 0.05$). ns: not significant for the interaction or for the isolated effect of the factors. Figure S4. Roots of Eucalyptus benthamii seedlings as a function of organic fertilizer doses and biochar levels, without and with topdressing fertilization, at 140 days. Table S1. Paired *t*-test and effect size for the quality, root morphology and physiological variables of Experiment 1. Table S2. Effect size for quality, root morphology and physiological characteristics of Eucalyptus benthamii seedlings with topdressing fertilization, as a function of organic fertilizer doses and hydrogel levels at 150 days. Table S3. Paired *t*-test and effect size for quality, root morphology and physiological variables of Experiment 2. Table S4. Effect size for quality, root morphology and physiological characteristics of Eucalyptus benthamii seedlings with topdressing fertilization, as a function of organic fertilizer doses and biochar levels at 140 days.

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