

Glitter Interference On Photosynthetic Rates of A Submerged Macrophyte (*Egeria Densa*)

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Abstract

This study analyzed the photosynthetic rates (by the light and dark bottle method) of the submerged macrophyte *Egeria densa* in the presence of three concentrations of glitter: 0.0235 g (T1/T4), 0.0117 g (T2/T5) and 0.0058 g (T3/T6), as well in its absence (control treatment, CT1 and CT2). About 800 apical fragments of *E. densa* were distributed in 8 subtreatments (4 under light conditions and 4 in the dark to obtain respiration), with 100 specimens in each. The CT showed the highest net photosynthesis rate (P_N = gross photosynthetic (P_G) rate subtracted from respiration (R_D)) of *E. densa*, with 59.3%, 32.8%, 13.0% higher compared to T1, T2 and T3, respectively. At T3 it was observed the highest mean respiration rate (R_E) of *E. densa* and at T1, the lowest. Comparing P_N with R_D , we found that the photosynthetic process was, on average 3.5, 2.47, and 2.93 times higher in CT, T1, T2, and T3, respectively. The presence of glitter may have increased the reflectance of water, as it is a suspended particle and reflected light intensely, considering that it is a metal coated particle. Glitter reflects radiation, decreasing the light absorption process, compromising the use of underwater radiation by *E. densa*. The microplastic interferes with the absorption of light necessary for photosynthetic processes, reducing them, enabling an imbalance in the ecosystem.

1. Introduction

As considered a primary microplastic (Horton et al. 2017; Yurtsever 2019a), glitter is usually made of Mylar™, a specific plastic polymer of BoPET polyester (Yurtsever 2019a) and, aiming at high reflectivity, is coated with aluminum (Locher et al. 2018), bismuth, titanium or iron (Yurtsever 2019b). It presented a melting point of 260°C, a density of 1.38 g cm⁻³ and is insoluble in water (Locher et al. 2018). Glitter is produced and commercialized in a wide diversity of colors and shapes (hexagonal, triangular, stars, among others). It is applied in several products (e.g., handbags, shoes, EVA and jewelry), from which they tend to detach due to use or forwarded to landfills, being possible to contaminate the ecosystem (Yurtsever 2019a) if glitter remain in the objects.

Glitter sizes range from 50 µm to 6250 µm, with those of 100 µm and 200 µm being usually applied in cosmetics (Blackledge and Jones 2007). Easily adhered to the skin, either by static electrical force, small size or dermal oils, the removal of glitter is usually done by fluxing, resulting in direct discharge into wastewater treatment plants (Tagg and Ivar do Sul 2019; Yurtsever 2019a) and, as other microplastics, with likely release in the effluent to the aquatic ecosystem (Murphy et al. 2016) or accumulation in the sludge of sewage treatment plants (Tagg and Labrenz 2018).

Many aquatic species inhabiting inland waters, such as aquatic macrophytes that perform multiple ecological functions (Wagner et al. 2014; Lambert et al. 2014) may meet microplastics. According to Graneli and Solander (1988), aquatic macrophytes allow a constant flow of nutrients due to their uptake, accumulation and release, contributing organic matter in the food chain (Cunha-Santino et al. 2010); in which they play a primary role (Cellamare et al. 2012); amplify spatial heterogeneity (Catian et al. 2012)

due to the addition of structures in the environment (Weaver et al. 1997); act as limnological indicators, presenting a behavior that reflects the environmental conditions (Melzer 1999), among others.

Egeria densa (Planch) belongs to the Hydrocharitaceae family, popularly known as Brazilian eelgrass. It is a freshwater herbaceous, submerged, rooted perennial plant and native macrophyte from South America, being found in slow flowing water environments. This species is present in at least 27 countries around the world in both subtropical and temperate regions (Hussner and Lössch 2005; Yarrow et al. 2009). *E. densa* banks are often dominant in clear water with little radiation attenuation (Carrillo et al. 2006) and temperature ranging between 16 and 28 C (Barko and Smart 1981); these conditions were attended in the experiments carried out at this stage of the project.

Productivity and ecosystem can be subject to modifications due to changes in biomass or macrophyte composition, with impacts on the nutrient cycle, biota and physicochemical characteristics of the lake (Carpenter and Lodge 1986). According to Schwarz and Hawes (1997), water clarity can be a limiting factor in the occupied area and in the submerged macrophyte biomass, reducing both. Abiotic functions such as temperature, transparency, pH, alkalinity and conductivity (in decreasing order of relevance), besides nutrients, mediate in the distribution of aquatic macrophytes (Lacoul and Freedman 2005).

