

Organomineral fertilizer based on swine manure and *Trichoderma harzianum* improves the performance of *Eucalyptus grandis*

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Abstract

The productivity of forest plantations of the *Eucalyptus* genus in Brazil depends on applying large amounts of fertilizers. Controlled-release or organomineral fertilizers represent sustainable alternatives to traditional mineral fertilizers, allowing reduced production costs, higher growth rates, and environmental benefits. Additionally, using biological inputs such as fungi of the *Trichoderma* genus can increase forest productivity and improve plant quality and vigor. Therefore, this study aimed to characterize the influence of different types of fertilizers associated with *Trichoderma harzianum* on the morpho-physiological attributes of young *Eucalyptus grandis* plants. This study was conducted in a greenhouse using a completely randomized design, with six replicates in a 5 × 2 factorial scheme, considering four types of mineral or organomineral fertilizers and a control treatment, with or without *T. harzianum* inoculation. At 180 d after planting, the morphology of the aerial and roots and the aspects of the photosynthetic apparatus were evaluated using the Falker chlorophyll index and chlorophyll *a* fluorescence. For basic fertilization, *E. grandis* plants cultivated using a controlled-release fertilizer and an organomineral fertilizer based on swine manure showed the best results for the morphophysiological features evaluated. *T. harzianum* inoculation also improved the *E. grandis* development, especially when associated with controlled-release or organomineral fertilizers. Therefore, using swine manure to formulate organomineral fertilizers combined with inoculating *T. harzianum* in seedlings is a sustainable alternative fertilizer for young *E. grandis* plants.

INTRODUCTION

Commercial forest plantations occupy approximately 131 million hectares worldwide, with South America having the highest percentage (FAO 2020). In Brazil, the area of trees planted in 2022 was 9.94 million hectares, of which 76% is cultivated with species of the *Eucalyptus* genus (IBÁ 2023). *Eucalyptus grandis* W. Hill ex Maiden has the largest planted area and high productivity, adaptability, and wood quality (Campoe et al. 2014; Costa et al. 2017). *E. grandis* is economically important because of its various applications in raw materials such as cellulose, solid wood, and charcoal (Carocha et al. 2015).

The productivity of forest plantations is influenced by several factors, including water, nutrients, and light, which are managed to maximize cropping efficiency (Landsberg and Waring 2014). In Brazil, the high productivity of commercial eucalyptus plantations depends on applying large amounts of mineral fertilizers (Gonçalves et al. 2013; Silva et al. 2013), which increases the production costs and environmental concerns (Abreu-Junior et al. 2017).

Controlled-release fertilizers (CRF) have been tested to maximize plant growth and development (Silva et al. 2015; Adams et al. 2017). Gradually releasing nutrients reduces nutrient loss through leaching and the need for additional fertilizers, thus reducing plantation maintenance costs. Organic fertilizers are an alternative to conventional mineral fertilizers. In this context, the increase in global pork production (ABPA 2022) is directly related to waste generation (He et al. 2016). However, the waste generated can

be used for the recovery of nutrients, thus becoming a viable economic option in addition to minimizing the environmental liabilities generated by pig farming units.

Organic solid waste in its organic form mainly contains nitrogen, reducing the nitrate leaching capacity because the mineralization of organic nitrogen is slower than the nitrification with $\text{NH}_4^+\text{-N}$ (Sánchez-Báscones et al. 2019). However, applying fertilizers in excess in the fluid form can cause environmental damage, such as the contamination of water bodies and groundwater, in addition to being difficult to transport (Barros et al. 2021). To overcome these limitations, a technique has recently been developed to mix organic sources of nutrients and mineral fertilizers, creating so-called organomineral fertilizers (Morais and Gatiboni 2015; Borges et al. 2019; Mumbach et al. 2020). This composition can improve the efficiency of nutrient use and facilitate the transport and management of organic waste (Morais and Gatiboni 2015; Borges et al. 2019).

Using biological inputs such as fungi of the genus *Trichoderma*, ectomycorrhizae, and endomycorrhizae can be an effective strategy for enhancing forest productivity and improving the nutritional quality of plants (Vejan et al. 2021). *Trichoderma* spp. are the predominant opportunistic saprophytes and ubiquitous constituents of the soil microbiota found in all climatic zones (Rahman et al. 2023). *Trichoderma* spp. are robust endophytes that can colonize roots and modulate plant-soil-environment interactions directly or indirectly (Ghorbanpour et al. 2018). Compounds produced by these fungi, including antibiotics, can increase root resistance to pathogens, regulate root architecture, and stimulate the growth of primary and lateral roots, resulting in greater absorption and accumulation of nutrients by plants (Cai et al. 2013; Zin and Badaluddin 2020; Griebeler et al. 2021). Therefore, this study aimed to characterize the influence of different types of fertilizers associated with *Trichoderma harzianum* inoculation on the morpho-physiological attributes of young *Eucalyptus grandis* plants.

