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Nutrient limitation determines the suitability of a municipal organic waste for phytomanaging metal(loid) enriched mine tailings with a pine-grass co-culture

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HIGHLIGHTS

- Co-culture modulated plant performance in amended treatments.
- Co-culture with the grass *P.miliaceum* negatively affected pine (*P.halepensis*) growth.
- *P.miliaceum* showed higher competitive ability when co-cultured with *P.halepensis*.

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ABSTRACT

The suitable phytomanaging of mine tailings not only requires an improvement of soil fertility but also the assessment of the biotic interactions between the selected plant species. This study aimed to evaluate the effect of an organic amendment on the response of two plant species of contrasting habit, a tree, *Pinus halepensis* and a grass, *Piptatherum miliaceum* growing on a metal(loid)-contaminated substrate collected from mine tailings. Pots containing single plant individuals or their combination, with and without organic amendment (at 10% rate), were established and grown in a greenhouse for 13 months. Plant biomass, foliar ionome, leaf $\delta^{15}N$ and metal(loid) concentrations were measured at the end of the experiment. The amendment alleviated P deficiency in the substrate and strongly stimulated biomass production by both plant species (10-fold for pine; 90-fold for the grass), leading to more balanced N/P ratios in leaves (especially for the grass). Co-culture with the grass negatively affected pine growth, decreasing total biomass and leaf $\delta^{15}N$ values and inducing severe N deficiency (leaf N/P ratio<10). In contrast, co-culture with pine improved the nutrient status and growth of the grass, but only under non-amended conditions. Needle metal(loid) concentrations in *P. halepensis* were affected by both amendment addition and co-culture with the grass. High biomass growth with low metal(loid) concentrations in *P. miliaceum* leaves for the amended treatment makes this grass species suitable for the phytomanagement of metal(loid) polluted tailings, since it achieves high biomass production together with low concentrations of metal(loid)s in edible/senescent parts.

1. Introduction

Plant interactions may play a critical role in the phytomanagement of polluted ecosystems, whether by favouring plant establishment (facilitation) or constraining it (competition) (Callaway and Pugnaire, 2007). Phytomanagement by phytostabilisation consist of the implementation of a sustainable plant cover to prevent or minimise wind and water erosion from polluted soils, resulting appropriate for the specific case of mine tailings (Robinson et al., 2009). For these sites, selected plant species should be tolerant to the unfavourable plant growth conditions of mine tailings, such as high salinity, high metal(loid) concentrations and

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low fertility (Parraga-Aguado et al., 2014a). However, most studies on phytomanagement of mine tailings lack an understanding of the interactions among the plant species involved and how soil amendments may alter those facilitation/competition interactions (which may determine the long-term sustainability of the systems). For instance, plant facilitation by employing nurse plant species or synergistic plant combinations have been pointed out as alternatives for the successful revegetation of mining degraded ecosystems, especially those under semiarid climates (Párraga-Aguado et al., 2014a; Domínguez et al., 2016). More specifically, the combination of pine trees-grasses might be pivotal under the limited resource conditions of tailings, especially in semiarid areas where water and nutrient scarcity become critical issues for plant growth: while the root system of the grasses provide surface protection to soil erosion, trees offer protection under their canopy and a deeper root system which does not compete for nutrient/water resources with the grasses (Parraga-Aguado et al., 2014a). In other non-polluted environments, these pine trees-grasses combinations have been shown to increase biodiversity and ecosystem productivity (Krapfl et al., 2016).

In previous research performed with mine tailings substrates, the co-culture of two grass individuals of the same species led to strong intra-specific competition that severely decreased biomass production (Parraga-Aguado et al., 2015), while the stimulation of biomass production was the main effect resulting from the co-culture of two pine seedlings of similar size and age (Martinez-Oró et al., 2017). When interspecific co-cultures of pines with grasses were evaluated, intense competition was found, with the grass being the dominant plant species (Martinez-Oró et al., 2017). However, no information is available on how the use of soil amendments may alter/modulate this interspecific interaction at the initial stages, when both root systems compete within the same soil depth, whether favouring/constraining pine establishment or increasing/decreasing the competitive role of the grass.

This study aimed at evaluating the effect of an organic amendment (municipal organic waste) on the response of *Pinus halepensis* Miller growing alone or in combination with the grass *Piptatherum miliaceum*(L.) Coss. for phytomanaging semiarid metal(loid) enriched mine tailings. For this purpose, a pot experiment was set up using a mine tailings substrate. Pots containing single plant individuals or combinations of both aforementioned plant species under unamended and amended conditions were placed in a greenhouse. Root and shoot dry weight (DW) yields, foliar nutrient status, leaf $\delta^{15}N$ composition and foliar metal(loid) concentrations were measured after 13 months. We hypothesized that interspecific interactions may modulate the benefits of the soil amendment for plant growth and alter nutrient/metal concentrations in both target species.

