RESEARCH ARTICLE



Barriers for plant establishment in the abandoned tailings of Nacozari, Sonora, Mexico: the influence of compost addition on seedling performance and tailing properties

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Abstract

Past mining activities have left a legacy of abandoned mine tailing deposits whose metal contaminants poses serious risks to ecosystems and human health. While the development of a vegetated cover in mine tailings can help in mitigating these risks, the local factors limiting plant establishment in these sites are not well understood, restricting phytostabilization efforts. Here, we explore some of the barriers that limit seedling establishment of two species (*Vachellia farnesiana* and *Prosopis velutina*) in a mine tailing deposit located in Nacozari, Sonora, Mexico, and assess whether compost addition can help in overcoming these barriers in pot and field experiments. Our field observations found 20 times more carbon and at least 4 times more nitrogen concentration in areas under vegetated patches than in non-vegetated areas, while a previous study found no difference in metal concentrations and other physicochemical parameters. This suggests that organic matter and nutrients are a major limitation for plant establishment. In agreement with this, species failed to establish without compost addition in the field experiment. Compost addition also had a positive effect on biomass accumulation, pH and microbial activity, but increased the substrate soluble concentration of As, Cu, and Zn. Nonetheless, only Cu, K, and Mo in *P. velutina* accumulated in tissues at levels considered toxic for animal consumption. Our study documents that compost addition facilitated plant establishment for the phytostabilization of mine tailings and help to prevent the dispersion of most metal contaminants via animal consumption. We encourage the use of complementary strategies to minimize the risk of dispersion of metal contaminants.

Keywords Mine tailings · Nutrients · Organic amendments · Phytostabilization · *Prosopis velutina* · Regeneration barriers · *Vachellia farnesiana*

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Introduction

Mine wastes are heterogeneous materials consisting of ore, gangue, rock, loose sediment, mill tailings, metallurgical slag, ash, processing chemicals, and fluids (Kossoff et al. 2014). However, mine tailings are usually the major component of mine waste after metal extraction (Lottermoser 2010). Tailings are composed mainly of silt or sand particles, usually lack nutrients and organic matter, have a wide range of pH (2-9) and metal(oid) concentration, have a relatively low water holding capacity when sand particles predominate and a poor microbial community (Li and Huang 2015; Lottermoser 2010; Moynahan et al. 2002). If mine tailings have high metal(oid) concentration and are left unreclaimed, they generally remain unvegetated for many years and particulate materials can spread over surrounding areas via eolian dispersion and water erosion (Mendez and Maier 2008). Thus, abandoned mine tailings can turn into a major source of local and regional metal(oid) pollution affecting ecosystems and human health (Meza-Figueroa et al. 2009).

Phytostabilization is a form of remediation that uses plants for in situ stabilization of tailings and its metal contaminants in the rooting zone with little or no accumulation in aboveground organs, preventing its dispersion to the surrounding environment and its entry to the trophic chain (Kennen and Kirkwood 2015). Suitable plants for tailings phytostabilization should be native species capable of germinating, growing, surviving, and reproducing in the particular physicochemical properties of tailings and the ecological conditions of the area, while limiting shoot metal accumulation and developing an extensive canopy cover (Mendez and Maier 2008). Furthermore, levels of shoot metal accumulation should be below the maximum tolerable levels for herbivores, to prevent impacts on animal health and minimize the transfer to the trophic chain and human consumers (Mendez and Maier 2008; NRC 2005). Surveys of native plants that naturally colonize mine tailings offer the opportunity to identify potential species (Ginocchio et al. 2017). Suitable species should be experimentally evaluated in controlled environments followed by field trials, in order to assess their performance prior to its implementation in remediation programs (Gil-Loaiza et al. 2016; Solís-Dominguez et al. 2012).

Establishing plants in mine tailings poses several inherent challenges. High metal concentration in tailings is probably the most commonly reported factor limiting plant establishment, also interacting with acidic pH and high salinity (Conesa et al. 2007; García-Carmona et al. 2019; Ginocchio et al. 2017; Ye et al. 2002). On the other hand, mine tailings should be considered a novel type of parent material rather than soil, being mineralogically and chemically more similar to volcanic ash than natural soils (Li and Huang 2015). This way, soil development processes such as the formation of organic matter, nutrient pools, soil structure and a microbial

community that naturally take hundreds or thousands of years. can also constrain the establishment of a self-sustainable plant community in tailings (Cross et al. 2019; Kato et al. 2005; Tardif et al. 2019; Tateno et al. 2019; Vitousek et al. 1993). Previous studies suggest that different factors can prevent plant establishment by strongly limiting either the process of germination, growth, or survival, depending on tailing properties and evaluated species (Gil-Loaiza et al. 2016; Novo and González 2014; Parra et al. 2016). Organic amendments such as compost have the potential to improve the organic matter and nutrient pools, soil microbial communities, and buffer some physicochemical properties of tailings, helping to overcome some of these barriers for phytostabilization (Bacchetta et al. 2015; Gil-Loaiza et al. 2016; Solís-Dominguez et al. 2012). However, studies have found that organic amendments can also increase metal availability or solubility, which can potentially increase the risk of dispersion of metal contaminants to the surrounding environment (Acosta et al. 2018; Asensio et al. 2013; Schwab et al. 2007). Efforts to identify the major barriers limiting plant establishment and strategies to overcome them, as well as potential undesired consequences derived from these strategies, are critically needed to allow the effective phytostabilization of metal contaminants in tailings.

Past mining activities in northern Mexico generated large amounts of mine wastes that have been abandoned and represent a serious risk to ecosystems and human populations. The Nacozari mining district in northeastern Sonora host several abandoned mine tailings. The Moctezuma Copper Company exploited several mines near Nacozari from 1900 to 1949, and left several million tons of waste distributed in three tailing deposits that cover 52 ha around the town (Alvarado and Volke 2004; De la O-Villanueva et al. 2013). Previous studies have described some physicochemical properties of these deposits such as mineral composition (Romero et al. 2008), texture (De la O-Villanueva et al. 2013), pH, electrical conductivity, and metal content (Meza-Figueroa et al. 2009). Although metal concentration in these tailings is relatively low in the context of the current Mexican legislation, the seasonal formation of efflorescent salts poses a serious risk, as these salts have a high metal concentration and are subject to eolian dispersion that transfer toxic metals into nearby residential soils (Meza-Figueroa et al. 2009). Forty-two plant species have colonized and formed four vegetation patches that cover less than 1% of one of the tailing deposits of Nacozari (Santos et al. 2017). The five most abundant plant species in the vegetation patches were Brickellia coulteri A. Gray, Baccharis sarothroides A. Gray, Pseudognaphalium leucocephalum (A. Gray) Anderb, Prosopis velutina Wooton, and Vachellia farnesiana Wight & Arn. These species were found to limit shoot accumulation in many of the most abundant metals in the Nacozari tailings (Cu, Fe, Mo, and Rb in all the species and also K, Mn, and Sr in some species, Santos et al. 2017).

