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Temporal Stability of Soil Microbial Properties in Responses to Long-Term Application of Compost Obtained from Tannery Sludge

Talyta Carine da Silva Saraiva [1], Sabrina Hermelindo Ventura [1], Eudemio Sousa Brito Junior [1], Sandra Mara Barbosa Rocha [1], Romario Martins Costa [1], Arthur Prudencio de Araujo Pereira [2], Ricardo Silva De Sousa [3], Jay Prakash Verma [4], Paul J. Van den Brink [5,6] and Ademir Sergio Ferreira Araujo [1,*]

[1] Soil Microbial Ecology Group, Federal University of Piaui, Teresina 64049-550, Brazil
[2] Department of Soil Science, Federal University of Ceara, Fortaleza 60416-030, Brazil
[3] Department of Agricultural Engineering and Soil, Federal University of Piaui, Teresina 64049-550, Brazil
[4] Plant-Microbe Interaction Laboratory, Institute of Environment and Sustainable Development, Banaras Hindu University, Varanasi 221005, India
[5] Aquatic Ecology and Water Quality Management Group, Wageningen University, P.O. Box 47, 6700 AA Wageningen, The Netherlands
[6] Wageningen Environmental Research, P.O. Box 47, 6700 AA Wageningen, The Netherlands
* Correspondence: ademir@ufpi.edu.br; Tel.: +55-86-998645449

Abstract: Successive applications of compost obtained from tannery sludge affected the soil microbial biomass and activity. However, the effect of this practice on the temporal stability of soil microbial properties is not known. This study evaluated the temporal stability of microbial biomass, respiration, and enzymes activities in soil with successive applications of compost obtained from tannery sludge. Soil samples (0–10 cm depth) were collected from sites with successive application of compost at the lowest (2.5 ton/ha) and highest (20 ton/ha) rates, including the control (0 ton/ha). Soil microbial biomass carbon (MBC) and nitrogen (MBN), respiration, dehydrogenase, and urease activities were evaluated at 0, 30-, 60-, 90-, and 150-days post-application. The soil microbial properties varied as per treatments and sampling time. The principal response curve showed higher variation of soil microbial properties in the treatment having highest rate of compost. This analysis showed dehydrogenase, urease, and MBC as the most responsive parameters. The temporal stability of soil microbial parameters showed highest values at the lowest rate of compost. This study showed that the successive application of compost contributed to a decrease in variation and increase in temporal stability of soil microbial properties at the lowest rate.

Keywords: metal contamination; soil microbial biomass; soil enzyme activities; waste management

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Citation: Saraiva, T.C.d.S.; Ventura, S.H.; Brito Junior, E.S.; Rocha, S.M.B.; Costa, R.M.; Pereira, A.P.d.A.; De Sousa, R.S.; Verma, J.P.; Van den Brink, P.J.; Araujo, A.S.F. Temporal Stability of Soil Microbial Properties in Responses to Long-Term Application of Compost Obtained from Tannery Sludge. *Sustainability* **2022**, *14*, 16736. https://doi.org/10.3390/su142416736

Academic Editor: Alberto Ferraro

Received: 14 November 2022
Accepted: 8 December 2022
Published: 14 December 2022

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1. Introduction

Tannery sludge is a solid waste generated by tanning industries that has been recommended to be applied in soils. Its successive application causes modification in the soil properties, mainly increasing soil pH, organic C, and chromium (Cr) content [1–3]. On the one hand, the increase in organic C improves soil properties, e.g., promoting better soil structuration, particle size, aeration, soil fertility and health [4]. On the other hand, high soil pH decreases the availability of nutrients [5], while Cr accumulation negatively affects the soil's microbial properties [6].

Regarding the effect of successive application of tannery sludge on soil microbial properties, more attention has been placed on soil microbial biomass [1], while no attention is paid to the effect of tannery sludge on temporal stability of soil microbial properties. The temporal stability of microbial properties is an important measurement for understanding context-specific responses of soil microbes to any disturbances [7], and greater temporal stability of microbial properties indicates higher soil functionality and resilience [8]. According to Atlas et al. [9], higher temporal stability can also confer to soils an increased buffering capacity to support disturbance.

