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The influence of the storage period on the suppressive capacity of composts enriched with *Trichoderma atroviride*

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ABSTRACT

Composts can be used to improve soil fertility while controlling soil diseases, contributing to the circular economy in agriculture. Biological control of soil diseases may be achieved by the activity of antagonistic microorganisms, that, by different ways, prevent the development of phytopathogenic microorganisms, being *Trichoderma* spp. one of the best-known antagonistic fungi.

In this work, the evolution of the suppressive capacity during a storage period of one year, was evaluated on two composts enriched with *Trichoderma atroviride*, against *Rhizoctonia solani*, *Clarireedia* spp. and *Sclerotium rolfsii* in turfgrass (*Agrostis stolonifera*). Both composts, obtained from agro-industrial residues, were enriched with *T. atroviride*, and stored at room temperature, in the dark. The composts were tested *in vivo*, at 0, 6 and 12 months of storage, as substrates for turfgrass (*Agrostis stolonifera* L. cv. T1) grown in pots. Plants were inoculated with the above-mentioned pathogenic fungi and the incidence and the severity of the diseases, AUDPC and BCI, were determined during the storage period. The highest biological control performance was achieved after 6 months of storage, especially on the composts enriched with *Trichoderma atroviride*.

1. Introduction

Composting converts organic residues into products, the composts, with improved characteristics that should be used in agriculture as fertilizers or on plant diseases control ([1–4]; Castaño et al., 2013; [5–8]). Recently, interest in composting has increased, due to social demand for environmentally friendly waste treatment methods, the need to increase circularity in agriculture, and the growing demand for organic farming products [9]. Plant disease biocontrol, through the microbial activity of composts, is an alternative and attractive approach that contributes to the agricultural circular economy. Being a sustainable and environmentally friendly option, composting allows the production of nutrient-rich products without the negative impact of synthetic agrochemicals on human and environment health ([10]; Stowell and Gelernter, 2001; [11]; Dempsey et al., 2012; Gan and Wickings, 2017; [12]).

The biocontrol of plant diseases depends on the microbiota, in particular, the presence of competitive species to pathogenic microorganisms [6]. Microbial activity is related to the organic matter characteristics, the host plant sensibility, and the environmental conditions [13,14]. Abiotic factors, such as pH, calcium, nitrogen and other nutrients, may also play an important role concerning the suppressive capacity of the composts [15].

Many soil-borne diseases are a consequence of the low organic matter content of soils and frequent soil tillage [16]. Compost applications, especially when enriched with antagonistic fungi, can be used in plant disease biocontrol, being *Trichoderma* spp. one of the fungi most frequently used [16–18].

Trichoderma species have proven to be effective in the biocontrol of many plant pathogens [19], including soil-borne fungi like *Rhizoctonia solani* [8,20], as well as other plant pathogenic fungi (Hermosa et al., 2000), such as *Fusarium oxysporum* [2], *Colletotrichum orbiculare* [21], *Phytophthora capsici* [22] and leaf pathogens, such as *Botrytis cinerea* [23]. During storage, compost characteristics may change, mainly its nitrogen content and microbial populations [24] affecting its suppressive capacity [5,16,25,26]. Therefore, evaluation the suppressive capacity of composts during storage is crucial, especially since composts are not always used immediately after composting. However, despite the recognized importance of compost storage, there is limited understanding of how storage duration and environmental conditions

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influence the persistence and effectiveness of suppressive microbial communities.

Although compost properties have been extensively studied, the specific impact of storage on the dynamics of disease-suppressive properties remains poorly understood. During storage, several changes occur in composts, which can influence their suppressive capacity (Rijn et al., 2007, [27]). Additionally, microbial populations, whose biomass typically declines [28], play a significant role in disease suppression. The suppressive capacity of composts is attributed to the activity of various microbial communities, which are key to understanding their disease control potential [29]. While the microbial populations responsible for suppressiveness may be altered during storage, the exact mechanisms behind these changes and their effects on disease control over time have not been fully explored [27,30].

This knowledge gap is critical, as understanding how storage conditions impact compost suppressiveness could lead to improved compost management practices. Such advancements would ensure more consistent disease control when composts are used. Therefore, this study aims to fill this gap by evaluating the suppressive capacity of two composts, at the end of the composting process (0 months) and after 6 and 12 months of storage. The changes in the suppressive capacity of compost with different age will be related to their changes in microbial communities, as well as physical and chemical properties of the different age composts.

The specific objectives were: (i) Evaluate the influence of age on the suppressive capacity of composts enriched with *Trichoderma atroviride* against the fungal pathogens *Rhizoctonia solani*, *Clarireedia* spp., and *Sclerotium rolfsii*. *In vivo* tests were carried out to determine disease control parameters.

(ii) To characterize the variation of enzymatic activity, microbial populations, and physical-chemical properties of composts during storage;
(iii) To assess the influence of compost origin, thermal treatment, and *Trichoderma* enrichment on disease suppression over time.

2. Material and methods

Plant growth experiments were conducted in a non-heated steel greenhouse at University of Algarve, *Campus* of Gambelas, Portugal (37°02′35.45′′N, 7°58′20.64′′W). The greenhouse was covered with low density polyethylene film and natural ventilation was provided through the roof and side walls windows. All the assays were carried out with two replicates.

2.1. Compost and substrates preparation

Three different plant wastes were mixed in two different proportions, in piles with manual turning, resulting in two composts, P1 and P2 [31]. In order to achieve a similar initial C/N ratio in both piles, the mix for compost P1 included mixing wasted oranges from a farmer's association; grass clippings from a golf course and grape marc from a wine house in a volumetric ratio of 0.9:1.1:1, respectively. Compost P2 mix included the same raw materials, and the addition of the wastes from the production of Shiitake mushroom (*Lentinula edodes* (Berk.) Pegler), with the volumetric ratio of 1.8:0.2:1.5:0.5, respectively. The composting process took place in wire mesh containers, covered with a plastic net to reduce heat and moisture losses, while allowing natural ventilation. Each composting pile had an initial volume of approximately 1 m^3. The composting of both residues mixtures was conducted twice, in two consecutive years. Chemical, physical, and microbiological variables were monitored during the composting process, following the methods described by Coelho et al. [18,31]. At the end of the composting processes, P1 and P2 were tested, using a commercial peat (P) (Hansa Torf Floragard, Germany) as a control substrate. These three substrates (P1, P2 and P) were tested for disease control as they were obtained and after submited to an heat treatment (t) and *Trichoderma* inoculation (E), resulting in treatments: P1t, P2t and Pt, and treatments P1tE, P2tE, and PtE, respectively. The heat treatment was applied with the aim of eliminating microorganisms and confirming the biological origin of the suppressiveness, these substrates were submitted to a thermical treatment (t) at 60 °C for seven days (referred as P1t, P2t and Pt). To study the effect of enrichment with the antagonist *Trichoderma atroviride*, the substrates P1, P2, and P were enriched with *T. atroviride*, referred as P1E, P2E, and PE. For the inoculation, a spore suspension was prepared by adding 50 mL of sterilized distilled water to each PDA Petri dishes where the fungus had grown for seven days. The concentration of conidia was determined using a Neubauer chamber, yielding a suspension of 1.33×10^6 conidia mL^{-1}. To achieve a final concentrations of 9.0×10^7 conidia L^{-1} in the enriched substrates, 68 mL of the conidia suspension, was thoroughly mixed into each substrate. The inoculated substrates were subsequently stored at room temperature, in the dark, during seven days. The heat-treated substrates (P1t, P2t, and Pt) were also enriched following the same procedure, resulting in P1tE, P2tE, and PtE [18].

The survival of *T. atroviride* was assessed by the mycelia growth on a semi-selective medium [32]. Serial dilutions of substrate samples were plated to estimate viable *Trichoderma* populations. Additionally, the growth of *T. atroviride* was visually monitored on PDA plates to confirm its colony-forming ability. To complement these methods, molecular techniques were employed to detect and confirm the presence of *T. atroviride*, providing a more sensitive evaluation of its persistence in the composts over time. All twelve enriched and heat-treated substrates were evaluated for their disease-suppressive capacity two weeks after enrichment (0 months) and after 6 and 12 months of storage.

2.2. Composts storage

To assess the suppressive capacity of the composts over time, the composts were kept in polypropylene tissue bags (to allow some aeration) and stored in a dry, dark place at room temperature, with the exception for the heat-treated substrates that were tested just after the heat treatment. Part of the substrates P, P1 and P2 were subjected to heat treatment at 0, 6 and 12 months after composting, resulting in treatments Pt, P1t and P2t respectively. These heat-treated substrates (t) were prepared just before the assays (no storage period after the heat treatment). At each evaluation period (0, 6 and 12 months), the properties of all the 12 substrates were evaluated, and tested for their suppressive capacity.