Santos et al. (2017) compared selected physicochemical properties of vegetated and barren areas in the Nacozari tailings, and suggested that pH, electrical conductivity, texture, and metal concentration do no limit plant establishment (Santos et al. 2017). Given that plants cover less than 1% of the tailing deposit area, in this paper, our goal is to identify other factors that restrict plant establishment. We explore the hypothesis that organic matter is the major barrier to plant establishment by limiting nutrient availability and microbial activity. To test the hypothesis, we (a) compared nutrients (N and P) and C (as an indicator of organic matter) in vegetated patches and adjacent barren areas within the tailings, (b) evaluated the effect of compost addition on the germination, establishment, growth, and patterns of metal accumulation (As, Ba, Cd, Cu, Fe, K, Mn, Mo, Ni, Rb, Ti, and Zn) in tissues in two of the most abundant species with phytostabilization potential in the vegetation patches (Vachellia farnesiana and Prosopis velutina) under pot and field experiments, and (c) assess the relative role of compost addition vs plants in modifying physicochemical properties and microbial activity of the tailings by comparing planted and non-planted compost addition treatments. If vegetated patches show greater concentration of nutrients and organic matter than barren areas, nutrient pools may function as limiting factors. If plant establishment fails when the growing substrate is just tailing material, phytostabilization should be assisted with organic amendments. If compost addition improves nutrient availability, microbial activity and pH, amendments could facilitate plant establishment and growth. Finally, if compost amendments reduce the accumulation of metals in above-ground plant tissues, established plants could minimize transfer of contaminants into local trophic chains.

Materials and methods

Study area

The Nacozari mining district is located in northeastern Sonora, in northwestern Mexico (Fig. 1). Regional climate is semi-arid with a mean annual rainfall of 578 mm and mean temperature ranging from 12.1 °C in January to 27.9 °C in June (SMN 2019). Regional vegetation is foothills thornscrub (Martinez-Yrizar et al. 2010). The Nacozari district has important ore



Fig. 1 Location of the abandoned tailings of Nacozari (30° 22' 2" N, 109° 41' 38" W, 1050 masl), in Sonora, Mexico, and the distribution of vegetated patches. Sampling scheme for C, N, and P beneath plants (BP) and areas between plants (ABP) inside and outside vegetated patches (OVP)

deposits, such as porphyry copper, breccia pipe, and veins with Cu, Mo, Au, Ag, and Zn (Alvarado and Volke 2004). The Pilares copper ore deposit (0.7–1.2% Cu) was exploited by the Moctezuma Copper Company for 50 years until the mine closed in 1949, producing an average of 3000 ton/day of copper. Large amounts of waste material were distributed into three deposits around the town of Nacozari and then abandoned (Alvarado and Volke 2004). The central tailing deposit (30° 22' 2" N, 109° 41' 38" W, 1050 masl) is of concern due to its proximity and risk to the Nacozari town (Meza-Figueroa et al. 2009).

The mineral composition of the central deposit is mainly quartz, gypsum, lepidocrocite, and copper sulfate (Romero et al. 2008), and 80% of the tailing materials is coarsegrained with large variation in particle size from coarse to fine silt (De la O-Villanueva et al. 2013). Mean pH is 3.8 ± 0.3 , while the mean electrical conductivity is $340.1 \pm 2 \ \mu\text{S/cm}$ (Meza-Figueroa et al. 2009). Common metals in the deposit are Fe $(31,739 \pm 381.9 \text{ mg/kg})$, Ti $(1508 \pm 365 \text{ mg/kg})$, Ba (423.1 ± 140) , Cu $(400.5 \pm 15.8 \text{ mg/kg})$, Rb $(298.4 \pm$ 5.6 mg/kg), and Mn (158.5 \pm 10.5 mg/kg); average values of As and Pb are 29.3 ± 4 and 39 ± 4.2 mg/kg respectively (Meza-Figueroa et al. 2009). However, given the semi-arid climate, some metals including Cu, Mn, Zn, and Ba reach very high values in efflorescent salts (170-230 times higher than mean values in tailings), that can be transferred to nearby residential soils and adjacent ecosystems (Meza-Figueroa et al. 2009).

Santos et al. (2017) described a set of 42 plant species from 16 families that have naturally colonized the central Nacozari tailing deposit. From this set, 15 were perennial species and 27 were annual species. A total of 872 individuals of perennial plant species were distributed in four patches that varied in size from 34 to 743 m² and covered 0.84% of the deposit. The most abundant perennial species were the trees *Prosopis velutina* and *Vachellia farnesiana*, the shrubs *Baccharis sarothroides* and *Brickellia coulteri*, and the perennial herb *Pseudognaphalium leucocephalum*. The distribution of perennial species in the tailings was not limited by properties such as pH, electrical conductivity, texture, and metal concentration (Santos et al. 2017).

C, N, and P content in vegetated patches and barren areas within the Nacozari tailings

To assess whether nutrients are the major limiting factor for plant establishment in the tailings, we compared total nitrogen (N), carbon (C), and available phosphorous (P) in the substrate among the four vegetated patches and adjacent areas lacking plants. In each patch, we collected samples beneath the canopy of individual plants (*V. farnesiana*, *B. sarothroides*, *B. coulteri*, and *P. velutina*), in open areas between plants within the patch and outside the patch, 50 to 100 m far from the patch (Fig. 1).

We collected three random samples from the surface tailings layer (0–10 cm) and three samples from the 10–20 cm layer in each of the three locations (beneath, between, and outside) of each patch. The three samples were mixed to form a composite sample in each patch. In the lab, samples were grinded and sieved through a 500 μ m sieve. Carbon and nitrogen were determined in 15 mg samples by complete combustion using a 2400 CHNS/O Perkin Elmer elemental analyzer, in CHN mode. We used total carbon as an indicator of organic matter. Available phosphorous was determined using 5 g samples through the Bray-P1 method (van Reeuwijk 1992).