MATERIAL AND METHODS

Study conditions and experimental design

The experiment was conducted in a greenhouse at the Forest Nursery of the Federal University of Santa Maria, Frederico Westphalen Campus, Rio Grande do Sul, southern Brazil (27°23'46" S 53°25'32" W) from January to July 2021. The climate of the region is humid subtropical, with maximum temperatures $\geq 22^\circ\text{C}$ and minimum temperatures between -3 and 18°C (Alvares et al. 2013). We used clonal seedlings of *E. grandis* obtained from the Agroforestry Nursery of the Brazilian Tobacco Growers Association (AFUBRA), which were produced in 50-cm³ tubes and were approximately 120 days old, with an average height and diameter of 39.8 cm and 3.17 mm.

The experimental design was completely randomized, with six replicates in a 5×2 factorial scheme, with five different types of fertilizers, with or without *Trichoderma harzianum* Rifai inoculation added to the substrate. The fertilizers used were: 1) controlled-release fertilizer (CRF: NPK 16-08-12 + micronutrients); 2) traditional mineral fertilizer (TMF: NPK 20-10-15); 3) organomineral fertilizer (OMF: NPK 8-4-6)

produced by mixing organic fertilizer (OF) based on swine manure and TMF; and 4) soil remineralizing organomineral fertilizer (SROF: NPK 8-4-6) formulated by mixing OF with rock dust and TMF. The total amount of nutrients (NPK) applied was the same for all treatments. A control treatment (without fertilizer) was also used.

OMF and SROF were produced in granulated form through extrusion and spheronization at the Organomineral Fertilizer Production Unit at UFSM-FW. Additional information on obtaining the FO and rock dust can be obtained from Barros et al. (2021) and Andriguetto et al. (2024).

The *T. harzianum* inoculum consisted of a commercial product at 1×10^9 UFC mL⁻¹ concentration. The concentrated solution was diluted in 1 L of distilled water (1:1 v/v), resulting in a final spore concentration of 5×10^8 CFU mL⁻¹. The inoculation was performed fractionally, with 6 mL of the solution applied around the root ball when the seedlings were planted and 6 mL applied 15 d after planting, according to the method proposed by Griebeler et al. (2023).

The seedlings were planted in 11-L pots previously filled with a clay soil, taken from an agricultural area, which was sieved through a 6-mm mesh and fertilized according to the treatments described to analyze the behavior immediately after planting (0–180 d after planting). The chemical characteristics of the soil were determined at the Soil Laboratory of the Integrated Regional University in Frederico, Westphalen (Appendix A). The seedlings were then inoculated. The *E. grandis* plants were kept in a greenhouse and received micro-sprinkler irrigation daily to maintain adequate water availability, with a water depth of 5 mm day⁻¹ being supplied.

Evaluation of morphological attributes

Plant growth was evaluated 180 d after planting. The height of the aerial part (H) was measured using a centimeter ruler from ground level to the shoot apex, and the neck diameter (SD) was measured at ground level using a digital caliper, with an accuracy of 0.01 mm.

To determine foliar dry (FDM), stem dry (SDM), and root dry (RDM) masses, the plants were sectioned into the aerial part and root system. The root system was washed under running water with the aid of sieves for removal from the soil. Subsequently, the material was packaged in Kraft paper and dried in a forced air circulation oven at $65 \pm 5^\circ\text{C}$ until it reached a constant weight. Finally, the samples were weighed on an analytical balance to determine the dry mass of each fraction. To determine the leaf area (LA), 20 leaves were randomly selected from each plant, distributed in a scanner containing a reference scale, and dried in a forced air circulation oven. The images obtained were processed using ImageJ® program. The LA of each plant was obtained using Eq. 1.

$$LA = \frac{LA_{20} * FDM}{FDM_{20}}$$

where LA is the leaf area (cm^2), LA_{20} is the leaf area of 20 leaves, FDM is the foliar dry mass (g), FDM_{20} is the dry mass of 20 leaves.

Physiological attributes

At 180 d after planting, the chlorophyll index and chlorophyll *a* fluorescence were evaluated. The chlorophyll index was determined using a portable meter (ClorofiLOG, CF 1030; Falker Automação Agrícola, Brazil) on healthy and completely expanded leaves from the middle third of the plant. One leaf per plant was analyzed (two readings per leaf, one on each side of the central vein). The results were expressed as the chlorophyll falker indices (ICF*a* and ICF*b*).

Chlorophyll *a* fluorescence analysis was performed using a modulated pulse fluorometer (Junior-PAM Chlorophyll Fluorometer Walz Mess-und-Regeltechnik) from 8:00 to 11:30 in the morning. Previously, a completely expanded leaf from the middle third was adapted to the dark using aluminum foil for 30 min. Then, the reading was performed to obtain the initial fluorescence (F_o), variable fluorescence/maximum fluorescence ratio (F_v/F_m), electron transport rate (ETR), the effective quantum yield of PSII [$Y_{(II)}$], and sequentially subjected to a saturating light pulse ($10,000 \mu\text{mol m}^{-2} \text{s}^{-1}$) for 0.6 s, thus determining the maximum fluorescence (F_m). The maximum quantum yield of PSII (F_v/F_m) was calculated using the ratio of the variable fluorescence ($F_v = F_m - F_o$) and the maximum fluorescence.