2.3. Substrates characterization

Chemical, physical and microbiological properties of the substrates were assessed at each storage periods (0, 6 and 12 months). Total fungi and *Trichoderma* populations were determined. Substrates samples were suspended in phosphate buffered saline (PBS), and serial decimal dilutions were prepared and inoculated into culture media suitable for each tested microorganism growth. Fungal enumeration was performed using Potato Dextrose Agar (PDA) (Biokar, France), inoculated by the spread plate technique and incubated at 25 ± 2 °C for 24–48 h (Coelho et al., 2012); *Trichoderma* spp. was cultivated in semi-selective culture media [32] and incubated at 25 ± 2 °C for 72 h. Additionally, the organic and mineral content [33], dry matter [34], C/N ratio, and total Kjeldahl nitrogen (TKN) were also assessed. pH was measured with a potenciometer (Crison Micro pH 2001, Spain), in an aqueous extract (1:2 w/v), and the electrical conductivity was determined in the same extract, after filtration, using a conductivity meter (Crison 522, Spain). With the API-ZYM™ system (bioMérieux Italia, Rome, Italy), enzymatic activity was determined in all the substrates, for 19 enzymes: alkaline phosphatase, esterase (C 4), esterase lipase (C 8), lipase (C 14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase,

β-glucosidase, N-acetyl-ß-glucosaminidase, α-mannosidase and α-fucosidase. Specific galleries were inoculated with 65 μL of a suspension containing 1 g of substrate and 9 mL of sterile saline solution (NaCl 9.0 g L^{-1}). The galleries, after being incubated at 37 °C for 24 h, were activated by adding 30 μL of Reagent ZYM A and Reagent ZYM B (bioMérieux Italia, Rome, Italy). A value ranging from 0 to 5 was attributed according to the colour developed in each enzymatic reaction, using the colour chart provided by the manufacturer. Results are expressed as enzyme relative activity per gram of dry weight of composts and peat.

2.4. Stored composts supressivity assessment

The effect of the storage periods on the enriched composts was evaluated by determining its suppressive capacity *in vivo*, on turfgrass (*Agrostis stolonifera* L. cv. T1) sown in 100 mL pots and grown in a greenhouse.

Pathogenic agents *Sclerotium rolfsii, Clarireedia* spp. [sin. *Sclerotinia homoeocarpa* [35] and *Rhizoctonia solani* were inoculated on plants by placing 6.5 mm diameter PDA disks with active mycelia grown for 7 days on the pots, when turfgrass had already completely covered the surface of the pots. For *S. rolfsii*, in addition to the mycelium disk, two *sclerotia* were added to each pot. Positive control consisted on the substrates subjected to thermal treatment and subsequently inoculated with the tested pathogens, whereas the negative control substrates subjected to thermal treatment but not inoculated with the pathogens. Twelve treatments were tested for each disease, with five pots per treatment and four replicates, making a total of 240 pots per disease, amounting to 960 pots in total. To increase and stabilize humidity and air temperature, the pots were kept under a tunnel of a lightweight geotextile, inside the greenhouse. Air temperature and humidity near the plants were daily recorded. The greenhouse trials with the composts obtained in two following years were conducted twice.

Plants were irrigated by overhead mist irrigation to maintain high levels of humidity and substrate moisture. Foliar fertilization was applied weekly using an organo-mineral fertilizer (Ret-Sul, Eibol, Spain) with 18 % N-total, 0.75 % Fe, 0.1 % Mn, 9 % free amino acids at the concentration of 150 mL 100 L^{-1} of water, according to manufacturer recommendations.

When the symptoms of each disease were detectable on the plants, the affected area was measured in all the plants, and subsequently every week, to determine disease incidence and disease severity. Disease incidence was determined by assessing the absence or presence of the disease on the plant. In total, 60 turfgrass pots were used for each replication and disease. Disease severity was evaluated on a symptom severity scale, adapted from Baayen and Van der Plas [36], where: 0 = asymptomatic plant (0 % disease); 1 = weakly infected plant (5 % disease); 2 = poorly developed symptoms (20 %); 3 = well developed symptoms (50 %); 4 = strong disease symptoms throughout the area (80 %); 5 = dead plants (100 %). Biological control index (BCI) and efficacy were calculated according to Byrne et al. [37]. The BCI was calculated to combine efficacy and consistency of disease reduction, according to the formula: $BCI = \frac{Efficacy}{Consistency}$ [37]. Efficacy is indicated by the mean disease reduction percentage, and consistency by the standard deviation of those means. The area under disease progress curve (AUDPC) was determined according to Campbell and Madden [38] and indicates the speed of the disease development. AUDPC per pot was calculated by integrating the disease severity between the onset of symptoms on the plants and the assay completion, divided by the total duration (in days) of the epidemic in each assay, according to the formula: $\sum_{i=1}^{n}\left(\frac{y_i+y_{i+1}}{2}\right)(t_{i+1}-t_i)$, where y = injury disease and t = time.

2.5. Statistical analysis

Physical, chemical, and microbiological properties of the substrates were compared using multifactorial analysis of variance (ANOVA) followed by Duncan New Multiple-Range Test for post-hoc comparisons. Data from the *in vivo* experiments, namely efficacy, AUDPC and the BCI, which were measured over multiple times points, were analyzed using repeated measures and Mixed ANOVA. Pairwise comparisons were performed using the Bonferroni New Multiple-Range Test. Disease severity was analyzed, and multiple comparisons (all pairwise) were performed using Kruskal-Wallis test. The normality of the sample distribution and the homogeneity of the variances were verified before ANOVA [39]. Additionally, linear regression and Spearman's correlation were conducted to examine how changes in the stored composts affected their suppressive capacity. A Principal Component Analysis (PCA) was performed to explore the overall patterns of variation in microbial and chemical parameters across storage period and treatments. All statistical analyses were performed using IBM SPSS Statistics ver. 26 (IBM Corp., 1989–2017, U.S.A.) over two consecutive years.

3. Results

3.1. Composts characterization

Compost enrichment at 0 months contributed to increase the organic matter content, as well as nitrogen content (Table 1). Additionally, the total fungi and *Trichoderma* spp. populations were higher in both enriched composts and peat (Fig. 1).

After six months of storage, P2 compost showed a significant total Kjeldahl nitrogen increase while P1 (P1 and P1E) composts showed a decrease, although the differences were not significant ($p < 0.05$) (Table 1). Moreover, organic matter content significantly decreased in P1E, P2E and P2tE ($p < 0.05$) (Table 1). Dry matter largely increased from 0 to 6 months ($p < 0.05$) in all P1 and P2 composts (Table 1), as the result of compost drying. *Trichoderma* spp. continued to show the highest populations in the enriched composts, P1E and P2E (Fig. 1). During this initial 6-month storage period, the microbial populations decreased.

After 12 months of storage, the nitrogen content decreased in all tested composts ($p < 0.05$) (Table 1), as well as organic matter, except in P1tE and P2tE ($p < 0.05$). *Trichoderma* spp. populations declined (Fig. 1) in the enriched composts. From 6 to 12 months of storage, the dry matter content increased. Nitrogen content significantly decreased ($p < 0.05$) in all P1 and P2 based substrates (Table 1). In this period, the microbial population decreased (Fig. 1).

3.2. Enzymatic activity

At 0 and 6 months of storage, there was a high enzymatic activity in substrates, except for those submitted to thermic treatment. After 12 months of storage the enzymatic activity decreased, namely in P1E and P2E, considering only the activity of nine enzymes (Table 2). The activity of N-acetyl-β-glucosamine was high at 0 and 6 months, namely in the enriched compost with *T. atroviride*: P1E and P2E (Table 2). However, after 12 months, the activity of this enzyme decreased, although with no statistically significant differences, with emphasis on P1E, P1tE and P2E (Table 2).

Lipase decreased in many substrates after the storage period of 6 months (Table 2). Leucine arylamidase increased after 6 months of storage and decreased after 12 months (Table 2).

A decrease in β-glucosidase was observed after 6 months of storage, and an increase after 12 months.

3.3. Composts suppressivity assessment

During greenhouse plant growing trials, the maximum temperature varied between 11 °C and 40 °C, and the minimum between 5.0 °C and 21.2 °C. The average maximum temperature was 26.4 °C and the average minimum, 13.2 °C. Humidity kept above 57 %.