Response of plants to compost addition

During the summer of 2015, we collected seeds from four perennial species from the Nacozari tailings: *V. farnesiana*, *B. sarothroides*, *B. coulteri*, and *P. velutina*. *Pseudognaphalium leucocephalum* did not produce seeds during that year and thus, we were unable to include this species in our study. Given that a previous study showed that plant performance with seeds collected within or outside tailings did not show significant differences when grown in pots with tailings material or local soil (Santos et al. 2017), we used seeds from both sites. We collected tailings material from the deposit and soil from an adjacent area in order to evaluate the response of plants to compost addition under semi-controlled conditions in pots and full sunlight. Soil samples correspond to a Leptosol (INEGI 2006).

Pot experiments in full sunlight

We set up an experiment with four treatments and five replicates in 1 gal pots (19 cm diameter and 15 cm height) over a bench in full light at the Institute of Ecology (UNAM) in Hermosillo, Sonora. Treatments consisted of composttailings concentration: 0, 2.5, 5, and 10% compost w:w and a control using soil adjacent to the Nacozari tailings. We previously prepared our own compost using local materials: cow manure (29%), wood chips (18%), leaf waste (41%), and local soil (12%). Materials were distributed in layers within a pit, saturated with water, covered with black plastic, and periodically watered and mixed for 4 months. The compost total C was 6.67%, total N was 0.58%, and C/N was 11.49 (measured with a 2400 CHNS/O Perkin-Elmer elemental analyzer, in CHN mode), and had a pH of 8.44 and EC of 4010 μ S/cm (measured with a WTW LF90 meter).

Each compost treatment was prepared, thoroughly mixed in a large plastic container, and used to fill each of the five-pot replicates. For the large-seeded *V. farnesiana* and *P. velutina*, we used three scarified seeds per pot, whereas for the small seeded *B. sarothroides* and *B. coulteri*, we used 50 seeds per pot. For *V. farnesiana* and *P. velutina*, if more than one seedling emerged, we kept just one seedling per pot. For *B. sarothroides* and *B. coulteri*, germination was poor and erratic and we were unable to obtain more than two seedlings per treatment and therefore, we excluded these species from our experiments. Pots were watered daily or every other day if they received rainfall. We recorded germination as the number of emerged seedlings from the planted seeds and establishment as the number of surviving seedlings from the emerged seedlings. Seedlings grew during 71 days (July–September) and their height (ground-apical bud) was measured with a ruler and then harvested, and a sample of the growing rhizosphere substrate was taken.

Seedlings were washed with a solution of distilled water and HCl (0.1%), separated in roots, stems, and leaves and dried at 65 °C for 72 h in an oven, and each plant was weighted to the closest milligram. Leaves were finely grinded, digested using a microwave-assisted digestion (EPA method 3052), using 9 mL of concentrated HNO₃ and 3 mL of HCl and finally, metal concentration was determined using ICP-OES. For quality control, we used apple leaf standard NIST-1515. The growing substrate was dried, grinded, and sieved through a 2 mm sieve, and then processed through the microwave-assisted acid digestion EPA-3051A method (Link et al. 1998), using 9 mL of concentrated HNO₃ and 3 mL of HCl and total metal concentration determined by ICP-OES. For quality control, we used inorganic sediment standard NIST-2702. From 20 analyzed elements, Zn was the only metal with an adequate recovery (85-115%) in the standard, and therefore we only kept data for this metal for further analyses.

The leaf/substrate metal concentration ratio, known as the accumulation factor, is a common indicator of plants capacity to accumulate metals in their above-ground tissues (values > 1) or whether plants have a limited transfer of metals from the substrate to their above-ground tissues (values < 1) (Fernandez et al. 2017; Mendez and Maier 2008; Santos et al. 2017). We calculated the accumulation factor for Zn in order to evaluate whether seedlings were accumulating or excluding this metal. In addition, we evaluated whether the leaf values of Zn reached the maximum tolerable levels (MTL) by domestic animals (NRC 2005).

Field experiment

We set up a field experiment (4.5 m \times 12.3 m) in a plain area of the Nacozari tailings in August 2016, after the start of the monsoon rainy season. We used three in situ compost concentration treatments in the tailings surface: 0, 10, and 20% w:w with 10 replicates in order to evaluate the influence of compost addition on seedling performance. We excavated cylinder-shaped holes (10 cm diameter by 30 cm depth) where we extracted tailings material that was used to prepare each of the compost treatments, using the previously prepared compost. Each compost treatment was prepared in the field, thoroughly mixed in a large plastic container, and used to fill back each of the 10 hole replicates. The distribution followed a completely random design using five columns and eighteen rows with a distance of 0.5 m between replicates. Given the germination problems detected for *B. sarothroides* and *B. coulteri* in the pot experiments, we only used *V. farnesiana* and *P. velutina* collected in 2015 in the field experiment. We planted (1 cm below surface) three scarified seeds in each replicate. After planting, we irrigated each replicate to field capacity.

We recorded germination and seedling establishment on weekly visits during the first month and bi-weekly after the first month. During field visits, we irrigated each replicate with 2 1 of water. Total rainfall during the experiment was 91.1 mm. Seedlings grew during a period of 42 days, after which height (ground-apical bud) was recorded and then were harvested. Given the low biomass reached by field seedlings, metal concentration was determined by mixing non-woody stems and leaves. Plant tissues were grinded into fine powder, cold digested for 8 h with HNO₃, heated for 15 min at 85 °C, followed by 2 h at 115 °C, cooled, brought to volume with HCl and the resulting solution was analyzed by ICP-MS at ALS (Vancouver) Canada using method ME-VEG41. Accuracy and precision $(\pm 10\%)$ were determined using NIST-1515 and NIST-1575a standards. In addition, we took leaf samples from adult individuals of the same species that were growing in the vegetated patches. These leaf samples were similarly processed and analyzed for metals using the same method ME-VEG41 at ALS Canada. Fifty-nine elements were determined using this method and most of them (except Cr and Pb) had adequate recovery in the standards. We also took samples from the growing rhizosphere substrate for soluble metal analysis. In this case, samples were analyzed using the deionized water leach method (ME-MS03) of ALS (Vancouver) Canada. Samples were dried, grinded, and leached using deionized water in a thermostat water bath (60 °C) for 2 h, centrifuged and the supernatant analyzed by ICP-MS. Accuracy and precision $(\pm 10\%)$ were determined using reference material OREAS-45d. In this case, we also had an adequate recovery for most elements in the standards. We limited our analysis to the elements that are of concern for the site, i.e., the most abundant metals in the tailings (Fe, Ti, Ba, Cu, Rb, Mn, Zn, in decreasing order of abundance, Meza-Figueroa et al. 2009), and elements whose concentrations reached toxic levels for plants (according to Kabata-Pendias (2011)) or animals. We evaluated whether leaf values reached the maximum tolerable levels (MTL) by domestic animals according to NRC (2005). The accumulation factor was calculated as the quotient leaf/substrate in

order to evaluate whether seedlings were accumulating or excluding metals and have phytostabilization potential.