Therefore, the photosynthetic process and primary productivity of submerged macrophytes can be limited mainly by light (Chen and Coughenour 1996). Considering this fact, this study assumes that the presence of glitter, regardless of concentration, results in interference with the paths of solar radiation (i.e., absorption and reflection) underwater and in effects similar to high turbidity conditions, and, consequently, a decrease in photosynthetic rates of *Egeria densa*.

2. Materials And Methods

The macrophytes used in the experiments were collected in the Experimental Garden of the Departamento de Botânica (21° 59' 00, 37" S 47° 52' 47, 42" W), in January and June, and the bioassays developed in the Laboratório de Bioensaios e Modelagem at the Departamento de Hidrobiologia/UFSCar, from January to September. In total, 800 individuals of *E. densa* were collected, being 100 used in each treatment (n = 8 treatments). Photosynthetic rates were analyzed by the light and dark flask method (Strickland 1960; Vinatea et al. 2010). The treatments contemplated were: macrophytes in the presence of glitter (0.0058 g, 0.0117 g, and 0.0235 g) with radiation (T1, T2 and T3, respectively) and without radiation (T4, T5, and T6, respectively) and glitter-free macrophytes subjected to radiation (control treatment, TC1) and isolated from it (control treatment, TC2). The glitter used in this study showed particles ranging in size from 0.063 mm to 0.71 mm.

The glitter concentrations were determined based on the same order of magnitude as the experiments carried out with the accumulation of micro and nanoplastics in oysters (Gaspar et al. 2018), and, therefore, do not represent the concentrations found in nature, being a laboratory simulation. For treatments with the presence of glitter (T1 to T6), an analytical balance (model M214Ai, brand Bel Engineering; precision 0.0001 g) was used to determine the masses of microplastic used in the

bioassays: ca. 0.0235g, 0.0117g, and 0.0058g, for this procedure a spatula with a microspoon was used. Recently, it was estimated a daily release of $2.7\text{--}3.0 \times 10^7$ and $2.5\text{--}2.7 \times 10^6$ glitter particles in activated sludge residue and effluent from a water treatment plant (Raju et al. 2020).

To remove debris from the branches of aquatic macrophytes (Pezzato and Camargo 2004), considering that these plants can retain several particles (Schulz et al. 2003), the macrophytes were gently washed with running water and then distilled. Apical fragments of 7 cm were selected and inserted in 300 ml volume biochemical oxygen demand (BOD) flasks, filled, according to Pezzato and Camargo (2004) and aiming to simulate the natural environment with greater similarity, with ambient water, ie, collected from the Monjolinho Reservoir (22° 00' S and 47° 54' W), at the Universidade Federal de São Carlos. After collection, it was necessary to remove the particulate matter from the water using a qualitative filter paper (ϕ pore size = 3 μm). According to Santos et al. (2010), in 2010 the Monjolinho Reservoir presented a varied pH, from acidic to basic (5.86 to 9.45), dissolved oxygen ranging from 4.89 mg L^{-1} to 14.43 mg L^{-1} , and high concentrations of nutrients such as nitrogen and phosphorus. The Reservoir it is a small, shallow, and eutrophic aquatic system (Regali-Selegheim and Godinho 2004).

In each incubation ($n = 800$), the initial dissolved oxygen concentrations of the water were analyzed in an oximeter (YSI, model 58) before inserting the *E. densa* fragments in the BOD bottles. Then, the bottles were incubated in a germination system for two hours (adapted from Pezzato and Camargo 2004) under the incidence of radiation ($47.25 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR (Machado et al. 2020; Wanderley et al. 2021); mean temperature: $23.3 \pm 1.8^\circ\text{C}$), conditions similar to Rodrigues and Thomaz (2010) (variation up to 200 $\text{mmol m}^2 \text{s}^{-1}$ RFA at 22°C), and gently constant stirring so that the glitter remains suspended, ensuring its homogeneity in the BOD flasks. Regarding the flasks without radiation (dark condition), these remained in the germination chamber simultaneously in the magnetic stirrer but were completely sealed with aluminum foil.

