Science of the Total Environment 954 (2024) 176750

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Recycling steel slag as fertiliser proxy in agriculture is good circular economy but disrupts plant microbial symbioses in the soil

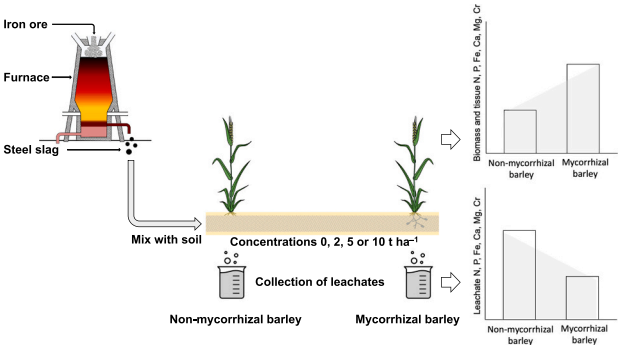
Vikrant Goswami [a], Sharma Deepika [a,b], Pulkit Sharma [c], David Kothamasi [a,d,*]

[a] Laboratory of Soil Biology and Microbial Ecology, Department of Environmental Studies, University of Delhi, Delhi 110 007, India
[b] Department of Botany, Zakir Husain Delhi College, University of Delhi, Delhi 110002, India
[c] Biodiversity Parks Programme, Centre for Environmental Management of Degraded Ecosystems, University of Delhi, Delhi 110007, India
[d] Strathclyde Law School, University of Strathclyde, Glasgow G4 0LT, United Kingdom

HIGHLIGHTS

- Reducing the 5 % share of fertilisers in global greenhouse emissions needs alternatives
- Slag, a nutrient rich by-product of steel making provides an alternative.
- The suitability of slag application in soil as a fertiliser alternative was tested.
- Slag raised yield but lowered crop sensitivity and dependence on arbuscular mycorrhiza.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Wei Shi

Keywords:
Agriculture
Arbuscular mycorrhizal fungi
Barley
Fertilisers
Mineral leaching
Nutrient uptake
Rhizophagus irregularis
Steel slag

ABSTRACT

Modern agriculture depends on synthetic fertilisers to ensure food security but their manufacture and use accounts for ~5 % of the global greenhouse gas emissions. Achieving climate change targets therefore requires alternatives, that while maintaining crop productivity, reduce emissions across the lifecycle of fertiliser utilisation. Steel slag, a nutrient-rich by-product of steel manufacture, offers a viable alternative. Being substantially cheaper than fertilisers, it is economically attractive for farmers, particularly in low-middle income countries of the Global South. However, slag application in agriculture poses risk of pollutant transfer to the human food chain and disruption of key plant-microbe symbioses like the arbuscular mycorrhizal fungi (AMF). Here, using barley as a model crop, we tested the suitability of slag as a fertiliser proxy. Mycorrhizal and non-mycorrhizal barley were grown in soils ameliorated with slag in concentrations of 0, 2, 5 and 10 t ha^{-1}. We analysed slag-mycorrhiza interaction and their combined effects on crop yield and risks to human nourishment. Slag increased grain yield by respective 32 and 21 % in mycorrhizal and non-mycorrhizal barley. Grain concentration of metal pollutants in mycorrhizal and non-mycorrhizal barley fertilised with slag were within the WHO recommended limits. But slag reduced mycorrhizal colonisation in barley roots and extraradical hyphal spread in the soil. The consequent decline in symbiont function lowered AMF-mediated plant nutrient uptake and increased mineral losses in leachates. AMF are keystone species of the soil microbiome. Loss of AMF function

* Corresponding author at: Laboratory of Soil Biology and Microbial Ecology, Department of Environmental Studies, University of Delhi, Delhi 110 007, India.
E-mail address: dkothamasi@es.du.ac.in (D. Kothamasi).

https://doi.org/10.1016/j.scitotenv.2024.176750
Received 14 January 2024; Received in revised form 3 October 2024; Accepted 3 October 2024
Available online 9 October 2024

presents long-term ecological consequences for agriculture and necessitates a careful evaluation of slag application to soil.

1. Introduction

Fertilisers are vital to sustain food security in modern agriculture. However, nearly 5 % of the global greenhouse gas emissions can be traced to their manufacture and application in agriculture (Gao and Serrenho, 2023). Realising climate change targets in agriculture, therefore, requires alternatives that can maintain crop productivity as well as reduce emissions across the lifecycle of fertiliser utilisation.