The influence of compost and plants on tailings properties

Besides to evaluating compost addition on seedling performance, the field experiment was also used to test the influence of compost and plant growth on selected tailings properties. For this objective, we also included holes filled with the same compost treatments (0, 10, and 20% with 10 replicates in a completely random design) where no seeds were planted. The planted and non-planted treatments were equally watered. We took substrate samples from each replicate of the planted and non-planted treatments at the beginning and at the end of the experiment, for analysis of pH, electrical conductivity (EC), metal concentration, and microbial activity. The samples were taken from the rhizosphere substrate of the planted treatments, and from the 0-10 cm depth substrate for the non-planted treatments. Samples were kept in black plastic bags at 4 °C until they were analyzed. The influence of plants on tailing properties was assessed by comparing planted to non-planted treatments, while the influence of compost alone was assessed by comparing tailing properties among compost addition levels in non-planted treatments.

For pH and EC, we put 3 g of each sample into a solution with deionized water (1:10 w/v), stirred for 18 h, and measured with a VWR symphony 830PCI pH and EC meter (previously calibrated according to Ponce de Leon-Hill et al. 2012). Heterotrophic microbial metabolic activity in substrate was inferred by analyzing carbon mineralization, through aerobic respiration by the Isermeyer method (Isermeyer 1952; Alef 1995). For microbial activity, we only analyzed two planted and non-planted compost treatments (0 and 20%), using 100 g per replicate during an incubation period of 13 days, changing CO₂ traps every 24 h. Briefly, this method measure CO₂ adsorption in a NaOH solution forming Na₂CO₃ where measured CO₂ is equivalent to consumed NaOH. Adding BaCl₂ leads to precipitation of Na₂CO₃ as BaCO₃ and the remaining non reacting NaOH titrated with HCl. Values were expressed in μ gC g⁻¹ accumulated during 13 days. For metal analysis, samples were dried, grinded, and leached using deionized water in a thermostat water bath (60 °C) for 2 h, centrifuged and the supernatant analyzed by ICP-MS.

Statistical analysis

Data were evaluated in order to test if they met the assumptions of parametric tests through the Levene and Shapiro-Wilk tests. When data met parametric assumptions, we used student t test for comparisons between two groups,

and ANOVAs with Tukey HDS post hoc tests for comparisons between more than two groups/treatments. When residuals did not meet parametric assumptions, they were transformed (natural logarithm and inverse). If after transformation, residuals did not meet assumptions, data were analyzed using the non-parametric Wilcoxon test for comparison between two groups, or Kruskal-Wallis test and Dunn test for multiple comparisons (Zar 2010) among more than two groups/treatments.

We used the above approach for evaluating (1) the influence of vegetated patches and depth on C, N, and P in tailings, (2) the influence of compost addition on biomass accumulation and seedling height on both species, and species differences for a given treatment of compost addition in the pot and field experiment, and (3) the effect of compost addition and plant growth over selected tailings properties (pH, CE, metal concentrations, and microbial respiration). We also compared germination and seedling establishment between compost addition treatments using χ^2 tests for the pot and field experiments. All statistical analyses used the JMP version 10 software (SAS Institute 2012), and plots were preparated in R version 3.5.3 (The R Foundation for Statistical Computing) and Inskcape version 0.92.

Results

C, N, and P content in vegetated patches and barren areas within the Nacozari tailings

When comparing C content over a single-depth layer of substrate, areas beneath plants, areas between plants, and outside vegetated patches showed significant differences at the 0–10 cm (F = 40.5, P < 0.0001) and 10–20 cm (F = 6.6, P = 0.01) depth layers. The differences between these areas were more pronounced in the 0-10 cm layer, where C content in substrate under plants was 11 times greater than sites between plants and 20 times greater than outside vegetated patches (Fig. 2a). Substrate N content showed significant differences between areas when compared at the 0-10 cm depth layer (F = 35.9, P < 0.0001), whereas no significant differences between areas were detected at the 10-20 cm layer (F = 1.2, P < 0.33, Fig. 2b). N under plants was about 4 times greater than areas between plants, but N outside patches was below the detection limit (< 0.017%) of the CNHS Perkin Elmer elemental analyzer. For C and N, significant differences between depths were detected only in areas beneath plants (C; t = 5.5, P = 0.001. N; t = 3.5, P =0.02) but not in areas between plants (C; t = 1.2, P = 0.3. N; t = 0.6, P = 0.6) or outside vegetated patches (C; t = 1.4, P =0.2. N: below detection limit). In contrast to C and N, no significant differences were detected for available P among



areas at the 0–10 cm (F = 4.1, P = 0.06) and 10–20 cm (F = 1.1, P = 0.34; Fig. 2c) depth layers.



Response of plants to compost addition

Pot experiment

Germination and seedling establishment of *V. farnesiana* and *P. velutina* for the different compost treatments varied from 60 to 100%. Germination between compost treatments showed significant differences ($\chi^2 = 17.5$, *P* = 0.04 for *V. farnesiana* and $\chi^2 = 21.2$, *P* = 0.01 for *P. velutina*) but there was not a clear correlation with increasing compost content. For seedling establishment, there were no significant differences between compost treatments ($\chi^2 = 11.2$, *P* = 0.26 for *V. farnesiana*, and $\chi^2 = 12.8$, *P* = 0.17 for *P. velutina*).

