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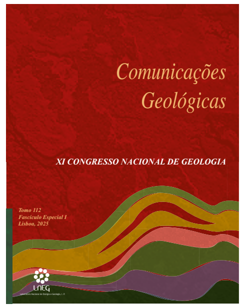
Effects of application of eucalyptus leaf biomass on soil quality: a field trial

Efeitos da aplicação de biomassa foliar de eucalipto na qualidade do solo: ensaio de campo



T. Teixeira[1*], P. Pato[1], A. Cachada[2,3], C. Patinha[1]

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Abstract: Approximately 30% of Portuguese forests are occupied by eucalyptus (*Eucalyptus globulus*). As this is a highly flammable invasive species, it is essential to control its proliferation. Thus, the PEST(bio)CIDE project, based on a circular economy perspective, aims to explore the phytotoxic potential of eucalyptus leaves, using its leaf biomass as an effective and environmentally safe biocide, and its use as a corrective to improve soil properties. It is also expected that the introduction of biomass into the soil will contribute to increase carbon reserves and soil fertility. Thus, it is possible to associate an economic benefit with better management of post-fire regenerated eucalyptus areas. Due to this, a field trial was carried out to test the effect of biomass application on the soil's physico-chemical properties. The results indicate that incorporating leaves has a positive effect on some soil properties, mainly in terms of increasing organic matter and available macronutrients.
Keywords: *Eucalyptus globulus*, physico-chemical properties, nutrients.

Resumo: Aproximadamente 30% da floresta portuguesa é ocupada por eucalipto (*Eucalyptus globulus*). Sendo esta uma espécie invasora altamente inflamável, é fundamental controlar a sua proliferação. Assim, o projeto PEST(bio)CIDE, baseado numa perspetiva de economia circular, visa explorar o potencial fitotóxico das folhas de eucalipto, usando a sua biomassa foliar como um biocida eficaz e ambientalmente seguro, e a sua utilização como corretivo para melhorar as propriedades do solo. Espera-se também que a introdução de biomassa no solo contribua para aumentar reservas de carbono e fertilidade do solo. Assim, é possível associar um benefício económico a uma melhor gestão das áreas de eucalipto regeneradas pós-incêndio. Para isto, foi realizado um ensaio de campo para testar o efeito da aplicação da biomassa nas propriedades físico-químicas do solo. Os resultados indicam que a incorporação de folhas tem um efeito positivo em algumas propriedades do solo, principalmente no aumento de matéria orgânica e macronutrientes disponíveis.
Palavras-Chave: *Eucalyptus globulus*, propriedades físico-químicas, nutrientes.

[1] Departamento de Geociências, Universidade de Aveiro, GeoBioTec, Campus de Santiago, 3810-193, Aveiro, Portugal.

[2] Departamento de Geociências e Ordenamento do Território, Faculdade de Ciências, Universidade do Porto. Rua Campo Alegre, 4169-007, Porto, Portugal, Portugal.

[3] CIIMAR-UP, Matosinhos. Terminal de Cruzeiros de Leixões. Av. General Norton de Matos, 4450-208, Matosinhos Porto, Portugal.

[*] Corresponding author / Autor correspondente: tiagofmteixeira@ua.pt

1. Introduction

It is estimated that eucalyptus arrived in Portugal at the beginning of the 19th century, and since the middle of the 20th century, the most common eucalyptus in Portugal, *Eucalyptus globulus*, has been planted as a source of wood to produce paper pulp of recognized quality. Since then, the eucalyptus plantation area has grown and, in 2019, it represented 845 000 hectares in Portugal, around a quarter of the national forest (26%), according to the Institute of Nature and Forest Conservation (ICNF, 2019).

This marked growth in the Portuguese forest area has led it to be considered an invasive and highly flammable species, which increases the risk of fire, making it essential to control its proliferation. In fact, the intrinsic characteristics of eucalyptus result not only in the rapid repopulation of burnt areas, but also in its dispersal to other areas. In this sense, the PEST(bio)CIDE project aims, through the valorisation of eucalyptus leaf biomass, to provide an answer for better management of areas regenerated after a forest fire.

Previous studies indicate that there is a phytotoxic potential of eucalyptus leaves, since compounds involved in tolerance against insects and pathogens (Batish *et al*., 2008) have also demonstrated herbicidal capacity against non-target species (Oerke, 2006). The PEST(bio)CIDE project therefore proposes, from a circular economy perspective, the use of eucalyptus leaf biomass as an effective and environmentally safe biocide. It is also expected that incorporating eucalyptus leaves into the soil will bring benefits for soil quality and contribute to carbon sequestration. That said, the aim of this work was to evaluate the effects of incorporating eucalyptus leaf biomass into some physico-chemical soil properties under real environmental conditions.