Industrial by-products like steel slag that are rich in Ca, Si, P, Mg and Fe (Ito, 2015; Das et al., 2020) may fit this role. Crops like barley, wheat, tomato, potato and rape fertilised with iron and steel slag have shown growth and yield improvements comparable or higher than synthetic fertilisers (European Commission: Directorate-General for Research and Innovation et al., 2017). Moreover, slag application in rice paddy fields not only increased grain yield but also reduced emissions of greenhouse gases CH_4 and N_2O (Wang et al., 2015; White et al., 2017; Gwon et al., 2018; Das et al., 2019; Das et al., 2020).

Recycling steel slag as fertiliser substitute in agriculture aligns well with the UN sustainable development goals which call for increased efforts from the states to mitigate climate change and increase resource efficiency by encouraging a circular economy (Hermassi et al., 2020; Mayer et al., 2022). Slag is a by-product of steel manufacture and accounts for 15–20 % of the total production (Li et al., 2022). With 161.4 Mt. crude steel produced in 2023 (World Steel Association, 2023), the steel industry is burdened with a substantial amount of slag that must be sustainably disposed. Channelling it as a fertiliser proxy in agriculture can provide returns from a waste that would, otherwise, have been dumped in landfills without remuneration and with considerable risk of mineral leaching. Being substantially cheaper than fertilisers, it is economically attractive for farmers, particularly in low-middle income countries of the Global South. But application of industrial by-products in agriculture as fertiliser proxies are rife with risk of transferring toxic pollutants to the human food chain (Goswami et al., 2023a). Moreover, they can damage soil health by disrupting the soil microbial community structure.

Decades of unsettling the soil nutrient balance by fertiliser application has reduced the ability of soil microbial symbionts to improve plant fitness and soil health (Ray et al., 2020). The intensification of nutrient inputs to soil from steel slag application may have similar impacts on the soil microflora. This disruption of microbial ecosystem multifunctionality can have long term consequences for agricultural soil health.

Arbuscular mycorrhizal fungi (AMF) are keystone organisms with a pivotal role in maintaining the soil microbial community. They are ubiquitous in agricultural soils, forming symbiotic association with over 90 % of cereal and vegetable crops (Posta and Duc, 2019; Diagne et al., 2020). AMF work synergistically with other microbes to promote plant growth, remove root pathogens, and improve tolerance to abiotic stresses (Fall et al., 2022). Their hyphae extend the root absorptive zone by over 100 cm from the root surface by penetrating soil pores that are otherwise inaccessible to plant roots (Zhang et al., 2018). This increase in absorptive surface area is instrumental in enhancing the uptake of mineral nutrients as well as reducing the volume of nutrients and pollutants leaching to below ground aquifers (Cavagnaro et al., 2015; Martínez-García et al., 2017; Goswami et al., 2023b).

However, the crop-AMF symbiosis is regulated by a mutualistic cost-benefit balance. In biological terms, this represents the ratio of photosynthetic costs incurred by the host to extract metabolic benefits from the fungal symbiont (Noë and Kiers, 2018). Like fertilisers, enrichment of the soil mineral nutrient content by steel slag addition reduces the cost-benefit ratio of outsourcing nutrient acquisition to AMF hyphae. Being obligate symbionts, a drop in mycorrhizal dependency of the crop may possibly remove AMF from agricultural soils. This can impact soil health due to the cascading effects of their loss on other plant beneficial microbial groups.

Here, using barley (*Hordeum vulgare*) as a model crop in a pot-based experiment, we quantified the impacts of steel slag application to soil on human nourishment and the viability of AMF symbiosis. Barley has served as a model crop for experiments analysing abiotic stresses because of its easy cultivability and robust ecological amplitude enabling tolerances to a multitude of environmental stresses (Dawson et al., 2015; Harwood, 2019; Rotasperti et al., 2020). We assessed the fertiliser replacement potential of slag by measuring its effects on crop growth promotion, crop mycorrhizal dependency, and the persistence of AMF role in promoting crop nutrient uptake and reducing mineral leaching.

2. Materials and methods

2.1. Steel slag, plant and fungal materials

Industrial grade steel slag (Supplementary S1) was obtained from the Indian Agricultural Research Institute, New Delhi. Barley (*H. vulgare* var. DWR 8160 Karan Maltsona) and *Rhizophagus irregularis* MUCL 41833 were used as the respective plant host and AMF inoculum.

