CHILEAN JOURNAL of AGRICULTURAL RESEARCH

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RESEARCH ARTICLE

Effects of arbuscular mycorrhizal fungi, poultry manure compost, and cadmium on plant growth and nutrient absorption of *Oryza sativa*

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Received: 4 April 2023; Accepted: 20 June 2023, doi:10.4067/S0718-58392023000600656

ABSTRACT

Compost produced with chicken manure and vegetable residues enriched with arbuscular mycorrhizal fungi (AMF) could become a sustainable agricultural strategy in biofertilization and bioremediation in rice (*Oryza sativa* L.) A completely randomized pot experiment was carried out to investigate the effects of AMF, humified composted poultry manure, and Cd on vegetative growth and elemental uptake of rice. After 90 d, rice treated with a combination of AMF and compost showed the highest plant growth. Overall, the best values in plant height, root length, chlorophyll content, and cation exchange capacity were obtained with the application of AMF and compost. In the absence of compost, Cd contamination significantly reduced AMF root colonization, while AMF alone increased root N, P, and K. Soil organic matter was higher when AMF, compost, and Cd were combined. Total soil P increased significantly with AMF inoculation (solely) compared to the rest of the treatments. The highest plant height (57.77 cm), root length (31.67 cm) and weight (167 g), shoot weight (74.67 g), and chlorophyll content (34.30 SPAD units) were achieved in the AMF-compost treatment. The highest values of root N (1.037%) and soil organic matter (2.66%) were obtained with the Compost-Cd treatment, which in turn presented lower Cd contents in roots and shoots. Cadmium inhibited morphological growth of the plant, biomass accumulation, and chlorophyll, but these parameters improved significantly with the application of AMF and compost, by facilitating the absorption of nutrients, particularly P.

Key words: Arbuscular mycorrhizal fungi, cadmium, *Oryza sativa*, synergistic effects.

INTRODUCTION

Rice (*Oryza sativa* L.) is a crop food of great nutritional value for mankind, being a critical crop in global food security. In Peru -and worldwide, rice is part of the essential family diet (Liu et al., 2014), and its cultivation is one of the main agricultural products: in the last 10 yr, the national production of paddy rice increased from 1 962 000 to 2 783 000 t, which represents an average annual increase rate of 4.2% (Neira et al., 2020). The Region of San Martín is considered the Peruvian region with the highest rice production, whose harvested area is 101 255 ha, with an average yield of 7 t ha^{-1} (MINAGRI, 2017). This extensive area planted with rice has demanded high levels of water use; likewise, agricultural soils contaminated by Cd, caused mainly by mining, electronic waste, and excessive use of N and P fertilizers, has been found in large-scale agricultural soils (Chen et al., 2019). Cadmium is absorbed by plants, accumulating in rice grains and entering human bodies due to its long half-life of up to 25 to 30 yr (Song et al., 2015), representing a serious threat to food security and human health (Li et al., 2019; Saikat et al., 2022). Therefore, soil contamination by heavy metals such as Cd not only inhibits plant growth, but also threatens human health, through food chain bioaccumulation (Zhang et al., 2019).

Cadmium content in rice soil has been reported up to 0.300-1.112 mg kg^{-1} (Song et al., 2015), while in grains the values were up to 0.062 ± 0.128 mg kg^{-1} (Song et al., 2017). Rice consumption has been considered the most important route of Cd exposure in humans (Li et al., 2017). Cadmium accumulation in rice is remarkably influenced by soil physical and chemical properties, such as soil organic matter (SOM), cation exchange capacity (CEC), pH, and other elements.

Faced with this problem, soil microbes play an essential role by affecting the characteristics of the soil and plant growth, which significantly influences the absorption of heavy metals. Soil microorganisms have been used over the last decades in order to increase plant growth, especially under soil limiting conditions. Spores of arbuscular mycorrhizal fungi (AMF) - beneficial soil microorganisms that form mutualistic symbioses with roots of most higher plant species, with the capacity to immobilize cadmium in the soil-plant system, have been used due to their strategies of immobilization and absorption of metals in the hyphae (Janeeshma and Puthur, 2020). They can increase root surface area for nutrient and water uptake (Liu et al., 2018). Likewise, compost prepared with manure has been considered as an important input to mitigate Cd toxicity in soils. Many studies show that composted manure immobilizes heavy metals in soil and reduces its uptake by plants; in turn, they improve soil conditions and plant growth, constituting a good alternative for heavy metal remediation. The main objective of this study was to elucidate the effects of AMF, poultry-manure compost, and Cd on plant growth and nutrient absorption in *Oryza sativa*.