2. Material and methods

2.1. Soil and plant species

The soil used in the pot experiment was taken from an abandoned disposal site of mine tailings at the Cartagena-La Unión Mining District (SE Spain, 0–392 m.a.s.l.; 50 km^2; 37°37′20″ N, 0°50′55″ W–37°40′03″ N, 0°48′12″ W) (Fig. SM1, Supplementary Material). Information about the environmental impacts in this former mining activity, currently discontinued, is available in Conesa and Schulin (2010). The selected organic amendment was a municipal organic waste (hereafter, amendment) provided by a recycling plant of urban metallic containers (Pedro Segura SL). Prior to lab analyses and pot experiment, both soil and amendment were separately homogenised using a concrete mixer and sieved through 2 mm (stainless steel sieve).

The main physico-chemical characteristics of the soil and organic amendment are shown in the Table SM1 (Supplementary Material). The mine tailings showed neutral pH (≈7.1), moderate electrical conductivity (2.4 dS m^{-1}), high sand percentage (>80%) and low concentrations of organic carbon (1.4 g kg^{-1}) and total nitrogen (0.1 g kg^{-1}). The amendment showed acidic pH (≈5.1), high electrical conductivity (4.1 dS m^{-1}), and high concentrations of organic carbon (162 g kg^{-1}) and total nitrogen (14 g kg^{-1}). Both the mine tailings and the amendment showed high metal(loid) concentrations: for the mine tailings *e.g.* 120 mg Cu kg^{-1}, 10,100 mg Mn kg^{-1} or 10,000 mg Zn kg^{-1}; for the amendment *e.g.* 2620 mg Cu kg^{-1}, 5380 mg Mn kg^{-1} or 7920 mg Zn kg^{-1}. The high metal(loid)s contents in the amendment does not allow its use for agricultural purposes but could be accepted as a soil conditioner in high metal impacted soils such as the case of mine tailings. Several authors have pointed out the suitability of municipal wastes, even containing moderate metal(loid) load, for the phytomanagement of metal(loid) enriched mining polluted soils (*e.g.* Lozano-Cerezo et al., 1999; Ciarkowska et al., 2017; Párraga-Aguado et al., 2017).

Two soil treatments were tested: i.e. the bulk soil and the soil amended at 10% (w/w; amendment/tailing) with the organic waste. The amended soil was homogenised and allowed to stabilise during two weeks (adding tap water till reaching half of field capacity).

For the experiment, two plant species were employed: the tree *Pinus halepensis* Miller, Aleppo pine, and the grass *Piptatherum miliaceum* (L.) Coss, smilo grass. *Pinus halepensis* is a woody plant species widely employed for restoring degraded semiarid ecosystems in the Mediterranean area (Querejeta et al., 2008). In addition, *P. halepensis* individuals are growing spontaneously on mine tailings in the studied area (Parraga-Aguado et al., 2014b). Their presence has been related with the improvement of plant biodiversity in these sites (Parraga-Aguado et al., 2014a). *Piptatherum miliaceum* is a widespread grass species usually found among the pioneer vegetation growing on mine tailings in SE Spain (Párraga-Aguado et al., 2015). It has a well-extended root system which provides protection against soil erosion (De Baets et al., 2007).

2.2. Pot experiment

Plastic pots of 20 cm diameter x 25 cm height were filled with ~3.5–4 kg of the bulk soil (15 pots) or the amended soil (15 pots). Then, all the pots were randomly arranged in a screened table. In February 2015, around one hundred seeds of *P. miliaceum* were germinated in plastic trays containing the unamended soil in a growth chamber (26 °C, 16/8 h light/dark). In March 2015, five individuals of four-week-old seedlings of *P. miliaceum* were transplanted into 10 pots of each soil treatment (see Fig. SM2) leaving 5 unplanted pots per treatment. After two months, *P. miliaceum* seedlings were manually thinned and only one plant per pot was left. During eight months, all the pots were irrigated with tap water to around half of field capacity. The purpose of this period was to mimic field conditions in which *P. miliaceum* acts as an early colonizer of tailings. Then, in December 2015, seedlings of *P. halepensis* were transplanted to the pots of the following four treatments: non-amended soil, non-amended soil with *P. miliaceum*, amended soil, and amended soil with *P. miliaceum*. Pots containing single individuals of *P. miliaceum* were also maintained for non-amended and amended soils. From this moment, plants were irrigated as before till the end of the experiment in December 2016 (13 months) for providing enough plant biomass at the end of the experiment. The number of replicate pots per treatment, after discarding pots with failure plants, was: n = 5 for non-amended soil with *P. halepensis* monoculture (Ph); n = 4 for non-amended soil with *P. halepensis*-*P. miliaceum* co-culture (Ph-Pm); n = 3 for non-amended soil with *P. miliaceum* monoculture (Pm); n = 4 for amended soil with