2.2. Experimental design

Plants were grown in plastic pots (10 cm diameter × 10 cm height). Air dried loam soil (USDA textural class: loam based on sand, silt and clay composition of 36, 48 and 16 % respectively) collected from an agricultural field in Jagatpur village (28°45′07″N - 77°12′37″E), Delhi, India was used as the growth substrate. Air dried steel slag was homogenised and mixed with the loam soil in concentrations of: (i) 0, (ii) 2, (iii) 5 or (iv) 10 t ha^{-1}, respectively. The slag-soil mixtures prepared above were autoclaved for 2 h at 121 °C. Around 420 g of soil amended with 0, 2, 5 or 10 t ha^{-1} steel slag was added to each pot. Each pot received 30 g of viable or autoclaved (121 °C for 2 h) *R. irregularis* inoculum containing 22 spores g^{-1}. Pots containing viable inoculum were labelled as mycorrhizal and those with autoclaved inoculum were labelled as non-mycorrhizal. After adding the inoculum, the pots were overlain with ~2 cm soil containing 0, 2, 5 or 10 t ha^{-1} slag such that the final weight of each pot was 500 g.

H. vulgare seeds were washed with liquid detergent and surface sterilised with 1.25 % sodium hypochlorite for 5 min. The surface sterilised seeds were rinsed with autoclaved double deionised water and planted in sterile 0.8 % agar. Five-day old seedlings were transferred to the pots prepared as above (five seedlings per pot) on 14th January 2023. Each non-mycorrhizal pot received 30 mL microbial wash prepared by suspending *R. irregularis* inoculum in double deionised water at a ratio of 1:6 and filtering through a series of sieves. The smallest sieve had a pore size of 8 μm. This step was performed to correct for possible differences in bacterial and non mycorrhizal fungal communities between the mycorrhizal and non-mycorrhizal pots (Koide and Li, 1989). The experiment followed a factorial design with two factors of four levels each: (i) 'mycorrhizal' *H. vulgare*; 'non-mycorrhizal' *H. vulgare*; and (ii) four concentrations of steel slag [0 (control), 2, 5 and 10 t ha^{-1}]. This resulted in a total of eight treatment combinations. With each treatment replicated five times, there were a total of 40 pots.

The pots were kept in a polyhouse at ambient temperature (7–35 °C) with a day-night cycle of 16–8 h. Soils were maintained at moisture levels of 15–20 % by weight. Plants did not receive additional nutrient

fertilisation at any stage during the experiment. Pots were randomised weekly.

Leachates were collected in beakers placed under each pot before watering on the 50th and 110th day. On the 50th day, prior to the collection of leachates, 5–6 cm shoot segments were excised and soil cores containing *H. vulgare* roots were extracted from a depth of ~7 cm from each pot using autoclaved soil corers (7 mm diameter). AMF colonisation and extraradical AMF hyphal length were measured in the extracted roots and soils. Fresh weights of the extracted shoots and root segments were recorded. A subset of the collected root samples was set aside to determine AMF colonisation. Dry weights of shoot segments and the remaining roots were measured after oven drying for five days at 70 °C. Leachates were acidified (pH < 2) with HNO_3 and their elemental chemistry was analysed immediately after collection.

2.3. Harvest

H. vulgare plants were harvested after 112 days on 5th May 2023. Plants were separated into grains, shoots and roots. The roots were rinsed under tap water to remove adhering soil particles. A subset of the roots was set aside to measure AMF colonisation. Following measurement of fresh weights; grains, shoots and roots were dried at 70 °C and weighed after five days to note the dry weights. The dry weights of the shoots and roots were corrected to account for the samples collected on the 50th day, as well as the subsets set aside from harvested roots for measuring AMF colonisation.

2.4. Root AMF colonisation

Roots were cleared at 90 °C in 10 % KOH and rinsed with tap water. AMF structures in the cleared roots were stained using a modified ink-vinegar method (Vierheilig et al., 1998). Root AMF colonisation was estimated following the modified line intersection method (McGonigle et al., 1990). Two hundred intersections were counted. The mycorrhizal dependency and sensitivity of barley to *R. irregularis* in each slag treatment were estimated as described previously by Goswami et al. (2023a, b).

2.5. AMF extraradical mycelium

The extraradical AMF hyphae were extracted and their lengths quantified as proxies for AMF biomass in soil following the aqueous extraction and membrane filter technique (Jakobsen et al., 1992). The extraradical hyphae were stained with ink-vinegar. Extraradical AMF hyphal lengths in soil were estimated using the modified Newman formula (Tennant, 1975). Two hundred intersections were counted.

2.6. Nutrient and elemental analyses

Plant tissues and the growth substrate were acid digested by H_2SO_4-peroxide digestion (Allen, 1989). NO_3-N, PO_4-P in plant tissues, growth substrate and leachates were estimated using the indophenol blue (Cataldo et al., 1975) and molybdenum blue (Chen Jr et al., 1956) methods, respectively. Available N and P in the growth substrate were estimated using the UV spectrophotometer and stannous chloride methods (APHA, 2000), respectively. Concentrations of Fe, Ca, Mg and Cr content in plant tissues, growth substrate and leachates were determined on an atomic absorption spectrophotometer (novAA 350i, Analytik Jena AG, Germany).