MATERIALS AND METHODS

Soil and organic compost characteristics

The agricultural soil used (a total of 72 kg sieved soil) comes from fields close to large extensions of rice planted in the San Martín region, Peru, at a 0-20 cm soil depth. The chemical characteristics of the soil were: Total N, 1%; available (Olsen) P, 5.12 mg kg^{-1}; available K, 71.23 mg kg^{-1}; soil organic matter (SOM), 2.46%; total Cd, 0.31 mg kg^{-1}; pH, 7.18; and cation exchange capacity (CEC), 396.20 μS cm^{-1}. The humified compost was made with free-range hen manure and organic field residues and at the same time, it was enriched with earthworm humus and had the following properties: Total N, 2.12%; available P, 1300 mg kg^{-1}; available K, 15900 mg kg^{-1}; organic matter, 28.63%; total Cd, 0.00 mg kg^{-1}; pH, 7.12; and CEC, 1789.23 μS cm^{-1}. For the treatments contaminated with Cd, an aqueous solution of $CdCl_2$ (5 mg kg^{-1}) was added to each pot containing 3 kg substrate. To help stabilize the contaminant, the Cd-containing soil was allowed to air-dry for 50 d, and it was agitated every 15 d (four times), following the methodology of Vallejos-Torres et al. (2022).

Experimental design

This experiment consisted of a 2 × 2 × 2 factorial design with the addition of Cd (0 and 5 mg kg^{-1}), addition of humified compost ('Compost' thereafter) (0 and 250 g $plant^{-1}$, w:w), and inoculation with arbuscular mycorrhizal fungi (AMF) (control without mycorrhizal fungi and inoculation with AMF at 8.33%) arranged in a completely randomized design with three replicates. The AMF inoculum consisted of 2000 spores isolated from the Mariscal Cáceres Province in the San Martín region, Peru. The mycorrhizal inoculum was composed of a mixture of spores of the following species: *Claroideoglomus* sp., *Microkamienskia peruviana*, *Microkamienskia* sp., *Diversispora* sp., and *Claroideoglomus etunicatum* (Vallejos-Torres et al., 2022).

The growth substrate consisted of a mixture of river sand and agricultural soil (1:2, w:w), which was sterilized by autoclaving at 120 °C for 2 h. The seeds of rice 'INIA 507 - La Conquista' were disinfected with 2.5% sodium hypochlorite and distilled water; then, four seeds were sown per individual pot, filled each with 3.0 kg growth substrate. The experiment was carried out in a greenhouse, located at the National University of San Martín in Tarapoto (Peru) (06°28'59.71" S, 76°21'18.90" W) from January to April 2022. During the experiment, the air temperature was 25-34 ºC. The plants were irrigated regularly with sterile water at 60% of the field capacity, without any fertilization treatment.

Sampling and measurements

After 90 d, a total of 96 plants (eight treatments × three replicates × four plants per replicate) were harvested. Plant roots were washed and weighed on an analytical balance to determine fresh biomass and Cd by the HNO_3/spectrum digestion method with atomic absorption (Isaac and Johnson, 1975). Both roots and shoots were extracted from each plant sample to quantify Cd concentration (digestion with HNO_3) using atomic absorption spectroscopy (Zhang et al., 2020), and another group of shoots were taken to the laboratory for foliar analysis of N, using the Kjeldahl method (Kjeldahl 1883). Phosphorus was measured by the digestion of HNO_3/spectroscopy UV-Vis (λ = 420 nm) method, and K by the HNO_3/spectrum digestion method with atomic absorption (Isaac and Johnson, 1975). Substrate pH was measured in an aqueous extract (1:5) using a pH-meter; for the determination of SOM, the Walkley-Black method (De Vos et al., 2007) was followed. The CEC was determined by potential acidity + sum of bases, and the available P was estimated according to the Olsen method (Olsen and Sommers, 1982). Rhizospheric soil was also collected for the measurement of mycorrhizal root colonization. The height of the plant was measured from the base to the highest tip of the leaf. Arbuscular mycorrhizal colonization of roots was measured by the grid line intersection method after cleaning roots in 10% (w/v) KOH and staining them, as described by Phillips and Hayman (1970). Mycelium lengths per unit weight of soil were determined using Newman's formula (Newman, 1966).