Evaluating the substrates at the three storage periods, some

Table 1
Physico-chemical properties of the tested substrates.

	ST	P1	P1t	P1E	P1tE	P2	P2t	P2E	P2tE	P	Pt	PE	PtE
N	0-M	2.05a	1.83a	2.22a	2.17a	1.78b	1.65b	1.95a	1.88a	1.18	1.13a	1.23	1.17
	6-M	1.94a	1.09b	2.06a	1.79b	2.18a	1.95a	2.00a	1.92a	1.17	0,99a	1.39	1.19
	12-M	0.94b	0.95b	0.94b	0.97c	0.90c	0.98c	0.91b	0.95b	1.54	0.91a	1.28	1.35
OM	0-M	72.0b	61.8a	77.0a	72.1 ab	83.5a	74.1a	86.9a	78.9a	93.5a	93.5a	94.7	95.7
	6-M	81.5a	62.8a	67.9b	66.3b	78.1a	74.6a	75.3b	69.0b	94.4a	94.1a	95.6	94.8
	12-M	62.3c	67.0a	65.3b	76.5a	69.9a	64.4a	70.7b	79.0a	94.4a	93.9a	95.3	95.3
DM	0-M	56.4c	56.9c	51.2c	59.6c	54.6b	54.1c	56.2c	53.1c	77.2a	76.5a	70.4b	76.4c
	6-M	83.3b	85.0b	85.4b	87.2b	82.9a	81.9b	81.3b	85.2b	35.8b	45.5b	84.8a	81.4b
	12-M	88.5a	88.8a	92.1a	90.7a	83.3a	85.6a	83.8a	87.9a	35.5b	40.8b	84.7a	91.0a
pH	0-M	7.08a	7.60a	7.08a	7.52a	7.35a	7.79a	7.23a	7.79a	5.00	4.93	5.05	4.77
	6-M	7.18a	6.71b	7.18a	7.11a	7.01b	6.71b	7.18a	7.18b	5.2	4.85	4.55	4.45
	12-M	7.16a	7.22a	7.04a	7.24a	7.08b	7.13b	7.19a	7.22b	5.51	4.28	4.40	3.93
EC	0-M	1.58a	1.71a	1.68a	1.90a	2.33a	2.27a	2.28a	2.21a	0.09	0.12	0.10	0.10
	6-M	1.75a	1.55a	1.77a	1.91a	1.62aa	1.75a	2.06a	2.18a	0.10	0.10	0.12	0.13
	12-M	1.88a	1.86a	1.87a	2.36a	1.65a	1.40a	1.51a	1,47a	0.09	0.10	0.08	0.13

P1, compost P1; P2, compost P2; P, peat; t, substrates submitted to a thermic treatment (60 °C for 7 days); E, substrates enriched with *Trichoderma atroviride*; ST, storage time; 0-M, compost stored for 0 months; 6-M, compost stored for 6 months; 12-M, compost stored for 12 months; N, nitrogen; OM, organic matter; DM, dry matter; EC, electrical conductivity. For each column, values with the same letter showed no statistical differences ($p < 0,05$), according to Duncan New Multiple-Range Test.

suppressive capacity was found in the tested composts. The stored composts kept their suppressive capacity, mainly in the enriched composts, with a tendency to present higher suppressive capacity after 6 months and a decrease after 12 months.

At 0 months of storage, compost enrichment contributed to the biological control of *S. rolfsii, Clarireedia* spp. and *R. solani*, compared to non-enriched composts. However, after 6 months of storage, the enriched composts showed the highest effectiveness in controlling the diseases under study. Against *S. rolfsii* the differences observed between substrates were: BCI and efficacy showed the highest value on P2E ($p \leq 0.001$) but with no differences when compared with P1E. *Clarireedia* spp. development was reduced in P1E and P2E, and these two substrates showed the highest efficacy ($p \leq 0.001$): BCI stood out P2E, with the highest values. For *R. solani*, P2E showed the best results on BCI ($p \leq 0.001$) and Efficacy ($p \leq 0.001$), but with no significative differences ($p > 0.05$) when compared to P1E (Figs. 2–4).

After 12 months of storage, the suppressive capacity decreased, but the composts kept some biological control against the studied diseases. The efficacy showed the best results in P2E, but with no significant differences respect to P1E ($p \leq 0.001$). Concerning *R. solani,* P1E and P2E showed no differences when compared with P1, P2 and PE (Fig. 4). BCI decreased significantly after 12 months of storage, being higher in the enriched substrates (Fig. 3).

When evaluating the performance of the substrates over storage time for the studied phytopathogens, it was observed that disease severity decreased after 6 months of storage (Fig. 2). For *Clarireedia* spp. according to Kruskal-Wallis test, it was observed $H\ (35) = 752.306$, $p \leq 0.001$; for *S. rolfsii* $H\ (35) = 839.727$, $p \leq 0.001$ and for *R. solani* $H\ (35) = 704.161$, $p \leq 0,001$. At the 6 months storage period, total fungi and *Trichoderma* spp. populations also decreased in the studied substrates ($p \leq 0.001$), according to ANOVA to repeated measures (Fig. 1). Treatment P1E showed the best results on *Clarireedia* spp.control at 0 and 6 months of storage (Fig. 2). Against *R. solani* and *S. rolfsii* the best results were obtained with treatment P2E at 6 months of storage. Disease severity increased at 12 months of storage.

Plants grown on the composts stored for one year, showed an increase in disease severity. The best control of *Clarireedia* spp. was observed with the P2E substrate (Fig. 2). The ANOVA with repeated measures showed that there is an effect of storage time on BCI and AUDPC, with differences over the three evaluation moments. For *Clarireedia* spp. it was observed $W = 0.437$, $p \leq 0.001$; for *S. rolfsii* $W = 0.259$, $p \leq 0.001$ and for *R. solani* $W = 0.313$, $p \leq 0,001$. For *Clarireedia* spp., AUDPC values showed differences over time, but with no differences between 0 and 12 months of storage ($p = 1.000$), which was expected since its value decreased at 6 months (where the substrates showed highest AUDPC), rising again at 12 months. For BCI the results were similar ($W = 0.744$, $p \leq 0.001$), with differences between 0 and 6 months ($p = 0.004$) and between 6 and 12 ($p \leq 0.001$). The BCI in the enriched composts, showed high values at 0 and at 6 months, with a marked decrease at 12 months (Fig. 3). The BCI values for *S. rolfsii* decreased from 6 to 12 months ($p \leq 0.001$) (Fig. 3).

For *R. solani*, AUDPC increased from 0 to 6 months ($p \leq 0.001$), and one year after enrichment, the composts still retained the ability to control this pathogen (Fig. 5). The AUDPC for *S. rolfsii* showed differences at all the assessment periods ($p \leq 0.001$), increasing at 6 months, and decreasing at 12 months but with differences for the 6 months assessment ($p = 0.803$) (Fig. 5).

Fig. 4 shows the efficacy of the enriched composts, with an increase at 6 months and a decrease at 12 months. However, the best results were achieved with the enriched composts P1E and P2E (Fig. 4).

According to the linear regression data and Spearman correlation at 12 months (Table 3), the biological control was strongly affected by the reduction of the nutritional resources and the decrease in *Trichoderma* spp. and total fungi populations. Despite its population decline, *Trichoderma* spp. continued to show a strong Biological control index, namely at 6 ($p \leq 0.001$) and 12 months ($p \leq 0.001$), only for *Clarireedia* spp. (Table 3).

4. Discussion

The biochemical and microbiological dynamics of compost during storage are intrinsically linked to enzymatic activity, organic matter decomposition, and nutrient cycling, with direct implications for microbial survival and substrate quality. As observed by Pang et al. [40], compost enrichment at 0 months increased the organic matter as well the nitrogen content. Changes in the microbiota, with higher populations, namely the increase in *Trichoderma* spp., may have contributed to the changes in pH, as described by Machado et al. [41]. Also Benitez et al. (2004) found that some *Trichoderma* strains can modify the external pH, adapting their metabolism to these conditions, reducing the phytopathogens virulence.