A new measurement of dissolved oxygen concentration was performed in each BOD bottle after incubation in light/dark. Then, the aquatic macrophytes were removed from the flasks and dried in the drying oven (ca. 45°C) until constant mass, and their masses were determined by gravimetry (model M214Ai, Bel Engineering; precision 0.0001 g). After obtaining the data, the photosynthesis and respiration calculations were performed using Equations 1 to 3 (Littler and Arnold 1985).

$$P_N = \frac{(c-i) \times v}{t \times DM} \text{ Eq. 1}$$

$$R_D = \frac{(i-d) \times v}{t \times DM} \text{ Eq. 2}$$

$$P_G = P_N + R_D \text{ Eq. 3}$$

where: P_N = net photosynthesis ($\text{mg O}_2 \text{ g}^{-1} \text{ DM h}^{-1}$); R_D = respiration ($\text{mg O}_2 \text{ g}^{-1} \text{ DM h}^{-1}$); P_G = gross photosynthesis; c = final O_2 concentration in the light flask (mg L^{-1}); d = and final O_2 concentration in the

dark flask (mg L^{-1}); i = O_2 concentration in the initial flask (mg L^{-1}); v = incubation flask volume (L); t = incubation time (h) and DM = incubated plant dry mass (g).

To verify the differences between the results of net photosynthesis and respiration of *E. densa* with the presence and absence of glitter, the homogeneity test (D'Agostino- Pearson) was performed. Since normality was not confirmed, the non-parametric Kruskal-Wallis test was applied. A p value < 0.05 was considered statistically significant.

3. Results

The results of net photosynthesis (P_N), respiration (R_D) and gross photosynthesis (P_G) in the 8 sub-treatments (6 with glitter and 2 without glitter) are indicated in Fig. 1. Regarding P_N , the specimens of *E. densa* that were not subjected to the presence of glitter demonstrated the second highest variation (minimum = $0.28 \text{ mg O}_2 \text{ g}^{-1} \text{ DM h}^{-1}$ and maximum = $22.54 \text{ mg O}_2 \text{ g}^{-1} \text{ DM h}^{-1}$) and highest value of photosynthetic rate ($7.60 \text{ mg O}_2 \text{ g}^{-1} \text{ DM h}^{-1}$), compared to the other treatments. In the T1, the P_N was the lowest of the four treatments (T1, T2, T3 and CT1); showing a variability about ca. half of the specimens that were not subjected to the presence of glitter (minimum of $0.51 \text{ mg O}_2 \text{ g}^{-1} \text{ DM h}^{-1}$ and maximum of $10.80 \text{ mg O}_2 \text{ g}^{-1} \text{ DM h}^{-1}$). The greatest variation among the four treatments (T1, T2, T3 and CT1) was presented in T2 (minimum of $0.37 \text{ mg O}_2 \text{ g}^{-1} \text{ DM h}^{-1}$ and maximum of $46.20 \text{ mg O}_2 \text{ g}^{-1} \text{ DM h}^{-1}$).

The normality test applied to the P_N rates revealed that only Treatment 3 (with 0.0235 g of glitter) presented $p > 0.05$ ($p = 0.2352$). When applied Dunn's Multiple Comparison Test, TC and T3 did not present a statistically significant difference, although a difference of 13% was registered. Among the other treatments were very and extremely important differences ($p \leq 0.001$). The P_N of specimens of *E. densa* that were not subjected to the presence of glitter was 1.5 and 2.5 times greater than the P_N of macrophytes with the presence of 0.0117 g and 0.0058 g of the microplastic, extremely significant differences of 32.76% and 59.34%, respectively. T3 presents P_N 1.31 and 2.17 times higher compared to P_N of T2 and T1, a very small difference ($p \leq 0.01$) of 23.85% and 46.05% respectively. The P_N of T2 was 1.65 times greater than the P_N of T1 (difference of 39.53%).

The mean R_D rates in the four treatments (T4, T5, T6 and TC2) were closer compared to the mean P_N rates (Fig. 1), with T4 showing the lowest value and variation, and T6 the highest value. None of these treatments obtained alpha value > 0.05 in the normality test. The mean respiration rate of TC2 was 1.74 times higher compared to T4; R_D of T6 was 1.83 times greater than T4; and R_D of T5 was 1.76 times greater than T4. Extremely significant differences were recorded between CT2 and T4 (CT2 being 1.74 times greater, and a difference of 42.66%), and between T4 and T6 (T6 being 1.83 times greater, and a difference of 45.42 %). T5 had R_D 1.76 times higher compared to T4, a significant difference ($p \leq 0.05$) of 43.18%.