Statistical analysis

All analyses were performed using the R programming language version 4.0.2 (R Core Team, R Foundation for Statistical Computing, Vienna, Austria). The normality and homoscedasticity of the data were verified using the Shapiro Wilk and Breush-Pagan tests, respectively. To analyze the effect of the treatments (AMF, compost, Cd, and their combinations) on the measured parameters, data were analyzed through ANOVA (using the R base function "aov") and Tukey-mean comparison tests with a probability of error of 5%, using the R base function "TukeyHSD".

RESULTS AND DISCUSSION

Effects on plant growth and chlorophyll

Root length and mycorrhizal colonization were significantly reduced when Cd was applied (Figure 1). As expected, root length was higher when both AMF and compost were combined (Figure 1a), while -and also as expected- AMF root colonization was higher when these fungi were applied alone (Figure 1b). Following the same pattern, a combination of AMF and compost resulted in significantly higher plant height (Figure 2a), shoot (Figure 2b) and root (Figure 2c) biomass, and chlorophyll content (Figure 2d). In all these cases, Cd applied alone resulted in trait values as low (or lower) as the control (Figure 2). The addition of compost and of compost + AMF to a Cd-contaminated pot, always resulted in significant increases in plant height, shoot and root biomass, and chlorophyll content (Figure 2). In most traits, except plant height, applying AMF alone resulted in increases compared to applying compost alone (Figure 2).

The interaction between AMF inoculum, poultry manure compost, and Cd had significant effects ($p < 0.001$) on plant height, root length, root biomass, and shoot biomass of *O. sativa*. Growth indicators and biomass characteristics also improved significantly with the application of compost made with chicken manure compared to the control. Chicken manure could provide essential nutrients directly to rice as indicated by Liu et al. (2021) when studying this waste material in maize, and its effects in plant growth. Without Cd contamination, root and shoot biomasses increased significantly. Cadmium contamination negatively affected root mycorrhizal colonization. This coincides with Wu et al. (2016), whom show negative effects of Cd (and Pb) toxicity on AMF and root endophytic fungi associated with wheat. Similar negative effects were shown by Vallejos-Torres et al. (2022) in cacao plants. Plant height and root length were higher under AMF, compost, and AMF + compost treatments, but were negatively affected by Cd application, as roots are the main way in which Cd enters to the plant, hindering its growth (Kanu et al., 2017). Cadmium content in rice roots was quite higher than in shoots, coinciding with Kanu et al. (2017), although the same authors indicate that Cd transport in the aerial parts of the plant can vary between cultivars. Similarly, Li et al. (2019) demonstrated that Cd significantly inhibited root length and dry weight in rice seedlings. Cd toxicity minimizes the mitotic division of meristematic cells, leading to a reduction in root growth and length and dry biomass (Barman et al., 2020).