During the studied storage period, the properties of the substrates changed, namely the content of Kjeldahl nitrogen, the content of organic matter, the content of dry matter, and the microbiota population. At 6 months, dry matter largely increased in all substrates, up to values above 80 %, contributing to the significant decrease of fungi populations. *Trichoderma* spp. continued to show the highest populations in the enriched composts, P1E and P2E. During this initial 6 month storage

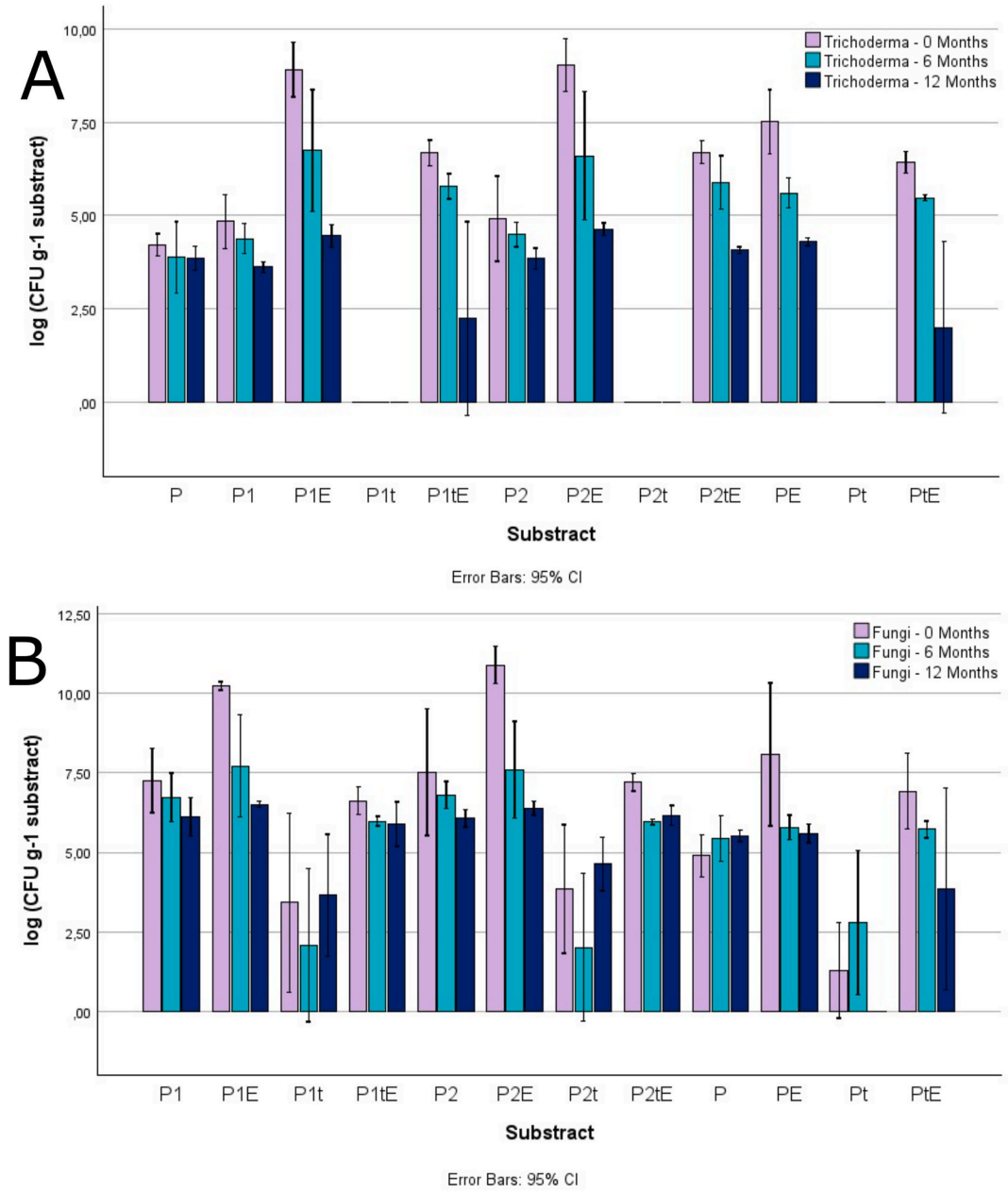

Fig. 1. Microbial populations at 0, 6 and 12 months of storage. A, *Trichoderma atroviride* population; B, Total fungi population; P1, compost P1; P2, compost P2; P, peat; t, substrates submitted to a thermic treatment at 60 °C for seven days; E, substrates enriched with *Trichoderma atroviride*. For each substrate bars depicting the same character had no statistically significant differences for $p > 0.05$ (Duncan Test).

period, microbial populations decreased, as observed by Saadi et al. (2010), likely due to a the reduction in composts moisture content. This is a crucial parameter, as microorganisms rely on water to transport nutrients and energy through the cell membrane [42]. A decrease in both moisture and organic matter leads to a reduction in the microbial activity [43].

At 12 months of storage, *Trichoderma* spp. populations declined in the enriched composts, although their levels remained higher compared to the other substrates. At this stage, moisture content was lower, which also affect nutrient availability for microorganisms [43]. A decrease in nitrogen content and organic matter was observed in all tested substrates, except in P1tE and P2tE. The reduction of carbon sources, likely driven by microbial activity [44] may have contributed to this decline. However, this decrease was not observed in the heat-treated and enriched composts, where a significant reduction of the microbiota had already occurred. From 6 to 12 months of storage, the increase in dry matter content created favourable conditions for the decline of microbial populations, along with a reduction in carbon sources [44]. The pH and nitrogen levels are key factors influencing the growth of *Trichoderma* spp. and its biocontrol capacity. Although *Trichoderma* spp. is known for its adaptability and resilience, changes in these environmental conditions can significantly impact its antagonistic activity. Thus, the observed decline in microbial populations and biocontrol performance could be linked to the variations in both pH and nitrogen availability during storage [45].

Nitrogen content significantly decreased in all P1 and P2 substrates, reaching values below 1 %. The shortage of microbial activity might have prevented the immobilization of $N\text{-}NH_4^+$ by microorganisms, consequently increasing NH_3 emission, causing nitrogen losses [44,46]. However, nitrogen losses are not common in mature and stabilized

Table 2
Enzymatic activity in the different substrates (enzyme relative activity.g^{-1}).

Enzyme[b]/Time		Substrates[a]											
		P1	P1t	P1E	P1tE	P2	P2t	P2E	P2tE	P	Pt	PE	PtE
1	0-M	4.0a	0.0a	4.0a	3.0a	5.0a	4.0a	5.0a	1.5a	0.0a	0.0a	2.0a	1.0a
	6-M	0.5a	0.0a	1.0b	1.0a	0.5b	0.5b	2.0a	0.5a	0.0a	0.0a	0.0a	0.0a
	12-M	2.0a	0.0a	3.5a	2.0a	0.0b	0.0b	2.0a	0.0a	0.0a	0.0a	0.0a	0.0a
2	0-M	4.0a	4.0a	4.0a	3.0b	4.0a	4.0a	4.0a	2.5a	1.0a	1.0a	2.0a	0.5a
	6-M	5.0a	4.0a	5.0a	4.0 ab	5.0a	2.5a	5.0a	3.5a	2.5a	2.0a	4.0a	0.0a
	12-M	4.5a	5.0a	4.5a	4.5a	4.0a	0.0a	4.0a	4.5a	1.0a	1.0a	4.0a	0.0a
3	0-M	4.0a	2.0a	4.0a	3.0b	4.0a	3.0a	4.0a	2.0a	0.0a	0.0a	2.5a	1.0a
	6-M	3.5a	1.5a	3.0a	3.0b	4.0a	3.5a	4.0a	1.5a	0.0a	0.0a	0.5b	0.0a
	12-M	3.0a	3.0a	4.0a	4.5a	3.5a	3.0a	3.5a	3.5a	0.0a	0.0a	3.0a	0.0a
4	0-M	4.5a	4.0a	4.0a	4.0a	4.0a	4.0a	4.0a	2.5a	1.0a	1.0a	4.0a	2.5a
	6-M	4.0a	4.0a	4.0a	4.0a	5.0a	4.5a	5.0a	4.0a	2.5a	2.0a	4.0a	4.0a
	12-M	4.0a	5.0a	4.5a	4.5a	4.5a	5.0a	4.5a	4.5a	1.0a	1.0a	5.0a	4.0a
5	0-M	4.0a	2.0a	4.0a	4.0a	4.0a	4.0a	4.0 ab	0.5b	1.0a	1.0a	1.5a	0.5a
	6-M	3.5a	1.5a	4.0a	3.0 ab	5.0a	1.5b	5.0a	4.0a	2.5a	2.0a	4.0a	3.0a
	12-M	3.0a	2.0a	3.5a	2.5b	2.5a	1.0b	2.0b	0.0b	1.0a	1.0a	3.0a	1.5a
6	0-M	2.5a	3.0a	3.0b	3.0a	4.0a	2.0a	4.0a	1.5 ab	0.0a	1.0a	2.5a	1.5a
	6-M	0.0b	2.0a	0.0c	0.0a	1.5a	1.5a	0.0b	0.0b	0.0a	2.0a	2.0a	1.0a
	12-M	3.5a	4.0a	4.5a	2.5a	3.5a	3.0a	4.5a	4.0a	0.0a	1.0a	0.0a	1.5a
7	0-M	5.0a	5.0a	5.0a	4.0 ab	5.0a	3.0a	4.0a	2.5a	1.0a	1.0a	3.0a	1.5 ab
	6-M	2.5b	3.0a	3.0b	3.0b	4.0a	4.0a	4.0a	3.5a	2.5a	2.0a	4.5a	3.0a
	12-M	4.0a	4.0a	4.0a	4.5a	4.5a	4.0a	4.5a	4.5a	1.0a	1.0a	3.0a	0.5b
8	0-M	4.0a	3.0a	4.0a	4.0a	4.0a	3.0a	4.0a	2.5a	0.0a	0.0a	3.0a	2.0a
	6-M	1.5a	1.5a	0.0b	0.0b	2.0a	1.5a	0.0b	1.5a	0.0a	0.0a	2.0a	0.0a
	12-M	4.0a	3.0a	3.5a	4.5a	2.5a	3.0a	4.5a	5.0a	0.0a	0.0a	4.5a	1.0a
9	0-M	4.0a	0.0b	5.0a	4.0a	4.5a	4.0a	5.0a	2.5a	0.0a	0.0a	4.0a	2.5a
	6-M	4.0a	2.5 ab	5.0a	4.0a	4.5a	3.0a	5.0a	4.0a	0.0a	1.0a	3.5a	1.0a
	12-M	4.0a	4.0a	3.0a	3.5a	4.5a	3.0a	4.5a	4.0a	0.0a	0.0a	4.0a	2.0a