When comparing P_N with R_D , it was found that the photosynthetic process was more efficient in terms of dissolved oxygen balance than R_D , being on average, 3.5, 2.47, 2.32, and 2.93 times higher in the treatments without and with glitter (ca. 0.0058 g, 0.0117 g, and 0.0235 g), respectively. The P_G is the sum between these two processes with no significant differences only between treatment without glitter and treatment with 0,0235 g ($p > 0.05$) and registering extremely significant differences among the other treatments.

The P_N rates, without the presence of the glitter, had greater variability compared to those submitted to the microplastic, as can be verified by the interquartile range and median. T2, however, presents the largest outliers. About 50% of the values of T1 and CT1 are above $2.5 \text{ mg O}_2 \text{ g}^{-1} \text{ DM h}^{-1}$ and below $12.5 \text{ mg O}_2 \text{ g}^{-1} \text{ DM h}^{-1}$, about 50% of the values of T2 are between 0 and $5 \text{ mg O}_2 \text{ g}^{-1} \text{ DM h}^{-1}$ and of T3 between 0 and $6 \text{ mg O}_2 \text{ g}^{-1} \text{ DM h}^{-1}$.

Regarding the R_D rates, the variabilities of the treatments with and without glitter were lowest, about 50% of the values in the range above $1 \text{ mg O}_2 \text{ g}^{-1} \text{ DM h}^{-1}$ and below $4 \text{ mg O}_2 \text{ g}^{-1} \text{ DM h}^{-1}$. The highest outliers remained related to T2, but T1 exhibited a higher amount of outlier compared to P_N , and both showed positive asymmetry (positive asymmetric data). The P_G rates, also, demonstrated high variability in the data and the highest number of outliers for *E. densa* submitted to the presence of 0.0117 g of the glitter. It was also found that for T4 and CT2 50% of the P_G values were above $5 \text{ mg O}_2 \text{ g}^{-1} \text{ DM h}^{-1}$ and below $15 \text{ mg O}_2 \text{ g}^{-1} \text{ DM h}^{-1}$, and for T5 and T6, 50% of the values were above $2.5 \text{ mg O}_2 \text{ g}^{-1} \text{ DM h}^{-1}$ and below $10 \text{ mg O}_2 \text{ g}^{-1} \text{ DM h}^{-1}$.

4. Discussion

Certain abiotic factors limit photosynthetic processes in aquatic macrophytes, among them, light, inorganic carbon, and temperature are the most relevant, but also oxygen concentration and current velocity (Madsen and Maberly 1991). The depth of the water body attenuates light exponentially (Pedersen et al. 2013), thus its penetration can be reduced by suspended particles, pigments in planktonic algae and dissolved organic matter (Staeher et al. 2012b). Considering that macrophytes are capable of transforming radiation energy into photosynthesis (Dale and Gillespie 1977), and that suspended particles reduce the penetration of light into the water body (Staeher et al. 2012b), the presence of glitter may have resulted in this decrease, since it behaves like a suspended sediment, and that the photosynthetic rates were lower in the incubations of *E. densa* with the microplastic. Despite resulting in effects similar to turbidity, the addition of microplastic glitter would possibly not result in the addition of nutrients, a situation similar to turbidity caused by soil particles (Machado et al. 2020).

In addition, it should be considered that on the surface of the water there is a possibility of reflectivity, transmission and absorption of incident energy, and the quantity and quality of suspended particles determine whether this will be absorbed, dispersed and transmitted. The reflectance of water varies

according to its surface and its interaction with direct solar radiation, its diffusion and transmission. Naturally, the water surface reflects some of the direct solar radiation (Novo et al. 1989), but the reflectance increases because of the elevation of suspended sediments (Bhatti and Nasu 2007). Furthermore, the smaller the particle size, the higher the reflectance (Holyer 1978). Thus, the microplastic glitter, acting as a suspended sediment, increased the reflectance of the water, reflecting radiation energy at a higher intensity and decreasing the absorption process, thus the underwater radiation may have been affected.

Considering that the reflectance of metals is highly intense (Stenzel 2016), and that glitter contains metal particles to achieve high reflectivity (Locher et al. 2018; Yurtsever 2019b), these microplastics also behaved as a reflective surface. According to Stenzel (2016), light can be absorbed or scattered in the volume or on the surface of the body, or else transmitted or reflected (partially or fully). The larger amount of microplastics may result in continued reflection of radiation energy between the glitter particles themselves, increasing the possibility of light remaining inside the bottle, which could explain the higher P_N and P_G rate in T3 (0.0235 g) than in T1 (0.0058 g). This effect can occur in aquatic ecosystems, at specific points with particle resuspension as a function of hydrodynamic flow or wind-induced resuspension (Bertrin et al. 2017), where *E. densa* (a macrophyte of submerged, rooted habit and wide global distribution) colonizes the backwaters of lotic environments (Pezzato and Camargo 2004) or the littoral zones of lentic environments (Vári 2013).