In contrast to germination and seedling establishment, compost addition clearly and significantly increased above-ground biomass and seedling height for both species (above-ground biomass: F = 52.1, P < 0.0001 for *V. farnesiana* and F = 26.0, P < 0.0001 for *P. velutina*; height: F = 23.4, P < 0.0001 for V. farnesiana and F = 8.4, P = 0.001 for *P. velutina*; Fig. 3a-b). As with total biomass, significant differences were also detected for leaf (F = 36.8, P < 0.0001 for V. farnesiana and F = 25.3, P < 0.0001 for *P. velutina*), stem (F = 43.3, P < 0.0001 for *V. farnesiana* and F = 20.6, P < 0.0001 for P. velutina), and root biomass (F = 7.1, P = 0.003 for V. farnesiana and F = 16.5,P < 0.0001 for P. velutina) (Fig. 3c-e). For V. farnesiana, differences between compost treatments and the treatment with just tailing materials without compost addition (0%)treatment were 2–3 times greater for height, 4 times greater for total biomass, 4-5 times greater for leaf, 4-5 times greater for stem, and 3 times greater for root biomass. However, differences between treatments with compost additions (2.5, 5, and 10%) were not significant (height: F =0.25, P = 0.78; leaf: F = 2.45, P = 0.12; stem: F = 1.33, P =0.30; root: F = 0.94, P = 0.41; Fig. 3a-e). For *P. velutina*, height and biomass accumulation increased as the level of compost addition increased, with differences between compost treatments and the treatment without compost addition (0%), being 2–3 times greater for height, 2–6 times greater for total biomass, and 2-9 times greater for leaf, 2-6, times greater for stem, and 2-5 times greater for root biomass



Fig. 3 Seedling height and biomass accumulation of *Vachellia farnesiana* and *Prosopis velutina* (n = 5) for the different compost treatments in the pot experiment. Parameters include seedling height **(a)**, above-ground biomass **(b)**, leaf **(c)**, stem **(d)**, and root **(c)** biomass.

Treatments include 0, 2.5, 5, and 10% compost concentration and natural soil control (NS). Asterisks indicate statistically significant differences between species within a given treatment, while letters indicate differences between treatments within a given species

(Fig. 3a-e). Even at the highest level of compost addition, biomass accumulation of *V. farnesiana* and *P. velutina* was substantially lower than their biomass accumulation under natural soil (Fig. 3a-e).

Field experiment

Compost addition to the growing substrate was necessary for seedling establishment under field conditions as no seedling survived under the treatment without compost addition (0%). For the 10 and 20% compost treatments, seedling establishment varied from 40 to 53%, 41 days after planting. For *V. farnesiana*, compost addition had a significant effect on germination ($\chi^2 = 15.9$, P = 0.01) and seedling establishment ($\chi^2 = 12.3$, P = 0.04). For *P. velutina*, compost addition had a significant effect only on seedling establishment ($\chi^2 = 14.9$, P = 0.02) but no significant effect on germination ($\chi^2 = 9.2$, P = 0.16). Given that no seedling from both species were able to establish under the treatment without compost addition (0%), data on biomass accumulation at the end of the experiment was restricted to the 10 and 20% compost treatments (Fig. 4a-e). For *V. farnesiana*, no significant differences were detected for seedling height between 10 and 20% compost (t = 2.2, P = 0.06) but for *P. velutina*, seedlings from the 20% treatment were taller (t = 2.4,

P = 0.04). Seedlings from the 20% compost treatment accumulated greater biomass than the 10% treatment, for V. farnesiana (t = 2.4, P = 0.04) and P. velutina (t =2.6, P = 0.02). The effect of compost addition was particularly evident for stem biomass in V. farnesiana, doubling the value from 10 to 20% compost. A similar effect was detected for P. velutina, doubling the values of leaf and stem biomass (Fig. 4c-d).



Treatments

Fig. 4 Seedling height and biomass accumulation of *Vachellia farnesiana* and *Prosopis velutina* (n = 10) for the different compost treatments in the field experiment at the abandoned tailings of Nacozari, Sonora. Parameters include seedling height (**a**), above-ground biomass (**b**), leaf (**c**), stem (**d**), and root (**e**) biomass. Treatments include just the 10

and 20% compost as no seedling survived under the treatment without compost addition (0%). Asterisks indicate statistically significant differences between species within a given treatment, while letters indicate differences between treatments within a given species

The influence of compost and plants on field tailing properties

рΗ

Adding compost to the tailings had a significant effect on pH, both at the beginning (F = 168.5, P < 0.0001) and at the end of the experiment (F = 731.8, P < 0.0001, Fig. 5a). Starting the field experiment, pH values changed from 5 under the treatment without compost addition (0%) to approximately 8 under the 10 and 20% compost treatments (Fig. 5a). At the end of the experiment, similar values of pH were detected for all treatments, except for the 20% compost treatment, where a slight increase (0.7) was recorded (Fig. 5a). Plant growth of *P. velutina* had no significant effect on pH. However, for *V. farnesiana* under 10% compost, a pH reduction of 0.5 was associated with plant growth (t = 4.08, P = 0.04, Fig. 5c).

Electrical conductivity

Adding compost to tailings had a significant increase in electrical conductivity (EC), at the beginning (F = 19.9, P = 0.002) and at the end of the experiment (F = 53.7, P < 0.0001, Fig. 5b). At the start of the experiment, the addition of compost caused EC to increase 3–5 times above the EC at the treatment without compost addition (0%). By the end of the experiment, EC on both compost treatments had decreased below the initial values, but remained above the EC of the control treatment (Fig. 5b). Plant growth had no significant effect on EC (t = 2.86, P = 0.08 for *V. farnesiana*; t = 1.47, P = 0.22 for *P. velutina*, Fig. 5d).

Microbial activity

Microbial activity was inferred from carbon mineralization in substrate from planted and non-planted 0 and 20% compost



Fig. 5 Effect of compost addition (**a-b**) and plants (**c-d**) over tailings pH and electrical conductivity in the field experiment at the abandoned tailings of Nacozari, Sonora. The figure includes data from 0, 10, and 20% compost treatments for non-planted subtrate (NP), for *Vachellia farnesiana* (Vf) and *Prosopis velutina* (Pv), and at the beginning and end of the field experiment. Points indicate observed values (n = 3) and bars represent the mean \pm standard deviation. In the upper plots (**a**, **b**),

different letters indicate significant differences between treatments, while asterisks indicate significant differences between the beginning and end of the experiment within the same treatment. For the lower plots (c, d), different letters indicate significant differences within a compost treatment, while asterisks indicate significant differences between treatments for a given species

treatments. Starting the experiment, carbon mineralization was 17 times greater in the 20% compost treatment than the 0% control (Fig. 6a). At the end of the field experiment, carbon mineralization was 6 times greater in the 20% compost treatment than the control. No significant differences in mineralization were detected between the 20% compost planted and non-planted treatments by the end of the experiment (F = 0.53, P = 0.61, Fig. 6b).