[a] P1, compost P1; P2, compost P2; P, peat; t, substrates submitted to a thermic treatment (60 °C for 7 days); E, substrates enriched with *Trichoderma atroviride*; ST, storage time.
[b] 1 – Lipase (C 14); 2 – Leucine arylamidase; 3 – Valine arylamidase; 4 – Acid phosphatase; 5 – Naphthol-AS-BI-phosphohydrolase; 6 – α-galactosidase; 7 – β-galactosidase; 8 – β-glucosidase; 9 – N-acetyl-β-glucosaminidase; 0-M, compost stored for 0 months; 6-M, compost stored for 6 months; 12-M, compost stored for 12 months. For each enzyme, in each column, values with the same letter had no statistical differences ($p < 0.05$), according to Duncan New Multiple-Range Test.

composts (Sikora and Szmidt, 2005). Nitrogen in composts is found mainly in the organic forms, while the predominant inorganic nitrogen is present as nitric (NO_3^-). Ammoniacal nitrogen, on the other hand, is typically associated with the thermophilic phase of composting (Sikora and Szmidt, 2005). For this reason, nitric nitrogen is often used as an indicator of compost stability (Chefetz et al., 1998). Nitric nitrogen is more susceptible to leaching [44], but this loss will have not happened in the studied composts, due to its low moisture content, as noted by Brito et al. [46]. However, the denitrification processes may have also contributed to N-NO_3^- losses. Ammonia volatilization may also cause losses of N-NH_3 in the gaseous form ([47,48]; Sikora and Szmidt, 2005; [46]). The characteristics of the composts at 12 months of storage contributed to the nitrogen losses by volatilization, contrary to what is mentioned by Hubbe et al. (2010), since a C/N ratio between 38 and 48 was reached, and very low moisture content (between 7.9 and 16.7 %). Although the composts were stored in polypropylene tissue bags, limited internal aeration may have occurred, contributing to nitrogen losses through volatilization. During compost storage, this possible lack of aeration, along with a decrease in moisture content, may have further contributed to the reduction of microbial populations and additional nitrogen losses. These nitrogen losses influenced the efficacy and resilience of *Trichoderma* spp., reducing mycelial growth and sporulation, which are promoted by nitrogen sources [45].

The activity of N-acetyl-β-glucosamine may be associated with high populations of *Trichoderma* spp., showing positive results in the biological control of the studied pathogens (Benitez et al., 2004, Köhl et al., 2019). At 0 and 6 months of storage, there was high activity of this enzyme, as decribed by Alfano et al. [4]. This activity was highest in the enriched composts (P1E and P2E), reflecting the enzymatic potential of *T. atroviride*, a known producer of N-acetylglucosaminidases. Nevertheless, the original composition of the composts also contributed, as enzymatic activity was detected, although at lower levels, in the non-enriched composts. This highlights the combined importance of microbial enrichment and compost composition in enhancing biological activity.

At 12 months of storage, enzyme activity decreased, as also observed by Li et al. [49], who reported a similar decline in N-acetyl-β-glucosaminidase activity over time in pine needle litter. The reduction in *Trichoderma* populations, due to less favourable environmental conditions, may have contributed to the decrease in N-acetyl-glucosaminidase activity, although no statistical differences were observed over time, with particular emphasis on P1E, P1tE, and P2E (Table 2). This reduction may explain the loss of suppressiveness in these substrates, as reported by Avilés and Borrero [50].

Trichoderma spp. are efficient producers of enzymes such as amylases, proteases, and lipases, which may have an industrial application. In nature, it is common to observe their activity on phytopathogenic microorganisms cell wall degradation [51]. These enzymes exhibit strong activity during composting (Goyal et al., 2005), with their activity being dependent on the degree of decomposition of the organic matter [52]. In our study, lipase activity decreased in many substrates after 6 months of storage, likely due to the decrease in microbial populations. This reduction in lipase activity can be attributed to the greater stability of the composts over time. Leucine arylamidase is involved in the release of N-terminal aminoacides from peptides, amides or arylamidases [53]. In this case, the enzymatic activity in compost P2E increased after 6 months of storage and decreased after 12 months. This change may have affected nitrogen loss, as mentioned by Müller et al.