Statistical analysis

The data were verified regarding the homogeneity of variances and normality of residuals using Bartlett and Shapiro–Wilk tests, respectively. To compare the data, analysis of variance (Factorial ANOVA) was used, with the Tukey test at a 5% probability of error ($p < 0.05$) used as a complementary test. The analyses were performed using the “ExpDes.pt” package (Ferreira et al. 2022) of the RStudio software (R Core Team 2021).

Complementarily, principal components analysis (PCA) was performed to verify the correlation structure and the degree of association of different variables investigated in this study. The analysis was performed using the Factoextra and FactoMineR packages from R software (R Core Team 2018), where only components 1 and 2 were considered.

RESULTS

The ANOVA demonstrated an isolated significant effect of different types of fertilizers on the H ($p < 0.000001$), SD ($p < 0.000001$), LA ($p < 0.000001$) and SDM ($p < 0.0000001$) attributes, no effect of the interaction between the study factors was verified. At 180 d after planting in the nursery, the average H of *E. grandis* plants subjected to the CRF treatment was 114.17 cm, an increase of 62.5% compared with the control treatment (Fig.1A).

For SD growth, the highest values were observed in plants managed with fertilizers, regardless of the type (Fig. 1B). Notably, the use of OMF (13.55 mm) increased growth by 57.4% in SD compared to that in

the control treatment (8.45 mm) (Fig. 1B). Regarding the morphological characteristics of LA and SDM, CRF and OMF provided the highest averages, which were statistically equal. CRF increased SDM and LA by 355.2% and 699.1%, respectively, compared with the control treatment (Fig. 1C and D).

The inoculation of *E. grandis* plants with *T. harzianum* significantly affected the H ($p < 0.000001$) and SDM ($p = 0.004382$) attributes. In H, *T. harzianum* provided an average growth of 103.9 cm at 180 d, which was 15.0% higher than that of plants grown without inoculation (Fig. 2A). SDM allocation increased by 13.1% when using *T. harzianum* in the cultivation of *E. grandis* compared to plants grown without inoculation (Fig. 2B).

We observed significant interactions ($p < 0.05$) between the study factors and the allocation of plant dry matter (FDM, RDM, and TDM). At 180 d after planting, using CRF, OMF, or SROF combined with *T. harzianum* inoculation had a higher average biomass of young *E. grandis* plants than in the other treatments (Fig. 3).

E. grandis plants grown using *T. harzianum* and CRF had TDM values 47.1% higher than those grown using TMF (Fig. 3C). The combination of *Trichoderma* inoculation and OMF increased 33.3%, 59.4%, and 45.2% in FDM, RDM, and TDM, respectively, compared to TDM. In contrast, plants in the control treatment showed significantly lower values than those in the other treatments (Fig. 3).

Regarding chlorophyll *a* fluorescence, using different fertilizers significantly affected F_v/F_m ($p = 0.01990$), $Y_{(II)}$ ($p = 0.00706$), and ETR ($p = 0.00726$). When CRF and OMF were used for base fertilization, *E. grandis* plants showed the highest averages of F_v/F_m and $Y_{(II)}$ (Fig. 4A and 4B). Using fertilizers, regardless of the type, was favorable for increasing ETR, providing the highest averages compared to the control treatment (Fig. 4C).

The PCA represented variability in the first two extracted components, explaining 77.6% of the total results obtained (Fig. 5). Among these, 65.0% of the variation was explained by component 1 (PC1) and 12.6% by main component 2 (PC2). No evident difference between the types of fertilizers used for *E. grandis* was observed, which agreed with the trend observed when the univariate variance analysis was performed.

PC1 separated the fertilizers into three groups corresponding to the observations of the control treatment (group 1), TMF and SROF (group 2), and CRF and OMF (group 3). The results indicated that plants grown under CRF or OMF showed similar behavior, with the highest H, SD, LA, TDM, ETR, F_v/F_m , and $Y_{(II)}$ values.

DISCUSSION

Our results showed that the *E. grandis* plant growth was enhanced when provided with CRF or OMF. Thus, the benefit of using granulated OMF made from pig manure as an alternative source of nutrients for forest plantations is one of the main results of this study. Generally, *E. grandis* plants exhibited the

best results in both morphological and physiological attributes when cultivated with CRF and OMF added to the soil at the time of planting.

Using CRF promoted an adequate supply of nutrients to *E. grandis* plants. Recently, other studies have highlighted the advantages of using CRF for the basal fertilization of forest species (Turchetto et al. 2019; Griebeler et al. 2023; Speetjens and Jacobs 2023). CRFs are water-soluble fertilizers enclosed in a semipermeable polymer coating that allows mineral ions to be available to plants at a controlled rate (Vejan et al. 2021). The regulated release of nutrients reduces leaching and improves the use efficiency compared with conventional non-encapsulated fertilizers.

This study demonstrated the high potential of OMFs based on pig manure for *E. grandis* cultivation. Plants cultivated with OMF had greater height and dry mass allocation than those cultivated with mineral fertilizer (MF). Other studies have shown similar or higher crop yields using organic fertilizers, such as pig manure or chicken litter, than with MF (Cassol et al. 2012; Corrêa et al. 2016). Adding pig manure compost enabled the better development of *E. grandis* seedlings grown in copper-contaminated soil (Negrini et al. 2022). Andriguetto et al. (2024) also found higher biomass allocation values in *C. americana* plants cultivated with OMF based on pig manure than the ones cultivated with MF.