Metal accumulation in plants

Pot experiment

For *V. farnesiana*, compost addition showed an increase in the rhizosphere concentration of Zn (for 0, 2.5, 5, and 10% compost treatments, respectively 41.28 ± 5.12 , 46.8 ± 3.13 , $50.5 \pm$



Fig. 6 Effect of compost addition (**a**) and plants (**b**) over tailings microbial activity (carbon mineralization) in the field experiment at the abandoned tailings of Nacozari, Sonora. In (**a**) microbial activity (n = 3) is compared at the beginning (B) and end (E) of the experiment on nonplanted treatments (NP), and in (**b**) comparison between non-planted treatments and those planted with *Vachellia farnesiana* (Vf) and *Prosopis velutina* (Pv). The mean and standard deviation at each incubation day is shown. Only values for the 0 and 20% compost treatments are included. Different letters in (**a**) indicate significant differences between beginning and end of the same treatment, while in (**b**) indicate significant differences to the other significant differences between planted and non-planted treatments

 $3.55, 56.76 \pm 1.56 \text{ mg/kg}; F = 9.95, P = 0.004$). In contrast, no significant differences were detected in foliar concentration among compost treatments (F = 2.94, P = 0.09). Tissue accumulation of Zn (7-34 mg/kg) was below MTLs for animals (300-500 mg/kg; NRC 2005) for the different compost treatments. For P. velutina, compost addition showed an increase in Zn concentration in the rhizosphere (0, 2.5, 5, and 10%)compost treatments, respectively 37.64 ± 8.56 mg/kg, 44.61 \pm 3.28, 47.83 \pm 5.29, 55.17 \pm 2.49; F = 5.37 P = 0.025). Foliar concentration of Zn showed significant differences among treatments but not strictly related to increasing levels of compost (F = 6.75, P = 0.013). For this species, foliar accumulation of Zn under different compost treatments (13-85 mg/kg) did not exceed MTLs for animals (300-500 mg/kg). Zn accumulation factors were < 1 under all treatments for V. farnesiana (0% compost: 0.47 ± 0.45 mg/kg; 2.5%: 0.72 ± 0.21 ; 5%: 0.21 ± 0.05 ; 10%: 0.12 ± 0.09) while for P. velutina only the 2.5% treatment had an accumulation factor > 1 (0% compost: 0.66 ± 0.31 mg/kg; 2.5%: 1.9 ± 0.63 ; 5%: 0.28 ± 0.03 ; 10%: 0.47 ± 0.47).

Field experiment

In this section, we focused our analyses on As, Ba, Cd, Cu, Fe, K, Mn, Mo, Ni, Rb, Ti, and Zn, as these elements are of concern at the Nacozari tailings (see the subsection "Response of plants to compost addition" in "Methods"). The analysis of metals in substrate and rhizosphere of planted and non-planted sites with compost addition treatments allowed us to isolate the effect of compost and plants over substrate soluble concentration of those metals. Compost addition in non-planted treatments increased soluble concentrations of Cu, As, and Zn, but did not modify the concentrations of the other metals (Online Resource). For both species, rhizosphere soluble metal concentrations showed no significant difference when compared to substrate metal concentrations in non-planted treatments in any of the analyzed metals (Online Resource).

Given that no seedling survived under 0% compost, we only report tissue metal accumulation data for the 10 and 20% compost treatments. In addition, only 50% of surviving seedlings of all treatments and none of the V. farnesiana replicates at 20% had enough biomass (leaves and non-woody stems) for metal analyses. In V. farnesiana, none of the studied metals exceeded toxic levels for plant tissue or MTLs for animals, except Cu which reached toxic levels for plants (2-20 mg/kg from Kabata-Pendias 2011, Table 1). In P. velutina, foliar accumulation of Cu reached toxic levels for plants (Kabata-Pendias 2011), while Cu, K, and Mo exceeded MTL for some domestic animals (Table 1). For adult plants growing in patches at the Nacozari tailings, foliar accumulation of Cu exceeded toxic levels for plants for both species and also Zn for P. velutina (100-400 mg/kg from Kabata-Pendias 2011), and Cu exceeded MTL for bovine and ovine on both

Species	Metal	Field compost treatments		MTL		
		10%	20%	Rodents	Bovine	Ovine
P. velutina	Cu	27.3 ± 6.5	33.8 ± 5.9	500	40	15
	Κ	8900.0 ± 1131.4	$13,750.0 \pm 1767.8$	10,000	20,000	20,000
	Mo	5.4 ± 0.6	5.0 ± 3.1	7	5	5
Vegetation patches						
V. farnesiana	Cu	52.37 ± 9.15		500	40	15
P. velutina	Cu	72.67 ± 37.12		500	40	15

Table 1Metal concentration (mg/kg) in plant tissues of (a) *Prosopisvelutina* (leaves and stems) grown under two compost treatments in theNacozari tailings and (b) adult plants of *Vachellia farnesiana* (leaves) and

P. velutina (leaves) growing in patches of the Nacozari tailings. For comparison, we include values of the maximum tolerable levels (MTL) by domestic animals (National Research Council 2005)

species (Table 1). Accumulation factors were > 1 in *V. farnesiana* for Cd, K, Mn, and Zn, and were < 1 in As, Ba, Cu, Fe, Mo, Ni, Rb, and Ti (Table 2). For *P. velutina*, accumulation factors were > 1 for Cu, Cd, K, Mn, Mo, Rb, and Zn, and were < 1 in As, Ba, Fe, Ni, and Ti (Table 2).