However, studies evaluating the temporal stability of soil microbial properties have mostly focused on land-use [10]. For example, Araujo et al. [11] assessed the temporal stability of microbial properties by comparing native and degraded lands, and they observed more temporally stable microbial properties on native land. Recently, Kostin et al. [10] assessed the temporal stability of microbial properties by comparing land-use types and degree of intensification, and they found higher stability in both extensive meadows and pasture than cropland. There is no study to date comparing the effect of solid wastes, and particularly tannery sludge, on the temporal stability of soil microbial properties.

In Brazil, a long-term study has evaluated the successive application of compost obtained from tannery sludge on soil properties, mainly microbial biomass [11]. The results from ten years of application have shown increased soil pH, organic C, and Cr in soil [1]. It is known that these soil properties are important drivers of soil microbial properties. Here, we hypothesized that these changes in soil pH, organic C, and Cr in soils with successive applications of compost obtained from tannery sludge could affect the temporal stability of microbial properties. Thus, this study evaluates the temporal stability of microbial biomass, respiration, and enzyme activities in soil with successive applications of compost obtained from tannery sludge.

2. Materials and Methods

In this long-term study [1], a compost obtained from tannery sludge was successively applied in a soil (Fluvisol) for twelve years at five rates (0, 2.5, 5, 10, and 20 ton/ha). The compost was produced by mixing tannery sludge with sugarcane straw and cattle manure (ratio 1:3:1; *v*:*v*:*v*), and the composting was performed using the aerated-pile method for 90 days. The compost presents the following chemical composition: pH ≈ 8.5, organic C ≈ 187 g/kg, N ≈ 1.7 g/kg, P ≈ 4.0 g/kg, K ≈ 3.3 g/kg, Ca ≈ 95.3 g/kg, Mg ≈ 6.8 g/kg, S ≈ 9.4 g/kg, and Cr ≈ 1815 mg/kg. This compost was amended into a field soil (0–10 cm) in all years, and its composition was assured regularly by laboratory analysis. In the twelfth year (2021), soil samples were collected monthly (0–10 cm depth) for 150 days (0, 30, 60, 90, and 150 days). In this study, we collected soil samples from the lowest (2.5 ton/ha) and highest (20 ton/ha) rates of compost, including the control (0 ton/ha). At each sampling time, temperature and moisture were estimated using a soil thermometer and moisture meter, respectively (Figure 1).

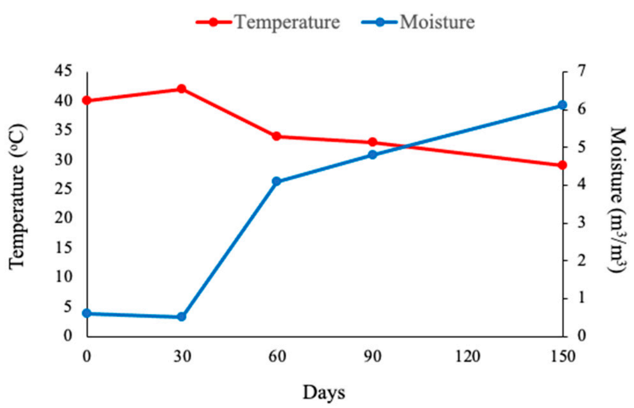

Figure 1. Variation of soil temperature and moisture for 150 days.

In addition, soil pH, organic C [12], and Cr concentration [13] were analyzed in all soil samples (Table 1). Soil microbial biomass C (MBC) and N (MBN) were determined according to Brookes et al. [14] with extraction of C and N from fumigated and unfumigated soils by K_2SO_4. An extraction efficiency coefficient of 0.38 and 0.45 were used to convert the difference in C and N between fumigated and unfumigated soil in MBC and MBN, respectively. Soil respiration (SR) was determined according to Alef and Nannipieri [15]. The respiratory quotient (qR) was estimated as the ratio between SR and MBC. Dehydrogenase (DHA) activity was determined according to Casida et al. [16] based on the spectrophotometric analysis of triphenyl tetrazolium formazan released by 5 g of soil after 24 h of incubation at 35 °C. Urease (URE) was measured using urea with the method developed by Kandeler and Gerber [17] using urea as substrate under incubation (1 h; 37 °C). The amount of ammonium produced was measured spectrophotometrically at 660 nm.