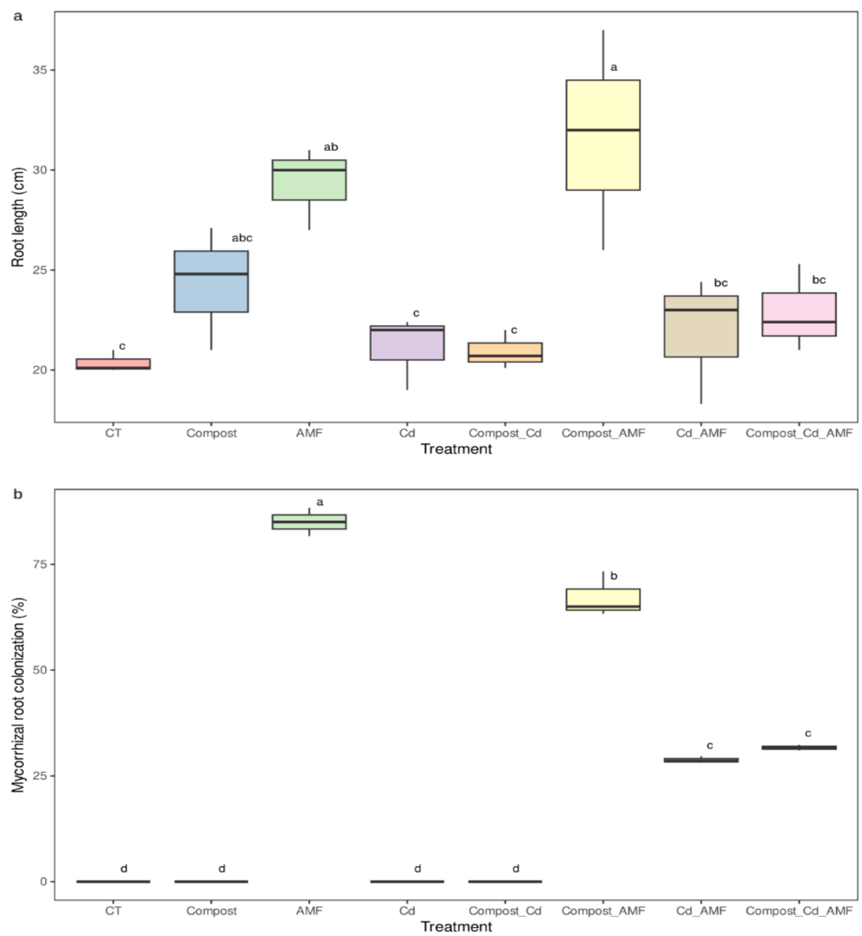

Figure 1. Effects of arbuscular mycorrhizal fungi (AMF) inoculation, humidified compost modification, Cd addition, and their combination on rice root length (a) and mycorrhizal colonization (b). Lowercase letters indicate significant differences between the different treatments.

The highest chlorophyll contents were found in the AMF and AMF + compost treatments; rice plants exposed to Cd showed inhibition of chlorophyll in their leaves (Herath et al., 2015). Accumulation of Cd in leaves could impair the chlorophyll biosynthesis and, therefore, reduce the chlorophyll content (Kanu et al., 2017). Furthermore, some authors such as Farooq et al. (2022) and Li et al. (2021) reported that Cd stress led to a substantial reduction in chlorophyll content in different rice varieties. The damage of chlorophyll pigments, photosynthesis, transpiration, stomatal conductance, and other physiological and photosynthetic processes, is due to deterioration in the thylakoid membrane and the formation of chlorophyll precursors (Vaculík et al., 2015).