Applying OMF to the soil had a similar effect as using CRF on the growth of *E. grandis* plants. According to Sánchez-Báscones et al. (2019), using organic solid waste reduces the leaching capacity of nitrate in the soil because it primarily contains nitrogen in its organic form. Furthermore, adding MF to organic compounds can improve the efficiency of nutrient utilization by plants (Morais and Gatiboni 2015; Borges et al. 2019).

The increased growth of *E. grandis* when cultivated with CRF or OMF was directly related to the improvement in the photosynthetic apparatus of the plants. CRF and OMF treatment resulted in higher values of the attributes generated by measuring chlorophyll *a* fluorescence. Chlorophyll fluorescence is sensitive to environmental stress, making it an indicator of the physiological state of plants owing to environmental or nutritional changes (Feng et al. 2015; Sim et al. 2021). In other words, lower chlorophyll *a* fluorescence emissions are related to lower nutrient restrictions by the plant.

Based on chlorophyll *a* fluorescence, the F_v/F_m ratio expresses the relative efficiency of light energy absorption by the PSII antenna complex, where higher values of F_v/F_m indicate greater efficiency in capturing energy from the PSII reaction center (Banks 2017). $Y_{(II)}$ represents the quantum yield of the photochemical energy conversion in PSII and is highly correlated with the photosynthetic rate (Baker 2008). The ETR, calculated as the product of PSII efficiency and absorbed light, is positively correlated with CO₂ fixation (Krall and Edwards 1992; Wong et al. 2014), as the flow of electrons through PSII is primarily consumed by carbon assimilation (Oberhuber and Edwards 1993).

Therefore, the performance in capturing energy by the PSII reaction center, electron transfer efficiency, and light energy conversion by the PSII reaction center of *E. grandis* plants were significantly improved with the application of CRF and OMF, which was most evident in F_v/F_m and $Y_{(II)}$. Consequently, the

enhanced photosystem performance provided more assimilation power (NADPH and ATP) for regenerating ribulose-1,5-bisphosphate, resulting in greater growth and biomass allocation in *E. grandis* plants.

Inoculation with *T. harzianum* also improved the *E. grandis* development, especially when associated with CRF or OMF. Griebeler et al. (2021) observed greater height values and improved photosynthetic apparatus in *Cordia trichotoma* (Vell.) Arráb. ex Steud., and *Cedrela fissilis* Vell. plants inoculated with *T. harzianum* and *T. aspereloides* compared to the control treatment. Chagas Jr et al. (2021) found that inoculation with *T. longibrachiatum* and *T. harzianum* promoted the highest initial growth of *E. urophylla* and *E. brassiana* seedlings.

Fungi of the *Trichoderma* genus can promote greater water and nutrient absorption, osmotic regulation, and the synthesis of metabolites involved in plant systemic resistance (Kafle et al. 2022; Corrales et al. 2022). Compounds produced by these fungi, including antibiotics, enhance root resistance to pathogens and their adverse effects (Zin and Badaluddin 2020). In a study on tomato plants, Cai et al. (2013) isolated and characterized a well-known butenolide metabolite, harzianolide, from the filtered fermentation broth of *T. harzianum*, which resulted in increased plant biomass.

Thus, secondary metabolites from *Trichoderma* can act as auxin-like compounds, positively influencing the growth of the colonized plants. Regarding the mechanism of action, RDM significantly increased in response to *T. harzianum* inoculation. Therefore, inoculation with *T. harzianum* influences the early stages of plant growth by improving root development. Greater root mass allows plants to explore a larger volume of soil owing to increased active absorption sites per unit area (Cai et al. 2013).

The present study demonstrated the benefits of using OMF based on pig manure and inoculating *E. grandis* plants with *T. harzianum* (Figs. 1 and 2). Due to necessity to feed a growing population while simultaneously reducing negative environmental impacts, pursuing sustainable and efficient agricultural practices has become increasingly relevant. In this context, using pig farming waste to formulate OMF, coupled with the use of bioinputs, such as *T. harzianum* inoculation, has become a promising alternative to a more sustainable forest production system. These practices offer substantial economic benefits, including reduced use of MF sources, increased forest growth and productivity, and income generation for producers. They also contribute to environmental conservation by promoting soil health and improving the quality of water resources.

CONCLUSION

Using CRF or granulated OMF based on pig manure maximized the growth and photosynthetic efficiency of young *E. grandis* plants. Therefore, using pig manure to formulate OMF is a sustainable alternative for fertilizing young *E. grandis* plants. Inoculation with *T. harzianum* combined with CRF or OMF based on pig manure was associated with a higher dry mass allocation in *E. grandis* plants.

Declarations

Author contributions Conceptualization: Felipe Turchetto; Methodology: Felipe Turchetto, Adriana Maria Griebeler, Breno Magno Silva dos Santos and Clovis Orlando Da Ros; Formal analysis and investigation: Felipe Turchetto, Breno Magno Silva dos Santos, Gabriel Coelho Waimer, Janaine Giombelli Jachi and Kellin Vanessa Andriguetto; Writing - original draft preparation: Felipe Turchetto, Breno Magno Silva dos Santos and Kellin Vanessa Andriguetto; Writing-review and editing: Tauã Osaida da Silva, Rodrigo Ferreira da Silva, Nilson Biesdorf and Pedro Mucin Montó; Supervision: Felipe Turchetto and Adriana Maria Griebeler

Availability of data and material Not applicable.