Table 1. Soil pH, organic C, and Cr content after twelve years of compost amendment.

Treatment	Soil pH (H_2O)	Organic C (g kg^{-1})	Cr Content (mg kg^{-1})
0 Mg ha^{-1}	6.3	8.7	2.5
2.5 Mg ha^{-1}	7.8	21.5	24.9
20 Mg ha^{-1}	8.9	41.8	158.1

Two-way split-plot ANOVA was used to test the effects of treatments, evaluation time, and their interaction on the soil's microbial properties. Treatments were considered as plots and the evaluation time as subplots. Data for MBN, qR, and urease were transformed into square root when normality of errors was not indicated by the Shapiro–Wilk test and homogeneous variance by the Bartlett test (Bartlett's K-squared). Tukey's tests ($p < 0.05$) were used for comparisons following ANOVAs, where appropriate. Analyses were conducted using R v 4.1.3 (R Core Team).

The principal response curve (PRC) [18,19] was used to show the temporal changes in microbial properties influenced by treatments as compared to the control (0 ton/ha) in one diagram and to quantify the contribution of each microbial property to the indicated differences between the treatments and the control. To make all parameters mathematically equally important in the analysis, they were standardized to zero mean and unit variance before analysis. Monte Carlo permutation tests were used to verify if the variation explained by the PRC diagrams, and the differences between treatments shown in the PRC diagram are significant [18]. The PRC was performed using CANOCO (version 5.3) [20]. The temporal stability of soil microbial properties was calculated as log10 coefficient of variance^{-1} (coefficient of variance = standard deviation/mean × 100) [21] and tested treatment effects using one-factorial ANOVA.

3. Results and Discussion

The soil's microbial properties varied according to treatments and the evaluation time (Figure 2). In the control and at the lowest rate of compost, MBC increased during the first 30 days and decreased at 60 days (Figure 2A). However, MBC increased again after 60 days. In contrast, at the highest rate of compost, MBC did not vary along 90 days and decreased at 150 days. In treatments with compost (lowest and highest rates), soil MBN showed highest values at the beginning (0 day) and decreased significantly at 30 days, maintaining low values over time (Figure 2B).

Soil respiration varied over time in treatments containing compost, while it maintained similar in the control (Figure 2C). However, in all treatments, the soil respiration increased at 150 days of evaluation. The respiratory quotient showed high variation over time in all treatments (Figure 2D). Comparing the lowest and highest rate of compost, the respiratory quotient presented lower values at the lowest rate and decreased over time. In contrast, the respiratory quotient increased over time at the highest rate of compost. Comparing treatments with compost, the activity of dehydrogenase showed lowest variation at the lowest rate of compost (Figure 2E). The activity of urease enzyme increased from 0 to 30 and 60 days, respectively, in all treatments, and decreased at 150 days, with the decrease being larger for the treatments with compost (Figure 2F).