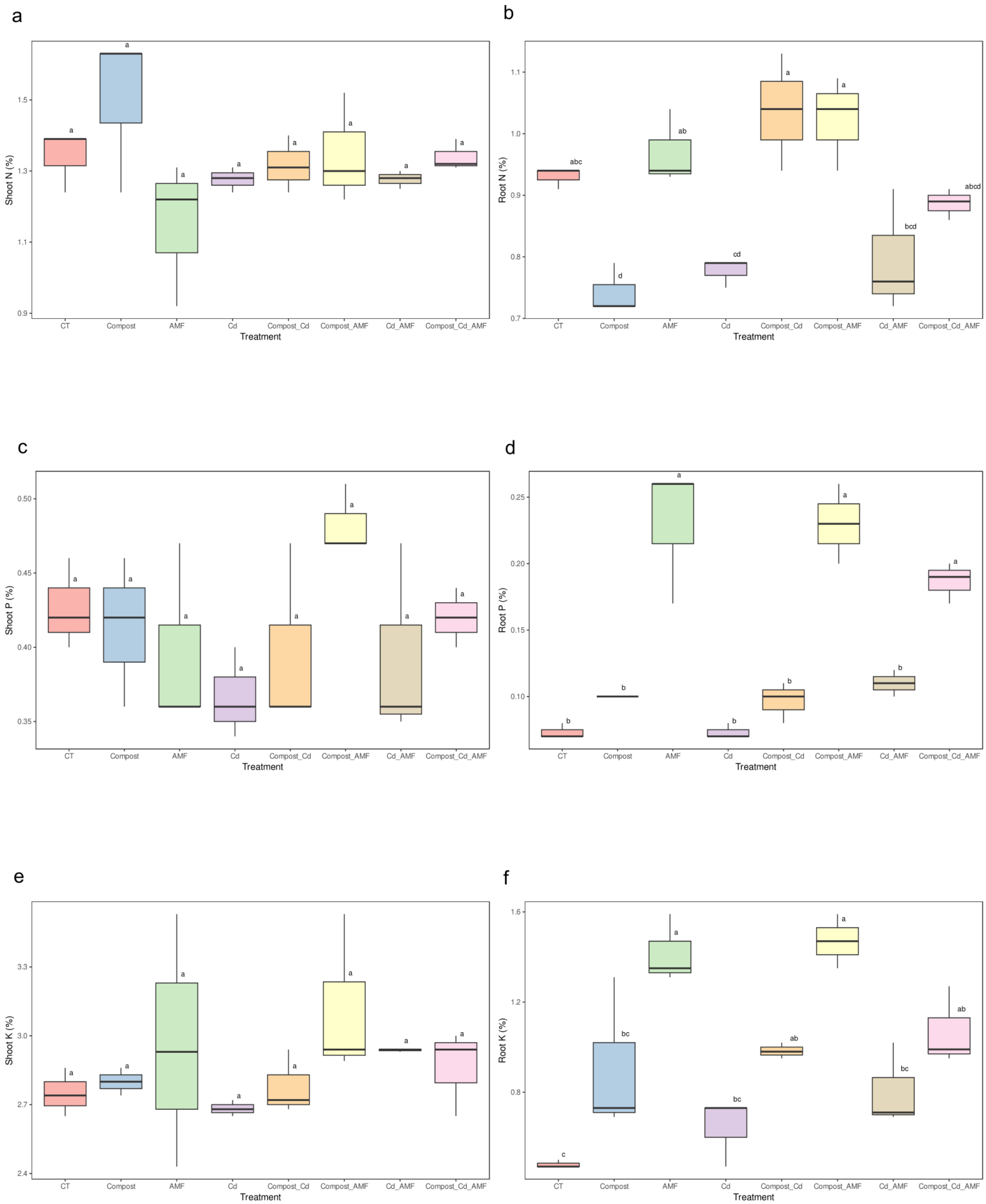

Figure 2. Effects of arbuscular mycorrhizal fungi (AMF) inoculation, humidified compost modification, Cd addition, and their combination on rice plant height (a) shoot biomass (b), root biomass (c), and chlorophyll content (d). Lowercase letters indicate significant differences between the different treatments.

Effects on nutrient absorption

Interestingly, and in contrast with the marked effects and patterns found for AMF, compost, and Cd application on rice root length and mycorrhizal colonization (Figure 1) and plant height, root and shoot biomass, and chlorophyll (Figure 2), such patterns were not found for rice shoot nutrient absorption (Figure 3). Overall, there were nonsignificant differences regardless of treatment for rice shoot N (Figure 3a), P (Figure 3c), and K (Figure 3e). When compost was combined with AMF and, unexpectedly, with Cd, higher root N values were obtained (Figure 3b). The AMF alone and combined with compost resulted in higher root P and K (Figures 3d, 3f). The Cd significantly reduced the efficiency of compost and AMF in capturing root N, P, and K (Figure 3).