Code availability Not applicable.

Conflict of interest The authors declare no conflict of interest.

Datta availability The experimental data used in the study are available from the corresponding author on reasonable request.

Humans and/or animals Not applicable.

Ethical approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

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Figures

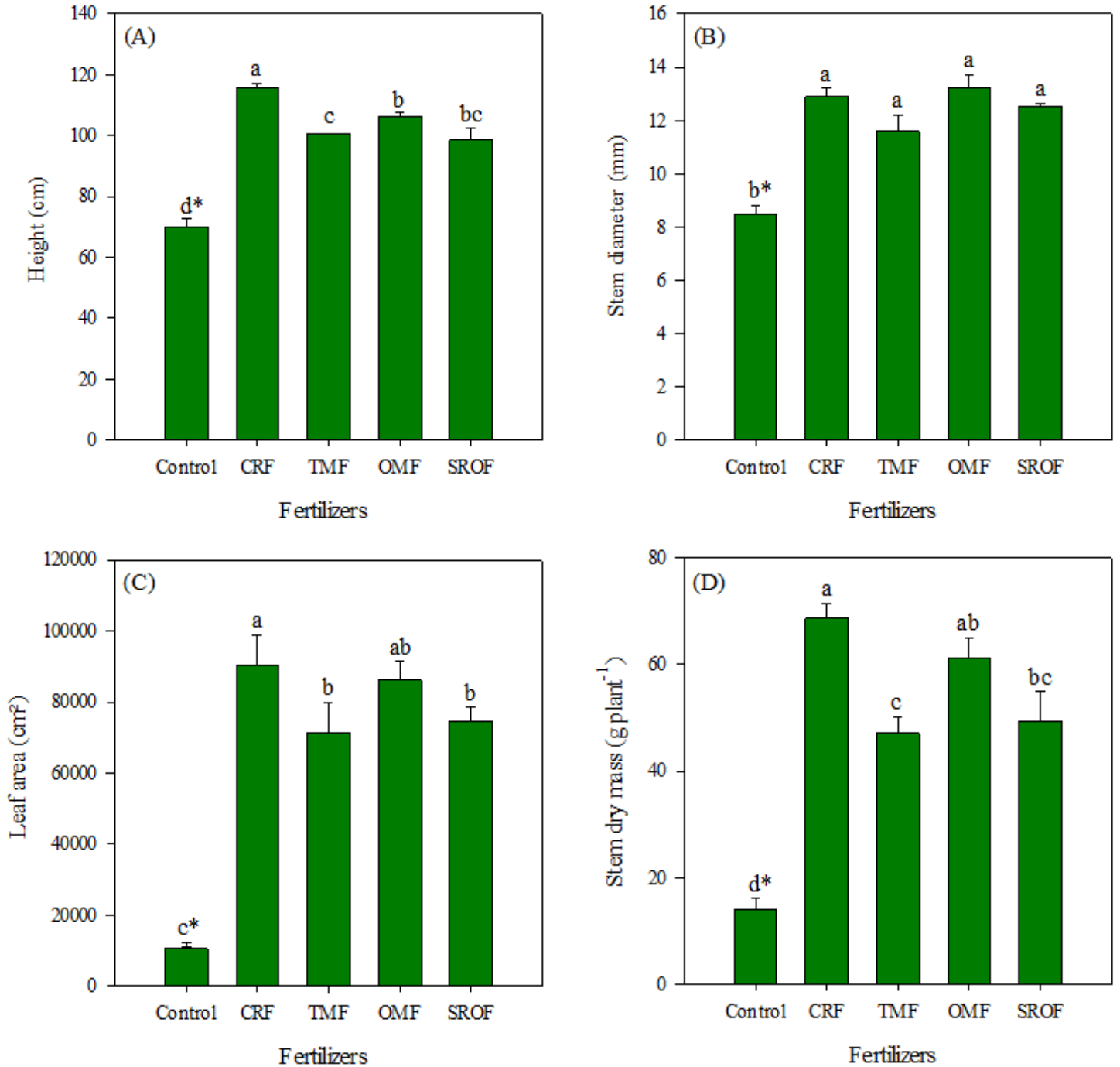


Figure 1

Height (A), stem diameter (B), leaf area (C), and stem dry mass (D) of *E. grandis* plants after using different types of fertilizers, 180 d after planting in a controlled environment. *Lowercase letters indicate the comparison between different fertilizers using the Tukey test at a 5% probability of error. Vertical bars indicate mean $\pm$ standard error. Where: Control is a treatment without fertilizers; CRF is controlled-release fertilizer; TMF is traditional mineral fertilizer; OMF is organomineral fertilizer; and SROF is soil remineralizing organomineral fertilizer