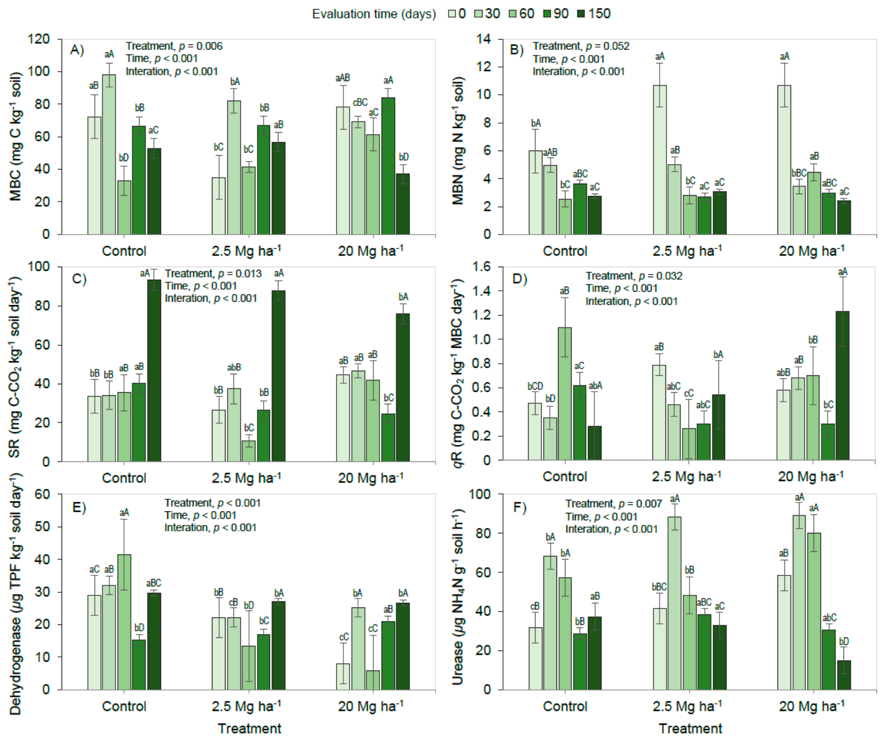

Figure 2. Variation of soil microbial biomass C (MBC; (**A**)) and N (MBN; (**B**)), soil respiration (SR; (**C**)), respiratory quotient (qR; (**D**)), dehydrogenase (**E**), and urease (**F**) at the lowest (2.5 Mg ha^{-1}) and highest (20 Mg ha^{-1}) rates of compost for 150 days. Lowercase letters compare treatments within the same time, while uppercase letters compare times within the same treatment by Tukey's test ($p < 0.05$).

We used a PRC analysis to show the variation of soil microbial properties according to treatments over time. As shown in Figure 3, the soil's microbial properties significantly varied over time in the treatment with the highest rate of compost, while the treatment with the lowest rate presented a lower variation. This PRC showed dehydrogenase, urease, and MBC as the most responsive parameters.

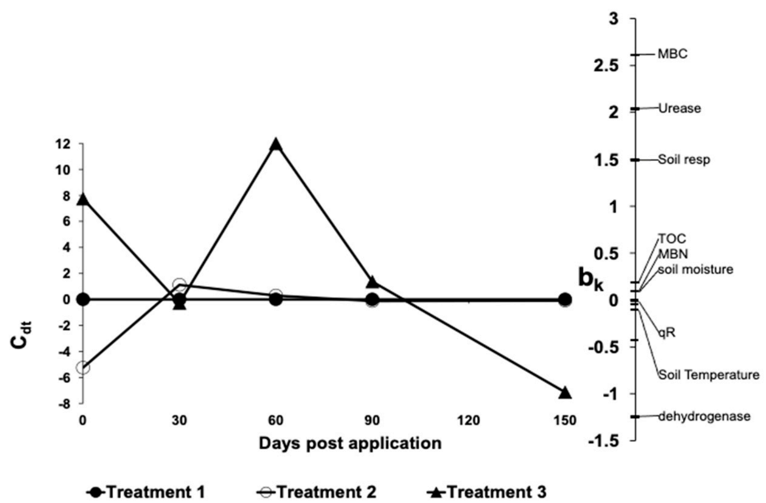

Figure 3. Principal curve response showing the variation of microbial properties over time. Evaluation time explained 84% of the total variation to microbial properties. Treatment explained 12% ($p < 0.001$), of which 50% is displayed in the PRC diagram. Treatment 1—control; Treatment 2—2.5 Mg ha^{-1}; Treatment 3—20 Mg ha^{-1}.

The temporal stability of soil microbial parameters showed distinct responses comparing treatments, mainly those with compost (Figure 4). Thus, the highest temporal stability of soil microbial parameters was found at the control and lowest rate of compost.