Discussion

Nutrients and organic matter limit vegetation development in Nacozari tailings

Previous studies have documented that the Nacozari tailings have a sandy texture, acidic pH (3.5–5), relatively low salinity (100–340 μ S cm⁻¹), toxic concentrations of Cu on the substrate (230–420 mg/kg that according to Mendez and Maier 2008 are

 Table 2
 Metal accumulation factors (leaf/substrate) for Vachellia

 farnesiana and Prosopis velutina grown under two compost treatments
 at the Nacozari tailing deposit

Metal	V. farnesiana 10%	P. velutina 10%	P. velutina 20%
As	0.09	0.31 ± 0.01	0.41 ± 0.10
Ba	0.24	0.74 ± 0.12	0.62 ± 0.07
Cd	2.25	20.5 ± 3.54	$\boldsymbol{9.10 \pm 1.27}$
Cu	0.45	5.58 ± 1.78	$\boldsymbol{2.58 \pm 0.46}$
Fe	0.17	0.69 ± 0.08	0.72 ± 0.14
Κ	2.38	$\boldsymbol{8.81 \pm 0.16}$	11.36 ± 1.46
Mn	4.78	35.25 ± 6.65	31.06 ± 2.90
Мо	0.19	1.32 ± 0.14	$\boldsymbol{1.00\pm0.7}$
Ni	BDL	17.00	13.06 ± 4.68
Rb	0.14	0.57 ± 0.09	$\boldsymbol{1.11 \pm 0.17}$
Ti	BDL	BDL	BDL
Zn	5.04	33.4 ± 2.32	$\textbf{22.48} \pm \textbf{1.81}$

BDL below the detection limit. Values in bold indicate a statistically significant difference

phytotoxic), and seasonal efflorescent salts, that further increase the levels of Cu and bring other metals (Mn, Ni, Zn) to toxic levels (Meza-Figueroa et al. 2009; Santos et al. 2017). Several studies over a broader range of mine tailings evidenced that vegetation development is mainly limited by high metal concentration (García-Carmona et al. 2019; Ortiz-Calderón et al. 2008; Ye et al. 2002) or colimited by high metal concentration and acidic pH (Conesa et al. 2007), coarse substrate texture (Ginocchio et al. 2017), high salinity (Parraga-Aguado et al. 2014), or poor nutrients pools (Shu et al. 2005). However, Santos et al. (2017) found no significant difference on texture, pH, EC, or metal concentration between barren and vegetated areas within the Nacozari tailings, indicating that none of these physicochemical factors limits vegetation development in the Nacozari tailings. In this study, we found that areas beneath plants have on average 20 times more carbon (likely due to litter input by already established plants) and higher nitrogen than barren areas (below detection limit on barren areas), but no difference in phosphorous (Fig. 2a-c). In agreement with our hypothesis, these findings confirm that the lack of organic matter and nitrogen is a major barrier for vegetation establishment outside vegetated patches. The formation of organic matter and nitrogen pools in tailings is a slow process, involving both the initial colonization by plants capable of establishing in nutrient-poor areas and development of microbial communities (Colin et al. 2019; Shu et al. 2005; Santini and Banning 2016). In line with our findings, other studies have shown that organic matter and nitrogen limits vegetation development in some tailings with high metal concentrations, while metal concentration is not the major limiting factor (Cross et al. 2019). Rehabilitation practices at the Nacozari tailings should focus on improving organic matter and nitrogen levels to facilitate revegetation, accelerate plant colonization, and promote vegetation self-sustainability.

We experimentally tested compost amendment to tailings as one of such practices to facilitate the establishment of two species previously identified as suitable for phytostabilization (Santos et al. 2017). This allowed us to determine which processes of plant development were limited by organic matter and nutrients, whose effects were more strongly evident on the field experiment. Compost addition improved the germination of V. farnesiana but not in P. velutina, and improved growth on both species by preferentially enhancing growth of above-ground organs (Fig. 4b-d). Most notably, none of the species was able to establish in the treatment with just tailing materials without compost addition (0%). These findings show that the low organic matter and nutrient levels on this tailing deposit strongly affect early-stage survival, followed by growth of above-ground organs and to a lesser extent germination on these species. As indicated by our field sampling, such conditions for the establishment of both species are likely to occur naturally below plants within the patches, where litter input has already enriched substrate organic matter and nutrients, and amendments like compost are needed to extend the area with suitable conditions for plant establishment. A previous study also found that early-stage survival is strongly inhibited under an unamended tailing treatment, but germination was less affected (Parra et al. 2016). In contrast, other studies had shown that plants fail to establish in tailings due to germination inhibition rather than early growth or survival (Gil-Loaiza et al. 2016; Novo and González 2014; Solís-Dominguez et al. 2012). Two years after setting our field experiment, most plants from the 10 and 20% compost addition treatments that were not harvested are still alive (F. Molina-Freaner, personal observation), implying that organic matter and nutrient enrichment to the tailings through compost addition can have a long-lasting effect on plant survival.

Feedbacks of compost and plants over tailings properties

Organic amendments and plants modify or ameliorate other tailing properties, besides improving organic matter and nutrient pools (Asensio et al. 2013; Solís-Dominguez et al. 2012; Touceda-González et al. 2017). In our study, only V. farnesiana had a minor effect over pH at the 10% compost treatment at this early stage but did not affect EC (Fig. 5d), microbial activity (Fig. 6b), or metal soluble concentration (Online Resource). In contrast to plant effects, compost addition had a much stronger effect. First, it increased EC (Fig. 5b), but the level of EC remained well below the range known to negatively affect plant or microbial processes (> 2000 µS cm⁻¹, Brady and Weil 2017). pH also changed immediately with compost addition from acid (5) to moderately alkaline (8), and this pH level remained stable until the end of the experiment (Fig. 5a). pH has a strong impact by shaping ionic interactions between organisms and the substrate they inhabit, nutrient uptake by roots, and is a strong determinant of microbial communities (Brady and Weil 2017; Valentín-Vargas et al. 2014). Moreover, compost addition immediately increased microbial activity, and its effect decreased through time but was still evident by the end of the experiment (Fig. 6a). Previous studies indicate that effects of organic amendments on the microbial activity and pH of tailings can last from several months to years (Solís-Dominguez et al. 2012; Valentín-Vargas et al. 2014; Zornoza et al. 2013), and these changes should contribute to a long-lasting effect of compost addition on vegetation growth in tailings.