However, it should be considered that the wave energy (formed by the fetch) is one of the limitations for the distribution of submerged macrophytes, with some species showing areas of preference for colonization where sediment suspension and water mixing is lower (Chambers 1987), such as *E. densa*, which has reduced distribution where water movement, and consequently sediment resuspension, is higher (Bertrin et al. 2017). Therefore, it is possible to assume that in natural environments, the photosynthetic response of the *E. densa* would be like T1, considering the reduced resuspension of microplastics and the larger dimensions of the water body compared to the bioassays used in this study, resulting in a wider space and distance between the resuspended glitter particles.

Thus, the glitter interfered in the radiation that could be absorbed in the photosynthetic processes. According to Morini and Muleo (2003), light regulates plant growth and development, relating directly with photosynthesis rates of submerged macrophytes (Menendez and Peñuelas 1993; Menendez and Sanches 1998), being extremely essential for photosynthetic processes (Freedman and Lacoul 2006). Therefore, the presence of microplastic may decrease photosynthetic rates in a monospecific bank of *E. densa*, however this process will not be determinant in the dissolved oxygen balance at these spots. However, it should be noted that reducing the photosynthesis rate can prejudice the macrophyte, since, as stated by Simpson and Eaton (1986) the efficiency of this process can determine the success of a specie.

The variation between photosynthetic rates in the same species may be due to the phenological stage of the plant, as well as the specific environmental conditions in which the plant develops (Rodrigues and Thomaz 2010), time of year, time when the plant is found in the water column (Pezzato and Camargo

2004), and temperature (Haramoto and Ikusima 1988). Therefore, considering that the specimens of *E. densa* used in the research had different phenological stages, to reproduce a real aquatic environment, and that the study was developed during different seasons of the year, such aspects may have influenced the large outliers of the treatments, like those presented by T2 and T5.

In general, although the application of glitter is not often on a daily occurrence, some single-use situations can result in a substantial amount in wastewater treatment plants and consequently in aquatic ecosystems, such as carnival festivals and other celebrations. However, there are few investigations considering glitter as a microplastic pollutant, possibly due to little understanding about its composition, and this concern is currently expressed by society (Tagg and Ivar South, 2019). About eight different studies have documented glitter particles found in samples taken from the environment, with variation in the sizes, shapes, and colors of the microplastics. The samples taken at the water surface in much of the assessments of the microplastic presence in aquatic compartments may be one reason for the small amount of glitter observed, taking into consideration that the microplastic would likely be in the sediments of the aquatic compartments and treatment plant sludge.

The glitter could also not be detected during density separation or flotation of the sediment, sludge, water, and soil samples aimed at extracting microplastics, or from dissolving the color coating in acidic solution (Yurtsever 2019b). It is interesting to note that the change in particle coating was only possible in an infrequent situation under natural conditions, and therefore, the PET would remain intact when deposited in the aquatic compartment, with a higher probability of interfering with the macrophyte's photosynthetic rates due to its metallic surface.

Although there are multiple restrictions and subsidies for decreasing the waste of disposable plastics, there are no targeted measures for glitter, which is introduced into the ecosystem already in small fragments that can accumulate in the environment as a pollutant. Therefore, it is important to formulate regulations and restrictions regarding the production, and use of this individual microplastic or in other products on the market (Yurtsever 2019a), since the continued introduction of microplastics in aquatic ecosystems will constitute a major problem in the future (Sarijan et al. 2020). It is necessary to consider glitter as a contaminating microplastic, capable of interfering in essential activities for the ecosystem, such as the photosynthesis of aquatic macrophytes, due to its microplastic structure and metallic surface, which can increase water reflectance and light reflection. The possibility of interference in the stomata is also considered, as reported by Dong et al. (2020).

5. Conclusions

Analyzing the data obtained, it is possible to state that the glitter interfered in the underwater radiation. The presence of the microplastic, in the amounts of 0.0117 g (T2) and 0.0058 g (T1), presented an extremely significant influence, reducing the net photosynthesis rates of *E. densa* in about 32.76% and 59.34%, respectively, in relation to the Control Treatment 1 (CT1). It is considered that reducing the photosynthetic process, and consequently the dissolved oxygen in the water, especially by more than 50%

can result in several imbalances in the ecosystem. The macrophytes that interacted with glitter also showed higher atypical values in both average net photosynthesis rate and respiration rate. It is assumed that, in a natural ecosystem, the effects of the microplastic glitter on *E. densa* would be similar to those that occurred in T1 and T2.