2.7. Statistical analyses

Statistical analyses were performed using R version 4.3.0. Normality of data was tested with a Shapiro-Wilk test. Data that did not follow Gaussian distribution were transformed using the R-package *bestNormalize* version 1.9.0 (Peterson, 2021). Differences in means were analysed with a one-way analysis of variance (ANOVA) followed by a Tukey's honest significant differences (HSD) post hoc test for pairwise comparisons. Relationships among AMF and plant parameters; and between extraradical AMF hyphal length and elemental concentrations in the leachates were explored using Pearson's correlation. The individual and interactive effects of slag additions in soil and inoculation with AMF on different plant-AMF metrics and elemental movement were evaluated using linear mixed effects models implemented with R-package *nlme* version 3.1–162 (Pinheiro et al., 2023). Total dry mass of plants, percent AMF root colonisation, AMF extraradical hyphal lengths, and concentrations of NO_3–N, PO_4–P, Fe, Ca, Mg and Cr in plant tissues and leachates respectively were included as response variables in the model. Concentration of steel slag and presence/absence of AMF were fixed factors. Time of plant tissue harvest and leachate collection was included in the model as a random factor. We implemented the linear mixed effects models on untransformed data following Schielzeth et al. (2020) who using simulations with violations of the normality assumptions showed that linear mixed effect models are robust even with unbalanced ecological data. They recommend avoiding transformations when faced with a trade-off between interpretability and conformance to model assumptions. Significance of all statistical tests was set at $p \leq 0.05$.

3. Results

3.1. Plant growth

Mycorrhizal and non-mycorrhizal plants had increased growth in soils with steel slag. Biomass of the mycorrhizal plants grown with respective 0 and 10 t ha^{-1} slag treatments was higher than that of the non-mycorrhizal plants (Fig. 1A–C). Grain yield of barley was higher when grown with steel slag. Grain biomasses of mycorrhizal and non-mycorrhizal plants grown in soils containing 10 t ha^{-1} steel slag were 32 and 21 % greater respectively than the plants grown in the control (0 t ha^{-1}). Analysis of variance (ANOVA) of the linear mixed effects model results showed that both, steel slag addition and AMF inoculation, significantly affected plant biomass (Table 1).

3.2. AMF colonisation of barley

Steel slag significantly impacted AMF colonisation of the roots and spread of the extraradical hyphae in the rhizosphere. Plants of the control had higher percent root colonisation than those grown in soils amended with steel slag at both harvests – 50th and 112th days respectively (Fig. 2A). Percentages of arbuscles, vesicles and hyphae in the roots of the control treatment harvested on the 50th and 112th day were 11.7, 8.16, 21.13 % and 20.6, 15.4, 43.76 % respectively. On the other hand, in plants grown at 10 t ha^{-1}, the respective arbuscle, vesicle and hyphal percentage colonisations in the roots harvested on the 50th and 112th days were 4.9, 4.9, 11.8 % and 11.2, 8.6, 21.3 % (Supplementary S2A—C).

A concentration dependent reduction in extraradical hyphal lengths was observed in pots amended with steel slag. Extraradical hyphal lengths were lowest in pots amended with 10 t ha^{-1} at both harvests (Fig. 2B). ANOVA results for the linear mixed effects model revealed that AMF inoculation had the largest significant effect on total root AMF colonisation and extraradical hyphal length, and the interaction of AMF inoculation with steel slag concentration was significant (Table 1).

3.3. Mycorrhizal dependency and sensitivity of barley to R. irregularis

Mycorrhizal dependency was low in all treatments, ranging from 17.2 ± 0.88 % in the control to 8.2 ± 0.83 % for plants grown with 10 t ha^{-1} steel slag (Fig. 3A). Mycorrhizal dependency correlated positively with total AMF colonisation (Fig. 4A) and extraradical AMF hyphal length (Fig. 4B). However, a negative relationship was observed