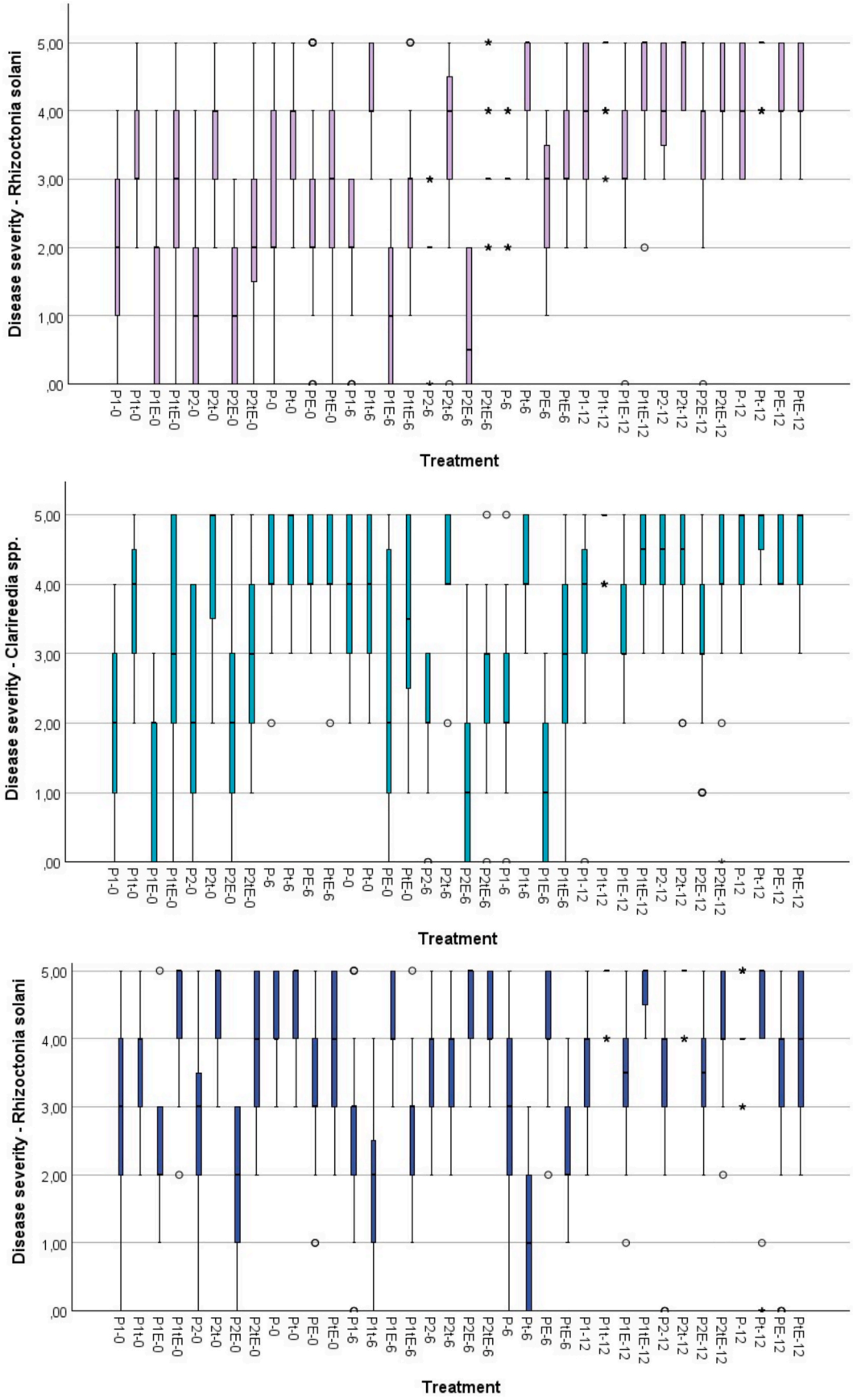

Fig. 2. Disease severity at 0, 6 and 12 months of storage. P1, compost P1; P2, compost P2; P, peat; t, substrates submitted to a thermic treatment at 60 °C for seven days; E, substrates enriched with *Trichoderma atroviride*. 0, substrates with 0 months; 6, substrates with 6 months; 12, substrates with 12 months.

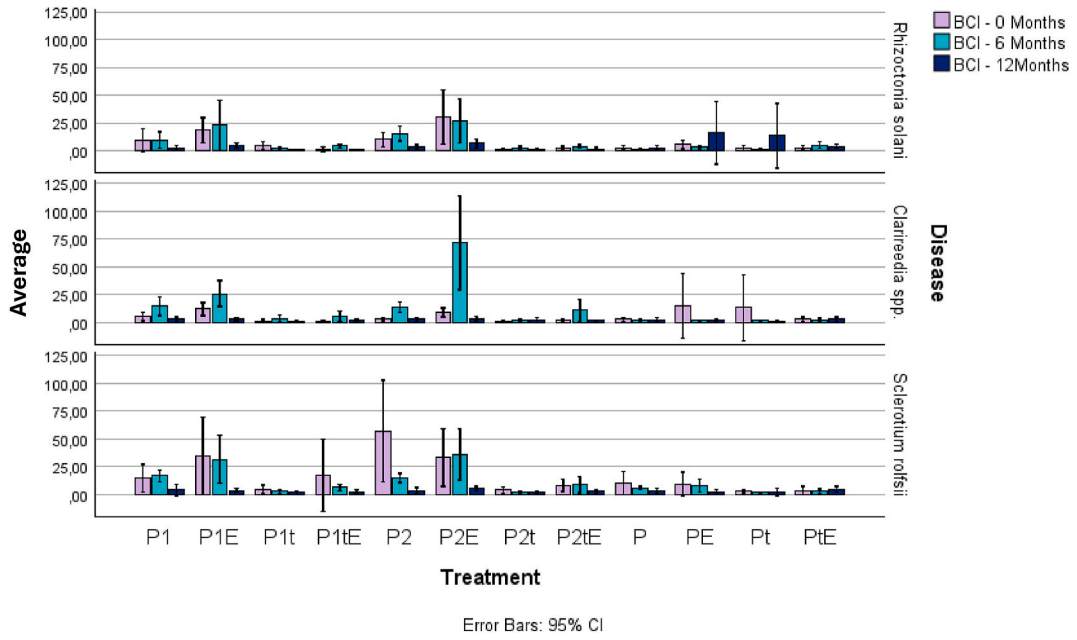

Fig. 3. Biological control index at 0, 6 and 12 months of storage. P1, compost P1; P2, compost P2; P, peat; t, substrates submitted to a thermic treatment at 60 °C for seven days; E, substrates enriched with *Trichoderma atroviride*. For each substrate, bars depicting the same character had no statistically significant differences for $p > 0.05$ (Duncan Test).

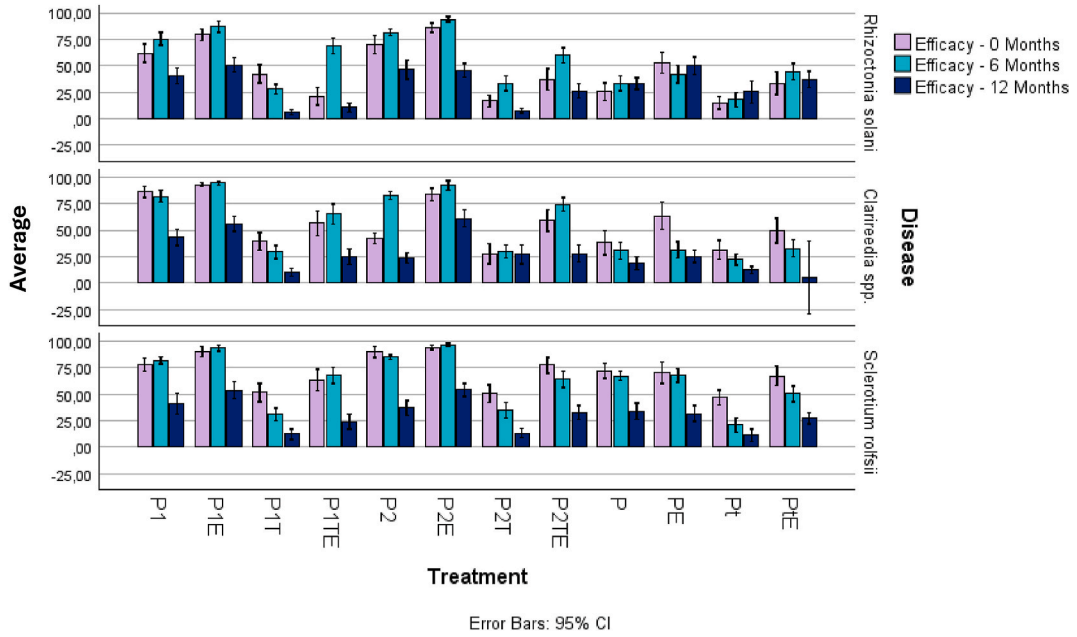

Fig. 4. Compost efficacy at 0, 6 and 12 months of storage. P1, compost P1; P2, compost P2; P, peat; t, substrates submitted to a thermic treatment at 60 °C for seven days; E, substrates enriched with *Trichoderma atroviride*. For each substrate, bars depicting the same character have no statistically significant differences for $p > 0.05$ (Duncan Test).

[54], who observed a correlation between this enzyme with the change of nitrogen in pine needle litter.

It was observed that β-glucosidase decreased at 6 months, followed by an increase after 12 months of storage. According to Borrero et al. [3], β-glucosidase may be an indicator of carbon compounds competition, showing a reduction of these resources at 12 months storage, when the lowest values of organic matter were recorded.

The temporal evolution of compost suppressiveness against *R. solani*, *S. rolfsii* and *Clarireedia* spp. reflected complex interactions between *Trichoderma* persistence, enzymatic activity and substrate maturation, with treatment-specific patterns emerging across storage periods. To analyse the suppressive capacity of composts, we observed that the stored composts kept their suppressive capacity, mainly the enriched composts, with a tendency to present higher suppressive capacity at 6 months, and a decrease at 12 months.