Although the difference was not significant between CT1 and T3, a small difference (13%) was recorded in the net and gross photosynthetic rates, in *E. densa* specimens submitted to the presence of the glitter (in the amount of 0.0235 g) are lower than those of macrophytes without the microplastic. Thus, the presence of the microplastic always results in a decrease in the photosynthetic rate of the submerged macrophyte. The study concludes that this effect may be due to the microplastic structure of the particles, which increase suspended solid particles, and the glitter has a metallic surface (high reflectance), decreasing the light energy of the incident light that returns to the bottle, in addition to increasing water reflectance, decreasing light absorption for photosynthetic processes. Therefore, the importance of further studies on the influence of this microplastic on the aquatic ecosystem and future investigations to assign an interference proportion of the two identified factors is emphasized.

Abbreviations

E. densa, *Egeria densa*; T1, Subtreatment 1; T2, Subtreatment 2; T3, Subtreatment 3; T4, Subtreatment 4; T5, Subtreatment 5; T6, Subtreatment 6; CT1, Control Subtreatment 1; CT2, Control Subtreatment 2; BOD, Biochemical Oxygen Demand; P_N , Net photosynthesis; R_D , Respiration; P_G , Gross photosynthesis.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The three authors declare that they have no competing interests.

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Author's contributions

LLY conducted the research and investigation process, developed the methodology and wrote the original draft. IB contributed to review and editing the manuscript. MBCS provided the study materials and other analysis tools, supervised the project, and reviewed and edited the manuscript. All authors read and approved the final manuscript.”

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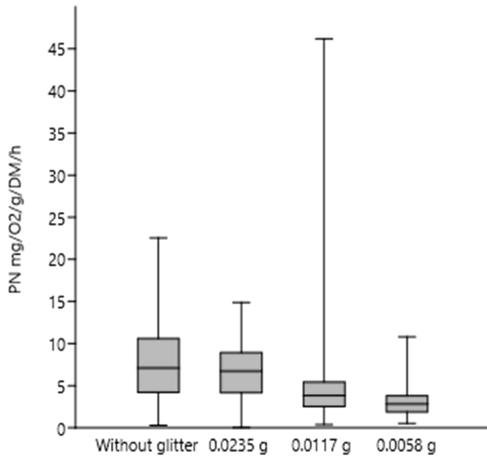
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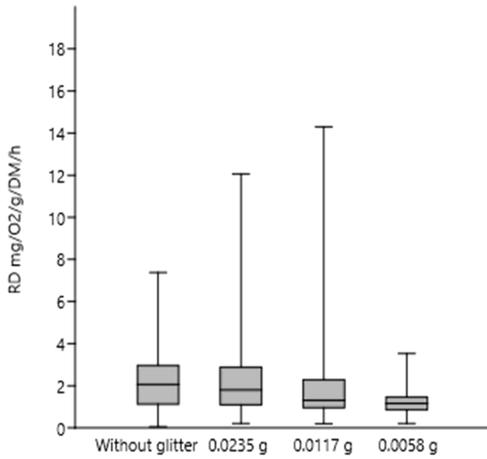
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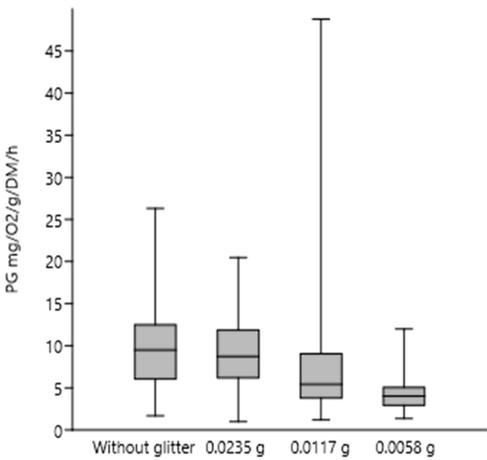
Figures



A



B



C

Figure 1

Box plot diagram of: (A) net photosynthesis (PN in mg O₂ g⁻¹ DM h⁻¹), (B) respiration (RD in mg O₂ g⁻¹ DM h⁻¹), (C) gross photosynthesis (PG in mg O₂ g⁻¹ DM h⁻¹) between the treatments without and with glitter.