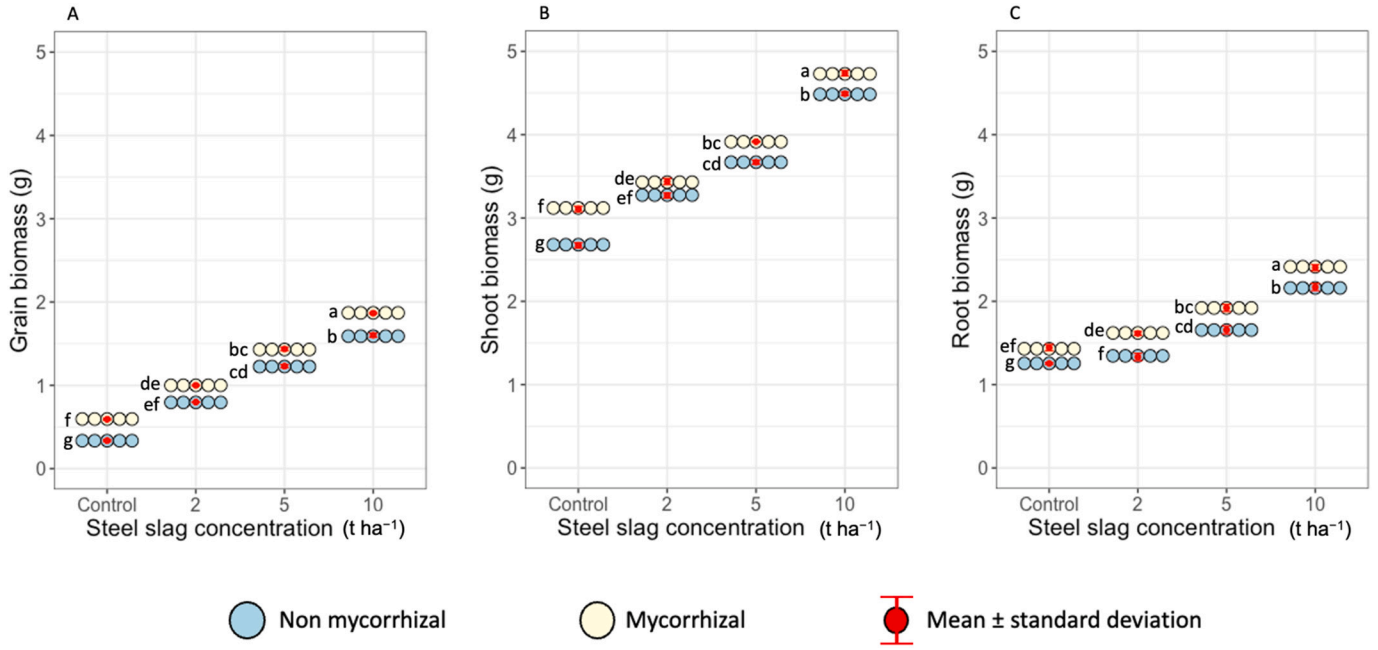


Fig. 1. Effects of steel slag and AMF inoculation on barley biomass. (A) grain biomass; (B) shoot biomass; and (C) root biomass. Each dot denotes one replicate. Red dot with error bars represents the average of five replicates ± standard deviation of the mean. Values that do not share an alphabet are significantly different ($p \leq 0.05$) in a Tukey's HSD post hoc test.

Table 1
Summary of model and ANOVA results of linear mixed-effects models analysing the individual and interactive effects of slag treatment and inoculation with *R. irregularis* (fixed factors) on AMF hyphal spread in the rhizosphere (extraradical hyphal length), mycorrhizal root colonisation and elemental concentrations in plant tissue and leachates collected on the 50th day and at harvest. Time of collection of tissue and leachate samples was included as a random factor. Asterisks next to the *F*-statistic indicate significance at: ***$p < 0.0001$; **$p < 0.001$; and *$p < 0.05$. *df*, degrees of freedom.

	df	Slag treatment	AMF	Interaction
		3	1	3
Plant total dry biomass	*F*	67.21***	24.32***	0.99
Root AMF colonisation	*F*	13.23***	679.31***	13.23***
Extraradical hyphal length	*F*	42.35***	997.12***	42.35***
Grain NO_3-N	*F*	21.899***	2.88	0.80
Shoot NO_3-N	*F*	144.97***	46.95***	2.97*
Root NO_3-N	*F*	59.62***	12.01***	1.39
Leachate NO_3-N	*F*	1.322	13.43**	4.09**
Grain PO_4-P	*F*	22.01***	4.24*	0.10
Shoot PO_4-P	*F*	625.38***	148.52***	0.61
Root PO_4-P	*F*	149.84***	47.08***	3.52**
Leachate PO_4-P	*F*	47.90***	24.31***	0.27
Grain Fe	*F*	21.14***	5.31*	0.74
Shoot Fe	*F*	86.32***	11.60**	1.20
Root Fe	*F*	222.07***	16.35**	0.17
Leachate Fe	*F*	42.02***	10.65**	0.81
Grain Ca	*F*	22.15***	3.60	0.30
Shoot Ca	*F*	290.94***	26.69***	1.14
Root Ca	*F*	379.55***	24.90***	1.27
Leachate Ca	*F*	74.45***	36.98***	5.49**
Grain Mg	*F*	19.46***	7.28*	1.71
Shoot Mg	*F*	389.00***	54.18***	10.16***
Root Mg	*F*	572.66***	56.46***	14.20***
Leachate Mg	*F*	174.94***	67.50***	9.22***
Grain Cr	*F*	21.15***	5.06*	0.63
Shoot Cr	*F*	432.13***	73.51***	18.38***
Root Cr	*F*	866.62***	115.72***	31.56***
Leachate Cr	*F*	43.73***	33.58***	4.99**