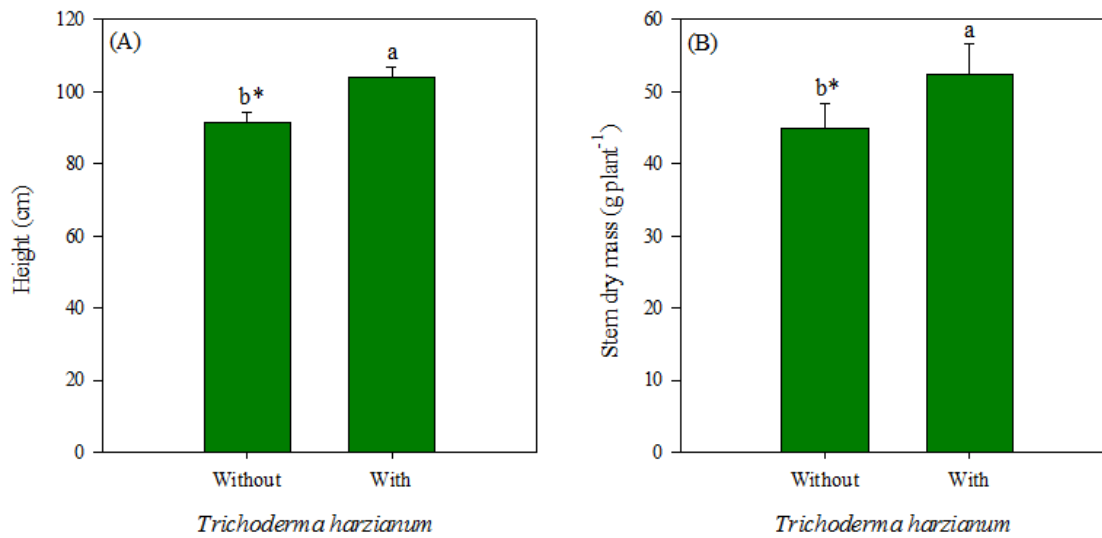


Figure 2

Height (A) and stem dry mass (B) of *E. grandis* plants, as a function of *T. harzianum* inoculation 180 d after planting in a controlled environment. *Lowercase letters indicate the comparison of means using the Tukey test at a 5% probability of error. Vertical bars indicate mean $\pm$ standard error