The composts showed suppressive capacity at the three evaluation periods, 0, 6 and 12 months, however total fungi and *Trichoderma* spp. populations decreased with time ($p \leq 0.001$), according to ANOVA to repeated measures. The enzymatic activity is an important variable to explain disease suppressivity. According to Martin [55], the presence of

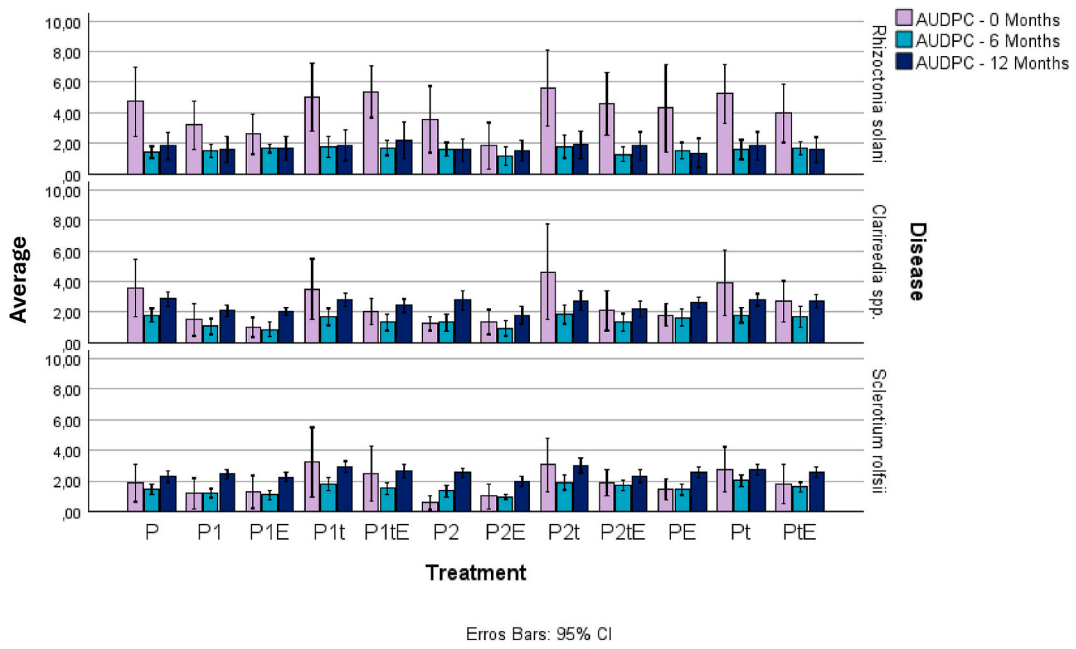

Fig. 5. Area Under Disease Curve Progress (AUDPC) at 0, 6 and 12 months of storage. P1, compost P1; P2, compost P2; P, peat; t, substrates submitted to a thermic treatment at 60 °C for seven days; E, substrates enriched with *Trichoderma atroviride*. For each substrate, bars indicate statistically significant differences for $p > 0.05$ (Duncan Test).

protease, chitinase, lipase and β-1,3-glucanase cell wall-degrading enzymes, may indicate a role of mycoparasitism. Lipase decreased during storage, but its activity was still observed in P1E after 12 months of storage.

Chitin, a polymer bound to β-(1,4)-N-acetyl-B-glucosamine (Brzezinska et al., 2014), and N-acetyl-β-glucosaminidase showed high activity at 0 and 6 months of storage. At 12 months their activity decreased, namely in P1E and P2E, but continued in all substrates with P1 and P2 composts. The activity of these enzymes may indicate the occurrence of mycoparasitism in the substrates, mainly in the enriched ones, where the registered activity was higher (Li et al., 2024). Disease severity increased at 12 months of storage while enzymatic activity decreased. Lorito et al. [56] also observed synergistic interactions between enzymes that degrade fungal cell walls and antifungal substances that inhibit spore germination. In the enriched composts, the highest *Trichoderma* populations likely enhanced the biological control, as well as the increased enzymatic activity, facilitating the degradation of the cell wall of the phytopathogenic agents [57].

For *R. solani* and *S. rolfsii*, the best results were observed with P2E at 6 months of storage. *Trichoderma* spp. produces lytic enzymes, that do not directly affect the phytopathogen. *Trichoderma* spp. can activate the chitinase genes and parasite *R. solani* when cellulose and glucose are in lower concentrations [55]. The suppressive capacity of composts against different phytopathogenic fungi is influenced by their level of maturation and stability [26]. Accordingly, the potential decline in carbohydrates concentrations may have contributed to the increased parasitism of *R. solani* at 6 months of storage, as previously reported by other authors como citado por outros autores [16,26]. In fresh organic matter, the synthesis of lytic enzymes involved in the biocontrol by *Trichoderma* spp. is repressed, due to its high glucose content [5]. In immature composts, *R. solani* still causes disease in plants, with no biological control due to the inactivity of *Trichoderma* spp. [26]. In stabilized or mature compost, with low concentrations of nutrients such as glucose, *R. solani* sclerotia are destroyed by the biocontrol agents [20]. However, in excessively stabilized compost the suppressive capacity may be lost if there are no food sources for microbial activity [5,25]. Concordantly with Danon et al. [58], at 12 months of storage it was observed that fungi populations decreased due to the lack of carbon sources, with a decrease in the suppressive capacity of the composts.

The substrate P1E showed the best results for *Clarireedia* spp. control at 0 and 6 months of storage.On composts with one year of storage, the plants showed an increase in the disease severity. Usually, total fungi and *Trichoderma* spp. populations decrease as dry matter content increase. However, it was observed that the enriched composts (P1E and P2E) exhibited the lowest severity, primarily due to the activity of the antagonists, particularly *T. atroviride*, which was added to de composts. At 12 months of storage, the best control of *Clarireedia* spp was observed on compost P2E. The significant reduction of nitrogen content in P1E may explain the lowest biocontrol capacity observed with this compost at this time. On the contrary, in P2E, the reduction in nitrogen content was likely compensated by the largest *Trichoderma* spp. population, which may have promoted the control of *Clarireedia* spp, as observed by Liu et al. [59], contributing to the success of P2E in controlling *Clarieedia* spp. growth at 12 months of storage. The low suppressive capacity in the heat-treated composts highlights the importance of antagonists in the biological control of plant diseases [31,60]. When enriched with *T. atroviride*, these composts exhibit a higher suppressive capacity. The addition of antagonists to the heat-treated substrate provides evidence that *T. atroviride* has strong potential in the biological control of the studied diseases, according to Vitale et al. [45]. The microbiota present in the substrates has a strong impact on all parameters analised for the assessment of biological control. The BCI in the enriched composts, showed the highest values at 0 and at 6 months, when the enzymatic activity was highest, with a sharp decrease at 12 months. A similar trend was observed for the AUDPC. Sant et al. [61], when enriching grape marc compost, also observed enhanced biological control of *Fusarium* wilt in carnation. Similarly, Matias et al. [8], when enriching sand filters with *T. atroviride* to control *R. solani*, also reported improved pathogen control, with notably higher BCI values and reduced disease severity. According to the linear regression data and Spearman correlation at 12 months, the biological control was strongly affected by the reduction of nutritional sources and the decrease in *Trichoderma* spp. and total fungi populations. Principal component analysis showed that, despite the population decline over time, *Trichoderma* spp., continued to exhibit strong biological control capacity, particularly at 6 months. At 12 months, its influence was observed only in the control of *Clarireedia* spp.

Table 3
Principal Component Analysis to evaluate which drivers had a significant effect on disease supressivity by composts.

	Rhizoctonia solani			*Sclerotium rolfsii*			*Clarireedia* spp.		
Variable[a]		Coef	Sig		Coef	Sig		Coef	Sig
0 Months									
Efficac	Constant		0.010	Constant		0.010	Constant		0.006
	DM	−0.547	0.015	DM	−0.547	0.015	N	0.224	0.052
	N	0.366	0.016	N	0.366	0.016	Tric	0.258	0.026
	pH	−0.554	0.025	pH	−0.554	0.025			
	Tric	0.238	0.036	Tric	0.238	0.036			
BCI	Constant		0.000	Constant			–	–	–
	Fungi	0.768	0.000	Fungi	0.768	0.000			
DS	Constant		0.471	Constant		0.039	Constant		0.000
	DM	0.237	0.044	DM	0.255	0.027	N	−0.270	0.019
	Tric	−0.256	0.030	Tric	−0.294	0.011	Tric	−0.231	0.043
AUDPC	Constant		0.000	Constant		0.000	Constant		0.000
	N	0.237	0.012	Tric	−0.243	0.029	EC	−0.295	0.012
	Tric	−0.306	0.006	C/N	0.331	0.003			
6 Months									
Efficacy	Constant		0.154	Constant		0.855	Constant		0.002
	DM	0.226	0.054	pH	0.382	0.001	OM	0.296	0.019
	pH	0.318	0.009	Tric	0.279	0.010	pH	0.763	0.000
	Tric	0.268	0.010				Tric	0.304	0.001
BCI	Constant		0.000	Constant		0.000	Constant		0.085
	Tric		0.000	Tric	0.406	0.000	pH	0.219	0.016
				C/N	−0.243	0.027	Fungi	−1.210	0.002
							Tric	1.738	0.000
DS	Constant		0.000	Constant		0.000	Constant		0.000
	Tric	−0.275	0.019	Tric	−0.371	0.001	N	−0.386	0.000
				C/N	0.360	0.001	Tric	−0.426	0.000
AUDPC	Constant		0.000	Constant		0.000	Constant		0.000
	Tric	−0.254	0.031	Tric	−0.243	0.034	Tric	−0.3443	0.003
				C/N	−0.318	0.006	N	−0.238	0.036
12 Months									
Efficacy	Constant		0.000	Constant		0.232	Constant		0.956
	N	−0.286	0.022	DM	0.431	0.000	DM	0.259	0.025
	Fungi	0.417	0.001	Tric	0.292	0.005	Tric	0.251	0.030
BCI	Constant		0.010	Constant		0.003	–	–	–
	pH	−0.242	0.041	OM	−0.250	0.021			
				Tric	0.394	0.000			
D	Constant		0.000	Constant		0.124	Constant		0.000
	EC	0.414	0.000	OM	0.326	0.001	Fungi	−0.411	0.000
				Tric	−0.543	0.000			
AUDPC	Constant		0.000	Constant		0.064	Constant		0.000
	OM	−0.262	0.010	C/N	0.413	0.000	Fungi	−0.400	0.001
	N	−0.497	0.000	Tric	−0.271	0.012			