While some studies have shown that organic amendments help in immobilizing metals in tailings substrate (Lee et al. 2014; Rodríguez et al. 2018; Zornoza et al. 2013), there is also evidence that some amendments can increase metal availability or leaching (Acosta et al. 2018; Asensio et al. 2013; Schwab et al. 2007). This issue should be assessed before phytostabilization actions are implemented in the field, as these effects may compromise the whole purpose of phytostabilization. In our field experiment, the addition of compost increased the soluble concentration of Cu, As, and Zn in the substrate (Online Resource). Several studies suggest that some compounds (such as fulvic acids, chelating agents, dissolved organic matter, bicarbonate, inorganic carbon) present in organic amendments have a major role increasing metal solubility and availability, by forming soluble complexes or colloids with metals at near-neutral to alkaline substrate pH (Asensio et al. 2013; Bolan et al. 2014; Schwab et al. 2007), and similar conditions occurred in our treatments with compost addition (Fig. 5a).

Notably, from the metals that increased its soluble concentration with compost addition, only Cu exceeded both plant leaf tissue toxicity limits (2–20 mg/kg from Kabata-Pendias 2011) and MTLs for animals (Table 1). However, Cu was also found at toxic levels for plants and exceeding MTLs in *V. farnesiana* and *P. velutina* growing within the vegetation patches (Table 1), suggesting that compost addition did not cause Cu to reach toxic levels. Other metals that reached toxic levels for animals in *P. velutina* (K and Mo) in the field experiment were not influenced by compost addition (Online Resource). These findings entail that compost addition did not have a significant impact on metal accumulation levels on plant tissues, but caution should be taken to reduce the risk of dispersion of metals to the surrounding environment through leaching processes.

Species performance for phytostabilization

Overall, our findings indicate that *V. farnesiana* had greater phytostabilization potential than *P. velutina*. According to metal accumulation factors, *V. farnesiana* had a limited transfer of the majority of the most abundant metals at the site (Fe, Ti, Ba, Cu, Rb, Mn, in decreasing order of abundance, from Meza-Figueroa et al. 2009) to above-ground tissues, except for Mn and As which are of environmental and health concern. *Prosopis velutina* had a limited transfer of some of those (As, Ba, Fe, Ti) and accumulated more metals than *V. farnesiana* (Table 2). However, most of the accumulated metals in above-ground tissues of both species did not exceed MTLs for animals, with the exception of Cu, Mo, and K in *P. velutina* (Table 1). Cu exceeded MTLs for animals in adult plants of both species growing in the vegetation patches (Table 1) even when its accumulation factor is < 1 (Santos et al. 2017). This finding reflects the high abundance of Cu at the Nacozari tailing that also increase during the seasonal formation of efflorescent salts (Meza-Figueroa et al. 2009), and its relatively high toxicity for animals (NRC 2005). Special attention should be paid to this metal during phytostabilization efforts at the Nacozari tailings.

It is noteworthy that none of the species had a significant effect over metal soluble concentrations in the substrate in our field experiment (Online Resource) and previous studies in the Nacozari tailings found no difference in metal concentrations between vegetated patches where those species are abundant and barren areas (Santos et al. 2017). This indicates that the differential performance of the species is not explained by its ability to modify soil physicochemical conditions, but due to their differences in the processes of mineral uptake and translocation.

Other practical implications

Based on our findings, we propose that phytostabilization efforts can take advantage of the differential performance of V. farnesiana and P. velutina under different scenarios. Vachellia farnesiana, showed more favorable accumulation factors and only one metal (Cu) exceeded MTLs for animals (Tables 1 and 2), and grew faster at the lowest compost addition treatment (2.5% compost) than P. velutina (Fig. 3a-c). Consequently, employing V. farnesiana at a low level of compost would minimize the risk of transfer of metal contaminants to the trophic chain, and at the same time would keep at minimal the increased risk of spreading contaminants to the environment through leaching caused by compost addition. On the other hand, P. velutina grew faster with increased compost addition, increasing both root and above-ground tissues (Figs. 3b-e, 4b-e). These traits of *P. velutina* would be appealing if a rapid colonization is desired and large amounts of compost are available. The rapid and potentially extensive growth of roots and canopy of P. velutina may be important attributes to help minimizing eolian and hydric dispersion of metal contaminants. It is worth noting that growth of P. velutina and also V. farnesiana may still be limited at tailings amended with compost compared to their growth potential under natural soils (Fig. 3a-e). Field experiments at larger scales at the Nacozari tailings should try to assess these tradeoffs, as well as provide evidence for compost-assisted plant colonization.

The few metals that exceeded MTLs, including K, Mo, and especially Cu, are of special concern, either because its

possible spread to the surrounding environment or its incorporation to local trophic chains. Previous studies suggest that mixing compost with products such as beringite, which has a strong capacity for metal immobilization, or with other organic amendments such as biochar, that also possess immobilization properties, could reduce metal availability and improve physicochemical properties (Forján et al. 2016; Rodríguez-Vila et al. 2014; Vangronsveld et al. 1996). Further studies and remediation practices at the Nacozari tailings should assess the utility of these approaches in conjunction with compost addition to minimize the risk of spread of K, Mo, and Cu. Furthermore, the use of other plant functional types is also encouraged (Navarro-Cano et al. 2019), to complement the phytostabilization roles of *V. farnesiana* and *P. velutina*, such as grasses, forbs, and shrubs.

Conclusions

We investigated the barriers that limit the establishment of two dominant plant species (Vachelia farnesiana and Prosopis velutina) that have been naturally colonizing a 50-year-old abandoned copper mine tailings in Nacozari, Sonora, Mexico, to inform future phytostabilization efforts. Our field observations at vegetation patches and barren areas within the tailings identified that low levels of organic matter and nitrogen in the substrate limit plant establishment. Our pot and field experiments with compost addition treatments showed that early-stage survival is the most affected process, followed by above-ground growth and germination, and that compost addition is an effective strategy to mitigate these barriers. Moreover, the added compost changed the pH from acidic to moderately alkaline and increased microbial activity, and these conditions improved species performance. Even when compost addition to the tailings increased soluble concentrations of several metals, most of these metals did not reach toxic levels for plant tissues or animal consumption. Furthermore, the experiments showed that V. farnesiana had better phytostabilization potential than P. velutina based on metal accumulation factors and plant tissue toxicity levels for animal consumption. Nonetheless, the increase in metal soluble concentrations in the substrate with compost addition, altogether with Cu, K, Mo tissue concentration levels in these species, requires further attention in future studies and phytoremediation plans to minimize the risk of spread to the environment and local trophic chains.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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