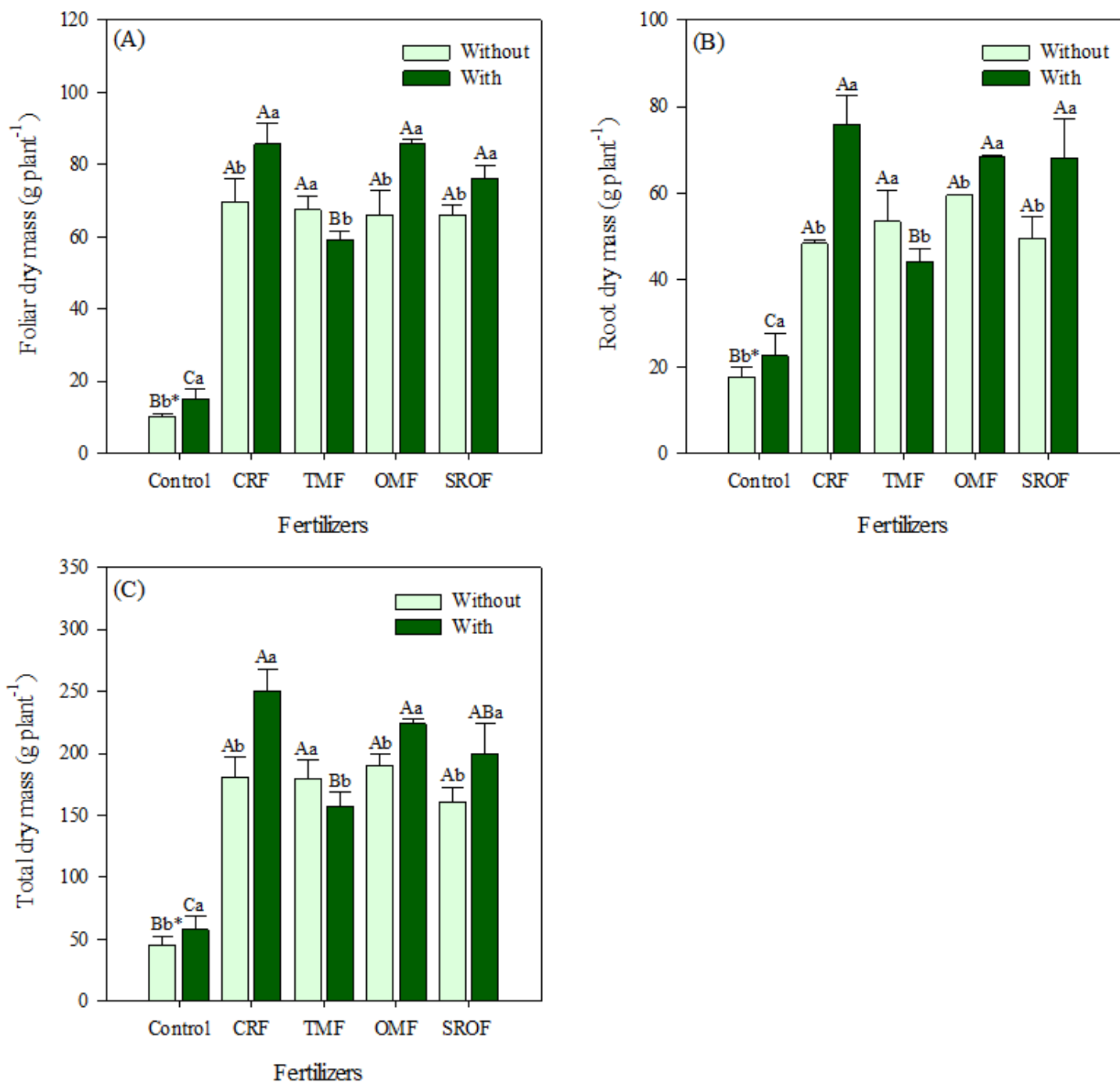


Figure 3

Foliar dry (A), root dry (B), and total dry (C) masses of *E. grandis* plants, as a function of using different types of fertilizers and combined with *T. harzianum* inoculation at 180 d after planting in a controlled environment. *Capital letters indicate the comparison of different fertilizers, while lowercase letters indicate the comparison of *T. harzianum* inoculation with each fertilizer, using the Tukey test at a 5% probability of error. Vertical bars indicate mean $\pm$ standard error. Where Control is the treatment without

fertilizers; CRF is controlled-release fertilizer; TMF is traditional mineral fertilizer; OMF is organomineral fertilizer; and SROF is soil remineralizing organomineral fertilizer

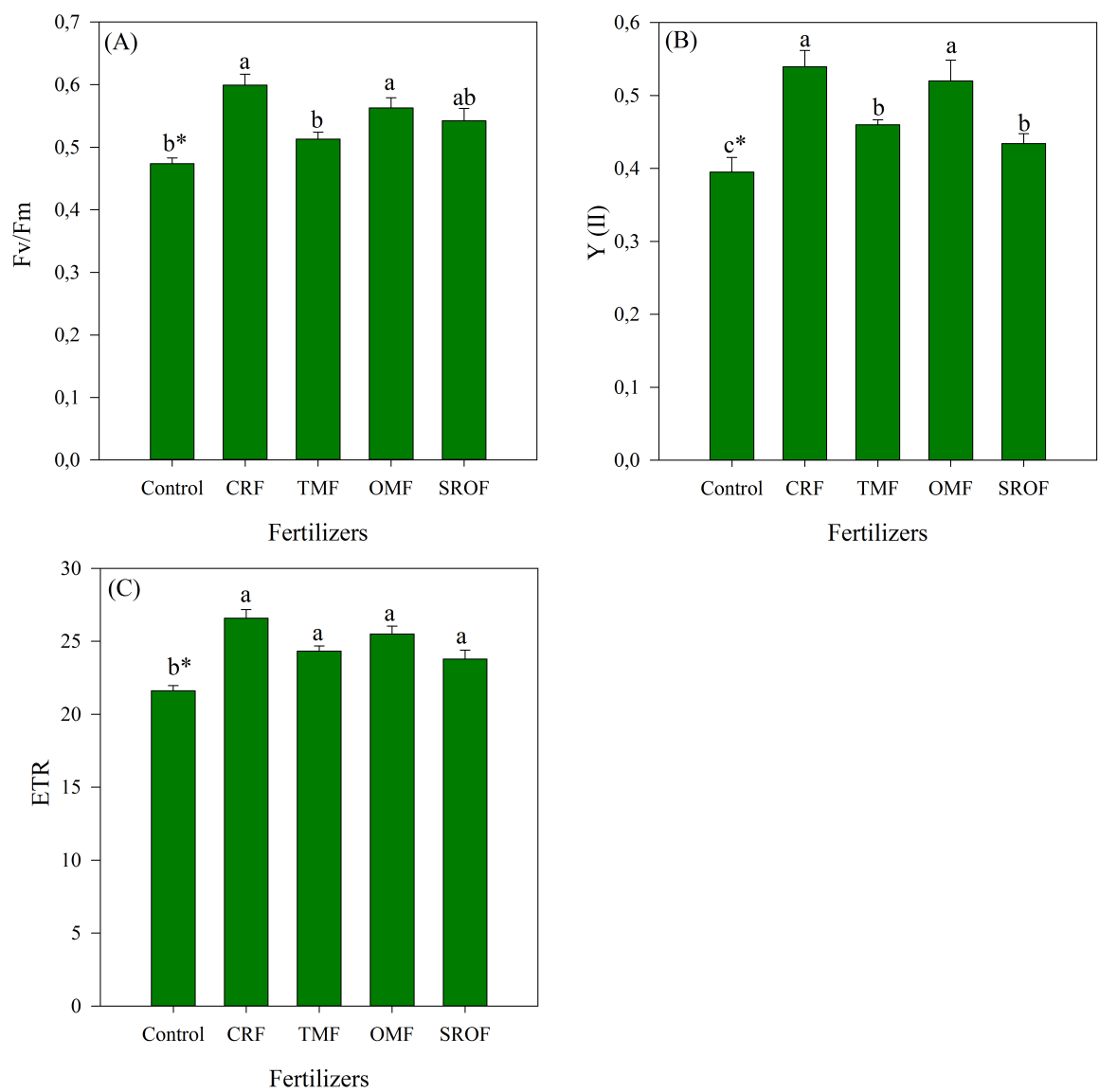


Figure 4

Quantum yield of photosystem II (A), effective quantum yield of photosystem II (B), and electron transport rate (C) of *E. grandis* plants as a function of different types of fertilizers at 180 d after planting in a controlled environment. Lowercase letters indicate the comparison of different fertilizers using the Tukey test at a 5% probability of error. Vertical bars indicate mean $\pm$ standard error. Where Control is the treatment without fertilizers; CRF is controlled release fertilizer; TMF is traditional mineral fertilizer; OMF is organomineral fertilizer; and SROF is soil remineralizing organomineral fertilizer