2. Material and methods

A field trial was set up in the Campus of Vairão of the Faculty of Sciences of the University of Porto. Initially, the soil (humic cambisol) was collected in order to characterize (physico-chemical analysis; major and trace elements and pesticides) before any intervention.

The concentration of major and trace elements was determined by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) (Agilent 7700) after digestion in a heating block (DigiPREP MS, SCP Science). For soils, a digestion with mixture of HNO_3: HCl (3:1), following the method 3051A from USEPA was performed, whereas eucalyptus leaves were digested using a mixture of HNO_3 and H_2O_2 (3:1). The quality control of the procedure was assured by the analysis of blanks, certified reference materials and replicates. The analysis of plant protection products residues (more than 500 active ingredients) was conducted by an external lab (LAB-SL, Spain).

The leaves of young eucalyptus were collected in the North of Portugal (Porto District), dried at 60 °C and fragmented. After ploughing and preparing the soil (June 2022), the area was divided into 30 squares of equal dimensions (50x50 cm) and ten replicates of three different treatments were applied in a random way: 5% m/m of eucalyptus (E); the pre-emergence herbicide S-metalochlor (H); and control (CTL). After one month (July-2022) maize was sown and in October-2022 it was collected.

Soil samples were collected from five squares of each treatment, at three different periods: one month after the beginning of the trial, when the maize was sown (July); two months and a half after the beginning of the trial (September); and when the maize was collected (October). The collected soils were oven-dried at 40 ºC and sieved at 2 mm for the following physicochemical analyses: water holding capacity (according to ISO 17512-1:2008 protocol; International Organization for Standardization [ISO], 2008); pH (1:5 v/v suspension in water and $CaCl_2$ according to ISO 10390:2021 ; International Organization for Standardization [ISO], 2021); electrical conductivity (measured in the 1:5 v/v suspension after 24h); organic matter (OM) contents through loss-of-ignition at 550 ºC (EN 15935:2021; European Committee for Standardization [CEN], 2021); total Carbon and Nitrogen content (LECO CN828). The available Mg, K, Ca, Na and P contents were also determined using the Mehlich 3 extraction procedure and determination by Atomic Absorption Spectroscopy (AAS) or colorimetric method.

Statistical analysis was performed using two-way ANOVA with repeated measures in GraphPad software.

3. Results and Discussion

The characterization of soil and eucalyptus leaves regarding its pseudo-total content on potentially toxic contaminants and macronutrients (Table 1) was crucial to evaluate the results observed in the field trial. Moreover, this characterization allowed to understand that the soil was not contaminated, since in the analyses of contaminants associated with pesticide residues no analyte was found above the limit of quantification nor high levels of potentially toxic elements, according to the Portuguese Environment Agency (APA) reference values for agricultural soils (Table 1; APA, 2022).

Also, it can be concluded that the incorporation of eucalyptus leaves will not contribute to an increase of potentially toxic elements in the soil as can be observed by the levels found (Table 1).

Table 1. Concentration of major and potentially toxic elements in soil and eucalyptus. * Within the APA - 2022 reference values for agricultural soils.

Tabela 1. Concentração de elementos maiores e potencialmente tóxicos em solos e eucalipto. * Dentro dos valores de referência da APA – 2022 para solos agrícolas.

Element	Soil (mg/kg)	Eucalyptus (mg/kg)
As	7.74 *	0.04
Pb	32.4 *	0.35
Cu	33.6 *	12.3
Zn	105*	22.9
Mg	1876	2413
K	2003	7979
Ca	831	7076
P	779	1236

When analysing the results regarding soil water holding capacity (WHC) (Figura1), it can be seen that values observed in the different treatments are very similar in the first sampling campaign (H=35.2 ± 1.6%; E= 35.8 ± 2.4%; CTL=34.4 ± 2.3%). Moreover, no major changes were observed over time, with no significant differences observed between treatments in all sampling campaigns. Although, the soil with the treatment where eucalyptus was applied shows a tendency to retain more water compared to the remaining treatments, being the difference between the 1st and the 3rd campaign statistically significant.