between total plant biomass and mycorrhizal dependency (Fig. 4C). The sensitivity of barley to *R. irregularis* was highest in pots with 2 t ha^{-1} and lowest in those supplemented with 10 t ha^{-1} steel slag (Fig. 3B). Mycorrhizal sensitivity, however, did not correlate with mycorrhizal dependency (Fig. 4D).

3.4. Elemental concentrations in plant tissues

3.4.1. N and P uptake

Mycorrhizal plants grown in the control had higher NO_3–N in the grains than the non-mycorrhizal plants ($F_{7,32} = 102.9$; $p = 0.0003$). Grain NO_3 content did not differ in mycorrhizal and non-mycorrhizal plants grown with 2 and 5 t ha^{-1} slag. However, in plants grown with 10 t ha^{-1} steel slag, grain NO_3 concentrations were higher in mycorrhizal plants compared to the non-mycorrhizal plants ($F_{7,32} = 102.9$; $p = 0.0004$; Fig. 5A). Shoots and roots of mycorrhizal and non-mycorrhizal plants grown with slag had higher NO_3 concentrations than those of the control. Plants grown at 10 t ha^{-1} steel slag had the highest tissue concentrations of NO_3. In tissues harvested on the 50th day, mycorrhizal plants in general had higher amounts of NO_3 than the non-mycorrhizal plants in all slag treatments (Supplementary S3A,B). In the 112th day harvest, mycorrhizal plants grown with control ($F_{15,64} = 265.9$; $p < 0.0001$), 2 ($F_{15,64} = 265.9$; $p = 0.0003$) and 10 t ha^{-1} steel slag ($F_{15,64} = 265.9$; $p < 0.0001$) had higher shoot NO_3 content than the non-mycorrhizal plants. In the case of roots, mycorrhizal plants of all slag treatments had higher NO_3 content than non-mycorrhizal plants at 50th day harvest. However, at 112th day harvest, NO_3 content was higher in mycorrhizal plants than the non-mycorrhizal plants in soils with 0 ($F_{15,64} = 203.7$; $p < 0.00001$) and 10 t ha^{-1} steel slag ($F_{15,64} = 203.7$; $p = 0.00001$). Mycorrhizal and non-mycorrhizal plants grown in 2 and 5 t ha^{-1} steel slag, did not exhibit any differences in root NO_3 concentrations (Supplementary S3B). Results of the linear mixed effects model show that NO_3 uptake by shoots and roots were significantly influenced by steel slag addition to the soil and inoculation with AMF. However, AMF inoculation did not affect NO_3 transfer to the grains (Table 1).

Grain PO_4–P content of mycorrhizal plants was higher than non-mycorrhizal plants in soils amended with 0 ($F_{7,32} = 104.3$; $p = 0.00033$) and 10 t ha^{-1} slag ($F_{7,32} = 104.3$; $p = 0.00031$). No significant