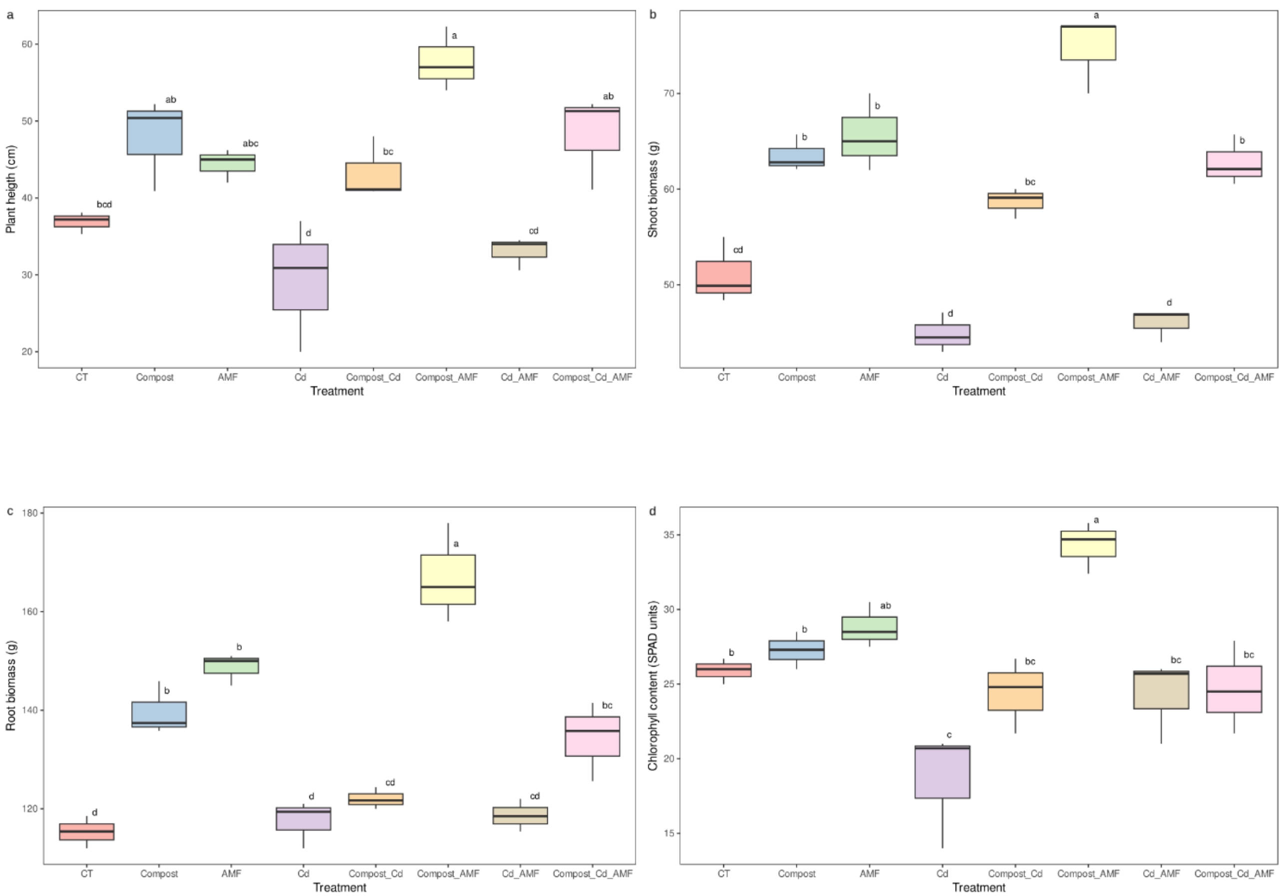

Figure 3. Effects of arbuscular mycorrhizal fungi (AMF) inoculation, humidified compost modification, Cd addition, and their combination on rice shoot N (a), root N (b), shoot P (c), root P (d), shoot K (e), and root K (e). Lowercase letters indicate significant differences between the different treatments.

The treatment combination of AMF and compost positively affected N, P, and K content in both rice roots and shoots. Previous studies have shown that AMF can facilitate the uptake of mineral elements by plants, particularly P (Liu et al., 2017). This is well established in mycorrhizal ecology, as hyphae explore a greater soil volume than roots by themselves (Liu et al., 2017). Root and shoot N, P, and K contents were higher than the control after applying compost, even in Cd-contaminated soils. Cadmium hindered the concentration of these nutrients in rice shoots and roots. The AMF inoculation in combination with the compost amendment had significant synergistic benefits, not only for nutrient uptake but also for the reduction in Cd uptake of rice in Cd-contaminated soils, results similar to those obtained by Zhang et al. (2019).