Regarding pH, when analysing Figure 2a it is observed that the samples from the different treatments presented very identical average values for the three sampling campaigns. Thus, apparently, this parameter is not affected either by the application of the herbicide or by the application of eucalyptus biomass to the soil. Electrical conductivity (EC; Figura 2b), which measures the amount of soluble salts in the soil, was higher in the eucalyptus treatment compared to the other two, especially in the first and third sampling campaign. There are statistically significant differences in the first sampling between the control treatment and the remaining treatments, and in the third sampling between the treatment with application of eucalyptus leaves, compared to the remaining treatments. Over time, it was observed a decrease in EC in plots treated with herbicide and eucalyptus, which can be related with the leaching of soluble salts due to precipitation events.

As expected, the average OM values are higher in the treatment where eucalyptus leaves were applied (Figura 3a) with emphasis on an evident increase in the third campaign.

The higher OM percentage values on the eucalyptus treatment, essentially in the 3rd campaign, could explain the increase in water holding capacity shown in Figure 1, since the greater presence of OM in the soil leads to a greater capacity to retain water (Bhadha *et al*., 2017). Similarly to OM, the treatment with eucalyptus leaves presents a higher percentage of carbon and nitrogen in the third campaign, with statistically significant differences compared to the other treatments (Figura 3b and Figura 3c).

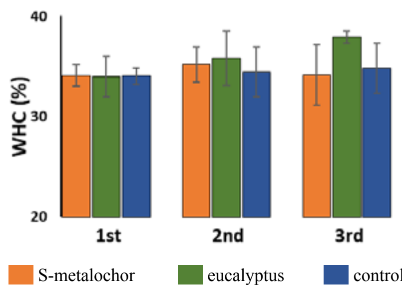

Figure 1. Average values of water holding capacity (WHC) in the different treatments for the three sampling campaigns. Error bars correspond to the standard deviation.

Figura 1. Valores médios da capacidade de retenção de água (CRA) nos diferentes tratamentos para as três campanhas de amostragem. As barras de erro correspondem ao desvio padrão.

The slight decrease in the C/N ratio in the eucalyptus treatment in the third campaign (Figura 3d) may indicate the existence of a higher rate of mineralization of organic matter.

The effect of adding eucalyptus on the availability of macronutrients was also evaluated, with Figure 4 showing the variation of these elements between treatments and over time. In general, Mg and Ca show a similar behaviour, with an increase in their content essentially in the eucalyptus treatment, although for Mg there seems to be a

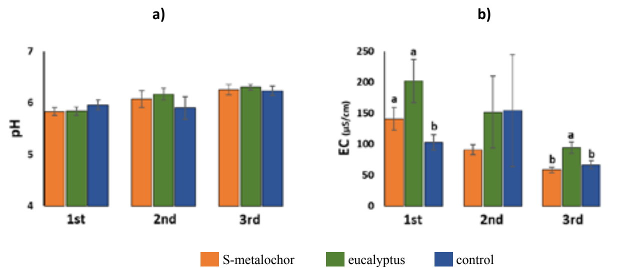

Figure 2. Average values of a) pH and b) electrical conductivity (EC) in the different treatments for the different sampling campaigns. Error bars correspond to the standard deviation. Statistically significant differences between sampling treatments, considering p ≤ 0.05, are marked with different letters above bars.

Figure 2. Valores médios de a) pH e b) condutividade eléctrica (CE) nos diferentes tratamentos para as diferentes campanhas de amostragem. As barras de erro correspondem ao desvio padrão. As diferenças estatisticamente significativas entre os tratamentos de amostragem, considerando p ≤ 0,05, estão assinaladas com letras diferentes por cima das barras.

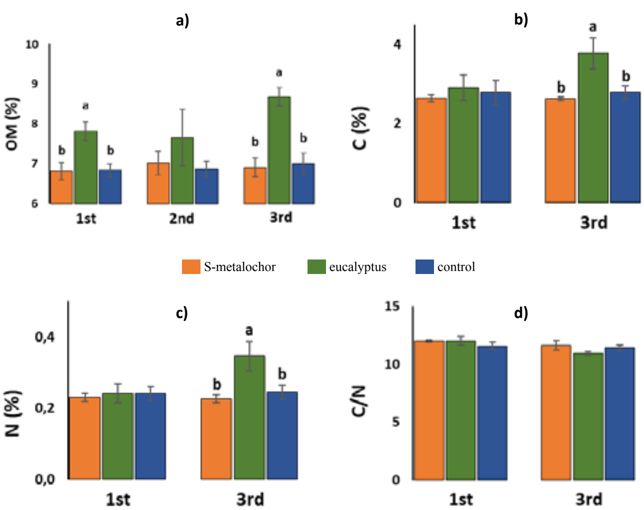

Figure 3. Average values of a) organic matter (OM), b) carbon content (C), c) nitrogen (N) and d) carbon/nitrogen ratio (C/N) in the different treatments for the different sampling campaigns. Error bars correspond to the standard deviation. Statistically significant differences between sampling treatments, considering p ≤ 0.05, are marked with different letters above bars.