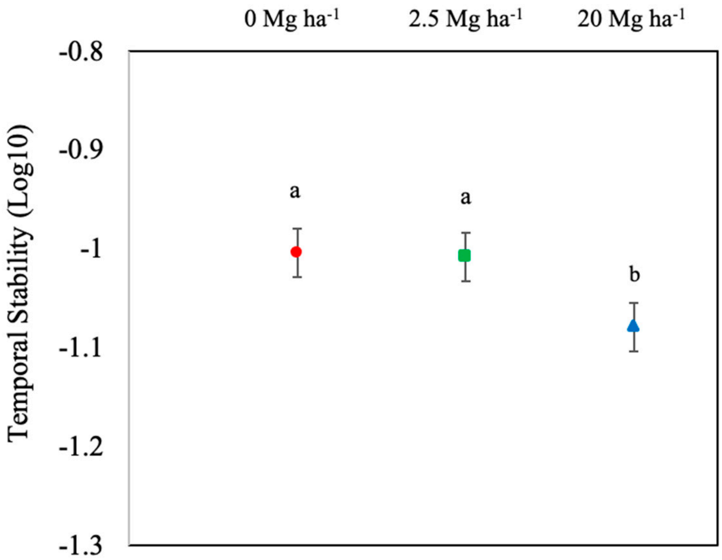

Figure 4. Temporal stability (log10 coefficient of variation^{-1}) of soil microbial properties as affected by application of compost. Bars with different letters differ significantly (Tukey HSD test, $p < 0.05$). Means with standard deviations.

This study assessed the impact of successive application of compost obtained from tannery sludge on temporal stability of soil microbial properties. Previous studies have shown that a decade of successive and permanent application of this compost changed the soil's chemical and microbial properties [1,22]. Positively, the application of compost significantly increased the soil organic C; while negatively, it promoted high soil pH and accumulation of Cr in soil (Table 1). Thus, this study addressed the hypothesis that these distinct chemical properties, i.e., soil organic C, pH, and Cr content, which found in soils under application of compost could influence the temporal stability of soil microbial properties. It is known that soil organic C conditions increase the stability of soil properties, mainly physical properties [23]. Particularly, soil organic C supports microbial biomass and the production of soil enzyme activity [24]. Thus, increased content of organic C after application of compost is very important for improving soil microbial properties, i.e., MBC, MBN, and soil enzyme activities. In contrast, high soil pH and accumulation of Cr can decrease the stability of soil microbial properties [25,26]. Based on these assumptions, our results have shown that although soil organic C increased at the highest rate of compost, it was not enough to promote the stability of soil microbial properties due to higher soil pH and Cr. On the other hand, the application of the lowest rate of compost increased soil organic C but promoted lower soil pH and accumulated less Cr content, which promotes higher stability of soil microbial properties. It could be possible to suggest that the lowest rate of compost promotes a buffering of microclimatic variations, for instance of soil temperature and moisture. Therefore, the soil's microbial properties, under application of the lowest rate of compost, seem to be less affected by environmental conditions.

Microbial C shows distinct responses with treatments and time as compared to microbial N (Figure 2A,B). In general, the microbial C increases over time in the treatment of the lowest rate of compost (Figure 2A). It differed from the responses of microbial C observed in the treatment with the highest rate of compost, in which there was a decreasing in microbial C over time. Microbial biomass C is the most active pool of soil organic matter [27], and the efficient use of C by microbes for increasing their biomass C, which is influenced by high soil pH and metals [28]. Thus, the higher pH and accumulation of Cr in soil under the highest rate of compost can contribute to decrease incorporation of C by microbes.

Indeed, Cr presents an inhibitory effect on C incorporation by microbes and this effect is concentration-dependent [29]. On the other hand, soil pH is an important driver that affects the efficiency of accumulating C into microbial biomass [25]. Therefore, increased soil pH and accumulation of Cr, after application of compost, contributed to lower MBC. On the other hand, microbial N showed similar responses in all treatments over time, which suggests no effect of chemical properties on this microbial pool (Figure 2B).