Effects on Cd and soil nutrients

All Cd-contaminated treatments had similar values of shoot Cd in rice plants (Figure 4a). Root Cd was the highest when Cd was applied alone, followed by when it was applied with AMF, then with AMF and compost, and finally, just with compost (Figure 3b).

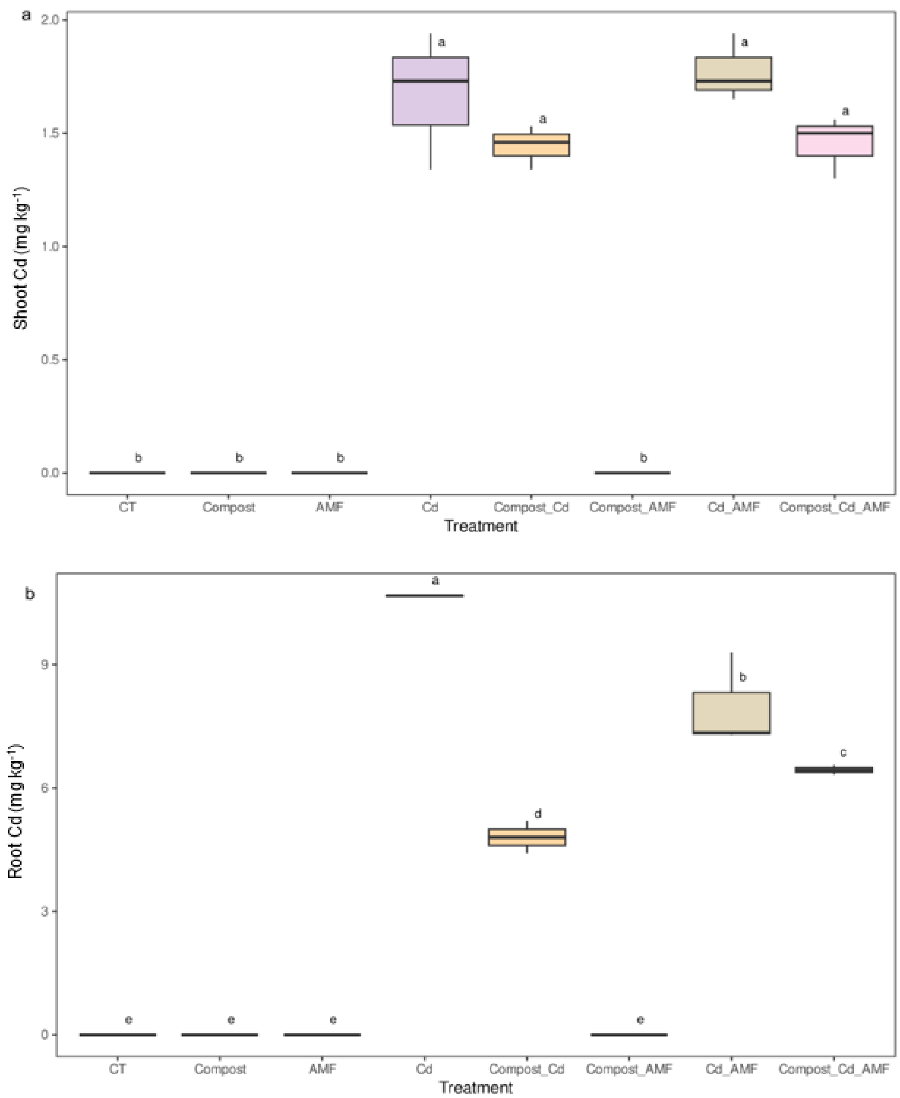

Figure 4. Effects of arbuscular mycorrhizal fungi (AMF) inoculation, humidified compost modification, Cd addition, and their combination on rice shoot Cd (a) and root Cd (b). Lowercase letters indicate significant differences between the different treatments.