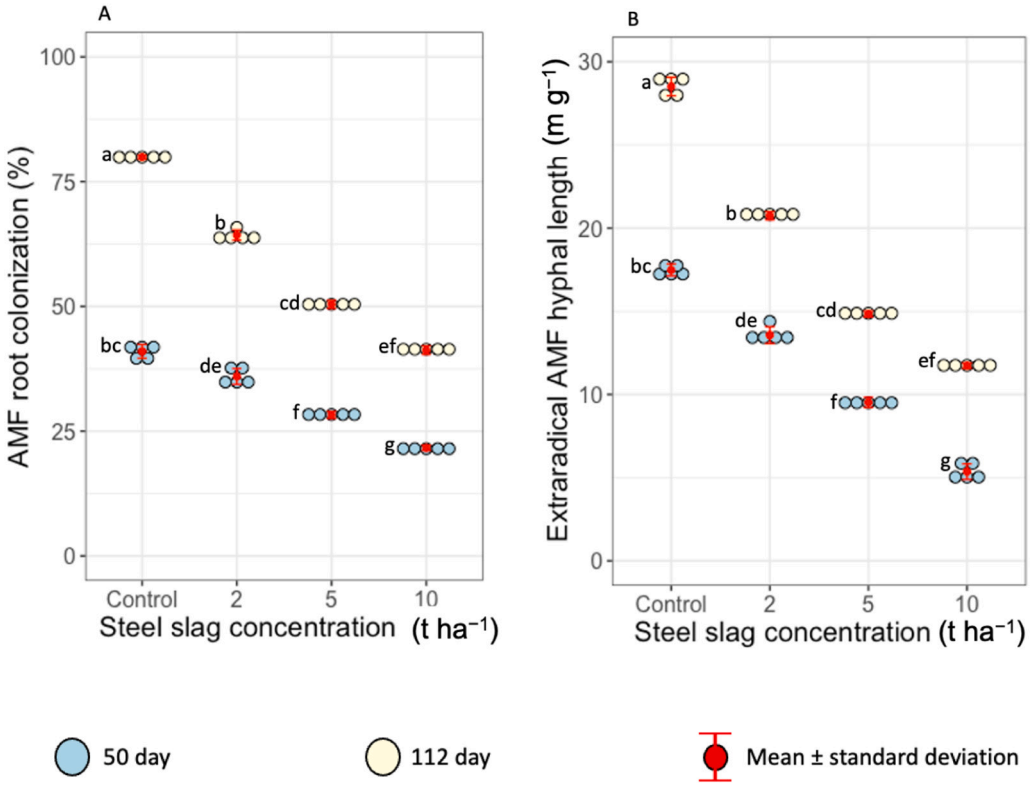


Fig. 2. Inhibition of AMF by steel slag. (A) percent root colonisation; and (B) extraradical hyphal length in soil. Each dot denotes one replicate. Red dot with error bars represents the average of five replicates ± standard deviation of the mean. Values that do not share an alphabet are significantly different ($p \leq 0.05$) in a Tukey's HSD post hoc test.

differences were observed in PO_4 concentration in grains of mycorrhizal and non-mycorrhizal plants in soils with 2 and 5 t ha^{-1} steel slag (Fig. 5B). In shoots harvested on the 50th day, only mycorrhizal plants of the control had higher PO_4 levels than the non-mycorrhizal plants ($F_{15,64} = 294.2$; $p < 0.0001$). At 112th day harvest, only mycorrhizal plants of 10 t ha^{-1} slag treatment had higher PO_4 levels than non-mycorrhizal plants ($F_{15,64} = 294.2$; $p < 0.0001$; Supplementary S3C). A similar trend was found for root PO_4 concentrations. While mycorrhizal roots had higher PO_4 than non-mycorrhizal roots in plants grown in the control, no significant differences in root PO_4 concentrations between mycorrhizal and non-mycorrhizal plants were observed in tissues harvested on the 50th day (Supplementary S3D). In tissues harvested on the 112th day, no significant differences were found in shoot and root PO_4 concentrations of mycorrhizal or non-mycorrhizal plants grown with control, 2 and 5 t ha^{-1} steel slag. However, in plants grown at 10 t ha^{-1} slag, the mycorrhizal roots ($F_{15,64} = 256.3$; $p < 0.0001$) and shoots ($F_{15,64} = 294.2$; $p < 0.0001$) had higher PO_4 concentrations than the non-mycorrhizal plants (Supplementary S3C,D). According to the linear mixed effects model, AMF inoculation and slag addition significantly influenced the uptake and transfer of PO_4 to roots, shoots and the grains of barley (Table 1).

3.4.2. *Metal uptake*

Grains of mycorrhizal plants had higher amounts of Fe and Ca than the non-mycorrhizal plants in soils with 0, 2 and 10 t ha^{-1} slag amendment (Fig. 5C,D). Mycorrhizal plants had higher grain Mg levels than non-mycorrhizal plants in the control ($F_{7,32} = 163.1$; $p = 0.000011$) and in soils containing 10 t ha^{-1} steel slag ($F_{7,32} = 163.1$; $p = 0.000018$; Fig. 5E). Grain concentrations of Cr were higher in mycorrhizal plants compared to the non-mycorrhizal plants in all four treatments (Fig. 5F).

In shoot and root tissues harvested on the 50th day, plants grown with steel slag had higher levels of Fe, Ca, Mg and Cr than plants of the control (Supplementary S3E–L). Shoots of mycorrhizal plants had higher Fe and Ca concentrations in plants grown at 0, 2 and 5 t ha^{-1} steel slag. Differences in shoot Fe and Ca concentrations were not significant between mycorrhizal and non-mycorrhizal plants grown in soils containing 10 t ha^{-1} slag. While differences in root Fe concentrations of mycorrhizal and non-mycorrhizal plants were not significant in any treatment, root concentrations of Ca were higher in the mycorrhizal plants compared to the non-mycorrhizal plants in soils with 0 and 2 t ha^{-1} slag (Supplementary S3H). Except for plants grown with 5 t ha^{-1} slag, significant differences in Mg concentration were not found in shoots of mycorrhizal and non-mycorrhizal plants harvested on the 50th day (Supplementary S3I). Apart from plants grown at 10 t ha^{-1} steel slag, Cr concentrations did not differ significantly in roots and shoots of mycorrhizal and non-mycorrhizal plants harvested on the 50th day (Supplementary S3K,L).