[a] BCI, biological control index; DS, disease severity; AUDPC, area under disease progress curve; N, nitrogen; OM, organic matter; DM, dry matter; EC, electrical conductivity; C/N, carbon – nitrogen ratio; Tric, *Trichoderma* spp. populations.

The control of *Clarireedia* at 12 months was achieved due to the presence of *Trichoderma* spp., as the nitrogen content had decreased. Nitrogen application is one of the cultural practices used to control the growth of this fungus [62]. In the ready-made composts (0 months) several variables were related to the suppressivity of the composts P1 and P2 enriched with *T. atroviride* and heat-treated, or only enriched. Substrates P1tE and P2tE showed the expected control of *T. atroviride* on the studied phythopathogenic fungi. The heat treatment will have eliminated the antagonists in P1t and P2t, thus eliminating their suppressive capacity. However, the addition of an antagonist restored its suppressive capacity indicating the fundamental role of *Trichoderma* spp. in the biological control, showing the potential of this fungus in the control of soil-borne diseases (Vitale et al., 2024). Several authors observed the positive effect of *Trichoderma* spp. in the control of *R. solani* ([11], Pugliese et al., 2008, [16]), *S. rolfsii* [63,64] and *Clarireedia* spp. with *T. gamsii* [65] and *T. atroviride* [31]. At 6 months of storage, a positive correlation was observed between *Trichoderma* spp. and the supressivity of the composts. This positive correlation was also observed for the BCI but it was negative for DS and AUDPC. Despite *Trichoderma* spp. populations decrease from 0 to 6 months, the composts kept their ability to control turfgrass pathogenic fungi, even better than at 0 months, in the composts P1, P2, P1E and P2E. On the other hand, the decrease in the nitrogen content showed a negative correlation with the disease severity caused by *Clarireedia* spp., highlighting the role of nitrogen as a cultural measure to control the development of this pathogenic fungi [62].

At 6 months of storage, the organic matter content remained high enough to support the activity of microorganisms, and the increase in dry matter will have been gradual, having no impact on their activity and nutrient available [43]. At 12 months, the decrease in organic matter content led to a reduction in total fungi and *Trichoderma* spp. populations, as described by Azim et al. [52]. likely due to a lack of nutrients, particularly carbon sources. This observation aligns with findings by other authors [16,44], who reported that the number of CFU. g^{-1} in composts is positively associated with enzymatic and respiratory activity. According to De Corato [16], organic matter has a fundamental role on the populations of microorganisms and their diversity, being considered as the natural support for suppressivity. De Corato [16] observed that hyperparasitism, particularly by *Trichoderma* spp. against *R. solani* and *S. rolfsii*, is strongly influenced by the level of organic matter decomposition, namely due to the presence of glucose and water-soluble nutrients which together repress enzyme production and lytic effect necessary to kill *R. solani*. Competition for nitrogen may also have been another mechanism of suppressiveness by *Trichoderma* against the pathogenic fungistudied [66], since nitrogen content

decreased between 6 and 12 months. However, microorganisms that are competitive saprophytes, facultative hyperparasites and symbionts can colonize and induce biocontrol if they are positioned at lower levels of the trophic chain, surviving in decomposed organic matter ([67]; Pal and Gardener, 2006).

After 12 months of storage, the decrease in microbial populations also led to a reduced enzymatic activity, namely the lytic enzymes such as β-glucanase, chitosiosis and chitinase verified by De Corato [16], and for N-acetil-β-glucosamine, leucine and Naphthol-AS-BI-phosphohydro lase. β-glucosidase increased from 6 to 12 months and should be an indicator of microbiostasis and evidence of competition for carbon sources between microorganisms with the reduction of organic matter [18].

For *R. solani*, it was found that the control efficacy increased at 6 months in P1, P1E, P2 and P2E, being higher in the enriched substrates that in non-enriched ones, and decreased at 12 months. When substrates were heated and enriched, the decrease at 12 months was much more accentuated due to the lack of the biological control agents, with a significant reduction of *Trichoderma* spp. For *S. rolfsii* the results were similar ($p \leq 0.001$). However, the effectiveness of substrates in controlling *Clarireedia* spp. from 0 to 12 months increased only in P2 and P2tE ($p \leq 0.001$) and decreased in all other substrates.

After one year of storage, disease suppressiveness remained higher in the enriched composts compared to thenon-enriched ones The use of organic composts for biocontrol is particularly effective when enriched with *Trichoderma atroviride*, as this promotes favourable environmental conditions for its persistence and activity. This finding underscores the significance of circular economy principles in agricultural sustainability, as highlighted by previous studies [68]. Given the seasonal availability of certain composting materials, such as grape marc, it is essential to ensure the long-term suppressive potential of composts designed for pathogen control.

In this context, the origin and composition of the composts gain particular relevance, especially when they derive from regionally abundant and underutilised waste streams. In the Algarve region, the leading citrus-producing area in Portugal, substantial quantities of orange processing residues are generated annually, representing a challenge and an opportunity for waste valorisation. At the same time, the Algarve stands out as one of Europe's top golf tourism destinations, with over 30 golf courses producing significant amounts of grass clippings, a nutrient-rich but highly perishable waste. By integrating these region-specific biowastes into composting processes, this work contributes to a territorial circular economy, reducing disposal pressures and transforming local organic by-products into valuable agricultural inputs.

Similar findings were reported by Chorolque et al. [69], who demonstrated the suppression of soil-borne phytopathogenic fungi via composting of onion agro-industrial waste. Their study illustrates how composting not only diverts waste from landfills but also enhances soil health and plant protection, aligning with circularity principles. In our study, suppressiveness was retained for up to 12 months, with peak effectiveness observed at six months. Maintaining suitable environmental parameters, particularly moisture, is crucial for the continued viability of *T. atroviride* and, consequently, for sustaining compost suppressiveness over time.

5. Conclusions

Composts P1 and P2 showed suppressive capacity against *Rhizoctonia solani*, *Clarireedia* spp. and *Sclerotium rolfsii* in turfgrass (*Agrostis stolonifera*). There was an increase in the biological control of the studied pathogenic fungi until 6 months of storage. At 12 months, the enriched composts showed higher suppressive capacity than the non enriched composts.

For both P1 and P2 composts the best disease control results were observed at 6 months of storage.

Substrates P2 and P2E were more effective in controlling *R. solani* and *S. rolfsii* in the three storage periods studied. For *Clarireedia* spp. control substrates P1 and P1E showed better results at 0 and 6 months. At 12 months, the control of *Clarireedia* spp was more effective on the P2E substrate.

According to these results, the enriched composts kept their suppressive capacity for at least 12 months, although the best control results were achieved at 6 months of storage.

CRediT authorship contribution statement

Luísa Coelho: Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Mário Reis:** Writing – review & editing, Supervision, Methodology, Investigation, Data curation, Conceptualization. **Lídia Dionísio:** Writing – review & editing, Supervision, Methodology, Investigation, Conceptualization. **Carlos Guerrero:** Writing – review & editing, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

I hereby declare that this manuscript has not been published, nor is it under consideration for publication elsewhere. All co-authors have reviewed and agreed with the content and consent to being identified as co-authors. Additionally, this submission does not infringe upon the copyright of any third party.

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Data availability

Data will be made available on request.

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