In general, the soil respiration increased at the end of the evaluation (Figure 2C), and it can be attributed to changes in microclimatic conditions, such as temperature and moisture observed in soils at 0 to 150 days of evaluation. As shown in Table 1, the soil temperature decreased, while moisture increased from 0 to 150 days of evaluation. However, we observed a variation in the soil respiration comparing the lowest and highest rates of compost. This can be explained by differences between soil organic C content found in these treatments. Several soil properties influence the soil respiration, being moisture, temperature, and organic C content, which are the most important soil factors [23]. In particular, soil temperature and moisture are important factors influencing microbial activity acting on organic C [29,30]. The respiratory quotient also varied according to treatments over time (Figure 2D). However, the comparing treatments with compost, the respiratory quotient decreases at the lowest rate of compost, while it increases at the highest rate of compost. These results confirm the effect of treatments on the efficiency in using C by microbes as observed in soil microbial biomass C. The use of C can be partitioned between incorporation (biomass) or losses (respiration) of C by microbes [31]. Thus, higher respiratory quotient, which represents losses of C from microbial biomass [32], means a decreased efficiency in using C by microbes. Thus, the highest rate of compost applied successively for years contributed to decreasing the efficiency of microbes to incorporate C into biomass.

The activity of dehydrogenase presented low variation in treatment with the lowest rate of compost (Figure 2E). In contrast, urease activity showed the same responses to both treatments with compost (Figure 2F). Comparing both enzymes, dehydrogenase is more sensitive to soil disturbance, mainly by metals [33]. Dehydrogenase is well-known as a sensitive indicator of microbial activity and is linked with microbial metabolisms [34]. Thus, the accumulation of Cr in the soil contributed to decrease soil microbial activity as also reported by Stêpniewska and Woliñska [35], who observed a reduction of dehydrogenase activity in soil with high content of Cr and a decrease in the oxidative capacity of the soil. In particular, Lukowski and Dec [36] found dehydrogenase more sensitive than urease to Cr contamination. Therefore, our results agree with these previous studies, which observed dehydrogenase more affected in treatment with high accumulation of Cr.

4. Conclusions

The present study represents the temporal stability of soil microbial biomass C and N, soil respiration, and soil enzyme activities (e.g., dehydrogenase and urease) in soil with continuous applications of compost from tannery sludge. The increased content of organic C after application of compost showed increased soil microbial properties, i.e., MBC, MBN, and soil enzyme activities. The increasing compost treatments as per time duration promotes soil pH and accumulation of chromium in soils, which affect soil microbial activities. Therefore, the compost application should be decreased in such way that the microbial stability for increasing soil fertility and health is maintained. As per the present study, the soil's microclimate variations such as moisture, temperature, pH, and Cr contamination showed more impact on soil microbial properties, i.e., MBC, MBN, and soil enzyme activities. The temporal stability of soil microbial parameters showed highest values at the lowest rate of compost. This study showed that the successive application of compost at the lowest rate contributed for decreasing variation and increasing temporal stability of soil microbial properties. In addition, this study suggests the evaluation and monitoring of Cr content in soils with amendment of tannery sludge since this metal could impact the soil's quality and human health.

Author Contributions: Conceptualization, A.S.F.A.; methodology, A.S.F.A. and R.S.D.S.; formal analysis, A.P.d.A.P. and P.J.V.d.B.; investigation, T.C.d.S.S., S.H.V., E.S.B.J., S.M.B.R. and R.M.C.; writing—original draft preparation, A.S.F.A., A.P.d.A.P. and R.S.D.S.; writing—review and editing, A.S.F.A., J.P.V. and P.J.V.d.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Conselho Nacional de Desenvolvimento Científico e Tecnologico—CNPq, grant number 305069/2018-1.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: This study was supported by Conselho Nacional de Desenvolvimento Científico e Tecnologico—CNPq (grant 305069/2018-1). Talyta C. S. Saraiva acknowledges FACEMA for her scholarship. Ademir S. F. Araujo acknowledges CNPq for his fellowship of research. Jay P. Verma thanks to SERB for providing SERB International Research Experience (SIRE) Fellowship 2022 (SIR/2022/000626).

Conflicts of Interest: The authors declare no conflict of interest.

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