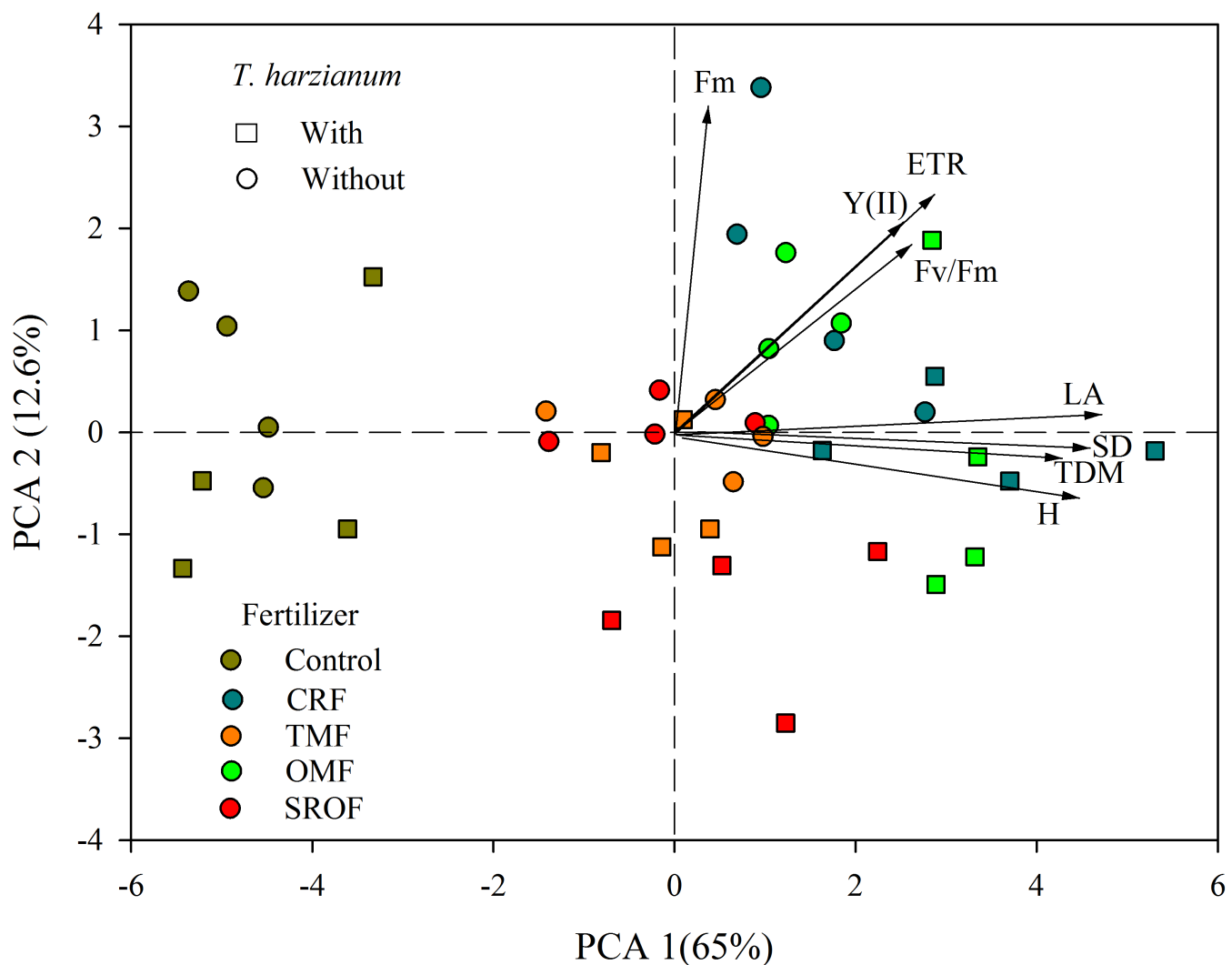


Figure 5

Relationship between Principal Component 1 (PC1) and 2 (PC2) of morphological and physiological attributes of *E. grandis* plants as a function of different types of fertilizers at 180 d after planting in a controlled environment. Where Control is the treatment without fertilizers; CRF is controlled-release fertilizer; TMF is traditional mineral fertilizer; OMF is organomineral fertilizer; SROF is soil remineralizing organomineral fertilizer; H is height; SD is stem diameter; LA is leaf area; TDM is total dry mass; Fm is maximum fluorescence; F_v/F_m is maximum quantum yield of PSII; and $Y_{(II)}$ is effective quantum yield of PSII

Supplementary Files

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