The results for the Tukey tests indicate that soil pH was lower in the control treatment; however, when AMF, compost, and Cd were added, they showed significant differences (Table 1). The SOM had similar results, showing the highest content when AMF + Cd + compost were combined, and the lowest SOM value was obtained by the control treatment. The highest CEC was presented by the treatment with the application of AMF and the application of compost, showing significant differences with the control treatment (lowest CEC value). Available P increased significantly by AMF inoculation compared to the control treatment; however, when Cd was added, it was reduced to a nonsignificant level.

Table 1. Tukey tests for soil chemical parameters across the different treatments. SOM: Soil organic matter; CEC: cation exchange capacity; AMF: arbuscular mycorrhizal fungi.

Treatment	pH	SOM	CEC	Olsen P
		%	meq 100 g^{-1}	mg kg^{-1}
Control	6.16 ± 0.21^{c}	1.29 ± 0.10^{c}	5.75 ± 0.03^{c}	4.95 ± 0.10^{c}
Compost	7.04 ± 0.08^{b}	1.85 ± 0.12^{bc}	8.15 ± 0.32^{a}	6.71 ± 0.15^{b}
AMF	7.44 ± 0.01^{a}	2.38 ± 0.15^{ab}	8.10 ± 0.06^{a}	7.33 ± 0.18^{a}
Cd	7.10 ± 0.05^{b}	1.92 ± 0.12^{bc}	7.70 ± 0.23^{ab}	6.84 ± 0.16^{ab}
Compost-Cd	7.43 ± 0.01^{a}	2.66 ± 0.12^{a}	7.70 ± 0.17^{ab}	7.24 ± 0.07^{ab}
Compost-AMF	7.57 ± 0.00^{a}	2.36 ± 0.14^{ab}	7.65 ± 0.26^{ab}	7.12 ± 0.00^{ab}
AMF-Cd	7.42 ± 0.02^{a}	1.42 ± 0.02^{c}	7.00 ± 0.00^{b}	6.89 ± 0.00^{ab}
Compost-Cd-AMF	7.49 ± 0.05^{a}	2.49 ± 0.02^{a}	8.00 ± 0.12^{a}	7.00 ± 0.07^{ab}

It is known that the addition of compost stabilizes soil pH through a liming effect that also favors plant growth (Ahmad et al., 2014). Compost addition significantly increased soil pH under AMF inoculation. Chicken manure is an organic material that has recently attracted attention for its higher levels of fertility (Wang et al., 2020). It is a stabilized soil amendment that can improve and restore SOM, improve water retention, and soil structure. In the same venue, CEC was higher under the AMF + compost treatment, and we think this treatment allows roots to growth by increasing pH, available P, and CEC, among other properties (Pandian et al., 2016). Likewise, mycorrhizal associations can contribute to reducing the transfer of heavy metals to plants by acting as an exclusion barrier (Cabral et al., 2015), which can react by binding heavy metals to the fungal hyphae (Gonzalez-Chavez et al., 2002).

CONCLUSIONS

Cadmium inhibited plant morphological growth and biomass accumulation in rice, probably caused by metal stress in rice roots. Inoculation with arbuscular mycorrhizal fungi (AMF) alone or with poultry manure compost significantly improved the rice growth. The AMF can facilitate plant nutrient uptake, particularly P. Likewise, Cd is known to inhibit plant nutrient uptake within the rhizosphere in rice. The results showed that Cd toxicity resulted in a substantial reduction of chlorophyll content while the addition of poultry manure compost significantly improved AMF colonization, and this in turn significantly increased root P concentration.

Author contributions

Conceptualization: G.V-T., N.G-J. Methodology: G.V-T., C.M. Software: G.V-T. Validation: C.M., L.A.O-S. Formal analysis: C.L., C.M. Investigation: A.A.A. Resources: A.L. Data curation: G.V-T., W.M-C., C.M. Writing-original draft: C.P. Writing-review & editing: J.S-R, C.M. Visualization: L.A., C.M. Supervision: N.G-J. Project administration: G.V-T. Funding acquisition: J.T. All co-authors reviewed the final version and approved the manuscript before submission.

Acknowledgements

G.V.T. thanks the agroforestry research center of Perú. C.M. thanks the Convocatoria Nacional Subvención a Instalación en la Academia Convocatoria Año 2021 + Folio SA77210019 (ANID-Chile).

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