Figure 3. Valores médios de a) matéria orgânica (MO), b) teor de carbono (C), c) azoto (N) e d) relação carbono/nitrogénio (C/N) nos diferentes tratamentos para as diferentes campanhas de amostragem. As barras de erro correspondem ao desvio padrão. As diferenças estatisticamente significativas entre os tratamentos de amostragem, considerando p ≤ 0,05, estão assinaladas com letras diferentes por cima das barras.

more evident difference between this treatment and the others, with statistically significant differences between the eucalyptus treatment and the remaining treatments in the first and third campaigns. Also, it was observed a statistically significant increase between the first and the last sampling campaign for all treatments, but particularly evident in the eucalyptus treatment.

Potassium (K) in the first sampling campaign presents higher levels in the eucalyptus treatment, when compared to the other treatments, however, over time the values tend to decrease in all treatments and become more similar. Throughout all studied period, P appears to be present at constant levels, with no differences between assays.

The introduction of eucalyptus leaves into the soil can explain the increase or decrease in the concentration of elements, especially if we consider the concentration of elements already present in the leaves (Table 1). The need for macronutrients for maize development may explain the decline in available K only over time (Das *et al*., 2012). The availability of macronutrients is influenced by environmental conditions,

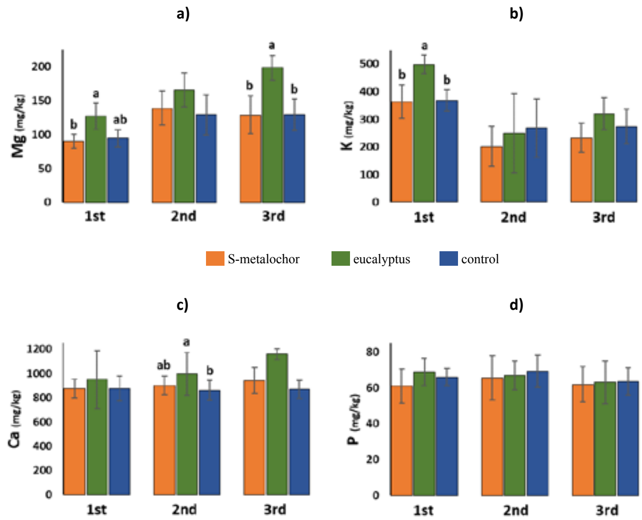

Figure 4. Average available (Mehlich 3) concentration values of a) magnesium (Mg); b) potassium (K); c) calcium (Ca) and d) phosphorus (P) in the different treatments for the different sampling campaigns. Error bars correspond to the standard deviation. Statistically significant differences between sampling treatments, considering p ≤ 0.05, are marked with different letters above bars.

Figura 4. Valores médios de concentração disponível (Mehlich 3) de a) magnésio (Mg); b) potássio (K); c) cálcio (Ca) e d) fósforo (P) nos diferentes tratamentos para as diferentes campanhas de amostragem. As barras de erro correspondem ao desvio padrão. As diferenças estatisticamente significativas entre tratamentos de amostragem, considerando p ≤ 0,05, estão assinaladas com letras diferentes acima das barras.

such as: variations in pH, microbial activity in the rhizosphere, relationships between nutrients (Ca, K and Mn, Al) (Huber & Jones 2013), factors that can explain variations in concentration of available macronutrients over time.

4. Conclusions

Given the significant growth of eucalyptus in the Portuguese forest area, and also the fact that it is an invasive and highly flammable species, it is important to control its proliferation. This control, namely through the use of eucalyptus leaves as a herbicide against non-target species, is being evaluated as part of the PEST(bio)CIDE project.

Incorporating eucalyptus leaves into the soil has a direct effect on the soil's physico-chemical properties. Through the analyses carried out, it was possible to observe that, during the time the field trial took place, the incorporation of eucalyptus leaf biomass into the soil does not seem to significantly influence the pH values in the soil; it causes an increase in electrical conductivity, when compared to the other treatments; it causes an increase in the content of organic matter, total carbon and nitrogen, as would be expected given the incorporation of leaves into the soil and it also causes an increase in the availability of some macronutrients that are essential for plant growth and development (namely Mg, K and Ca).

Acknowledgments

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