In the 112th day harvest, plants grown with slag had higher levels of Fe, Ca, Mg and Cr than the control (Supplementary S3E–L). Except for plants grown at 5 t ha^{-1} slag, concentrations of Fe, Ca and Mg in shoots of mycorrhizal plants were higher than those of the non-mycorrhizal plants. AMF inoculation had significant effect on Fe and Ca uptake by roots in all treatments except at 5 t ha^{-1} slag. No difference was observed in Ca concentration in mycorrhizal and non-mycorrhizal roots at 5 t ha^{-1} slag. Mg concentration differed significantly in mycorrhizal and non-mycorrhizal roots except at 2 t ha^{-1} slag. No differences were found in Cr concentrations of roots and shoots of mycorrhizal and non-mycorrhizal plants harvested on the 112th day except at 10 t ha^{-1} slag concentration. ANOVA results of the linear mixed effects models showed that steel slag amendments to the soil and AMF inoculation had significant effects on the uptake of Fe, Ca, Mg and Cr by barley plants (Table 1).

3.5. *Leachate elemental concentrations*

The concentrations of NO_3, PO_4, Fe, Ca, Mg and Cr were higher in

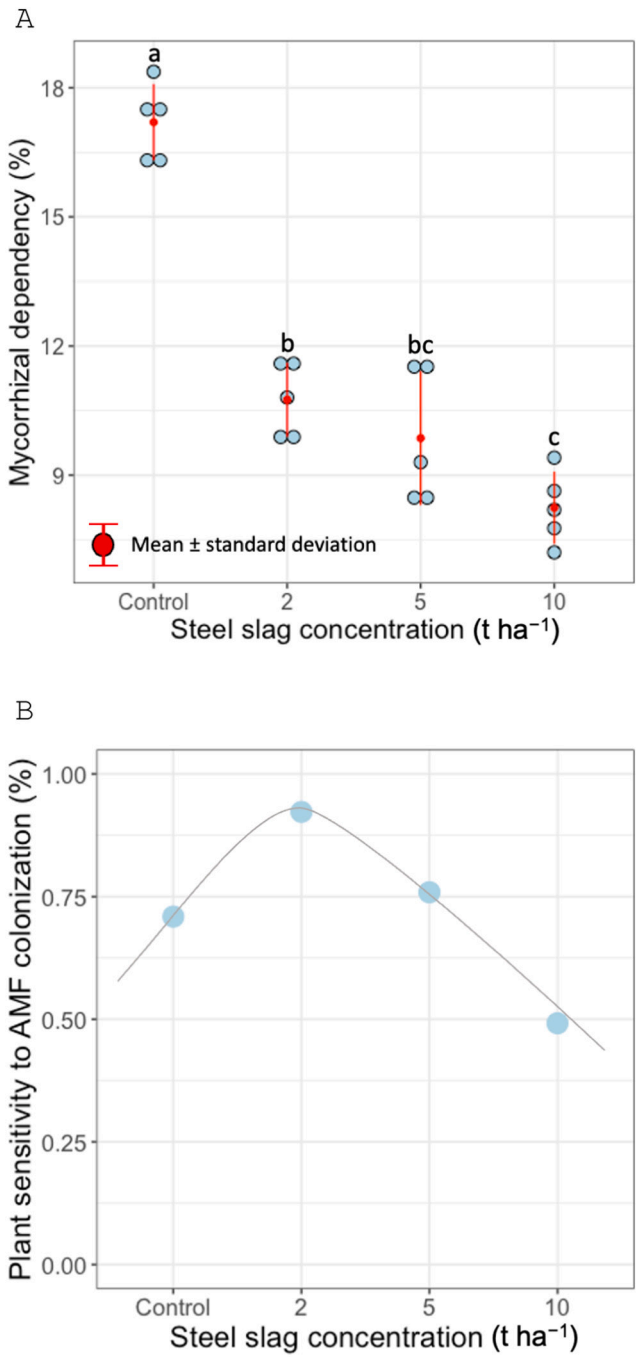


Fig. 3. Effects of steel slag on plant response to AMF. (A) Dot plot showing the mycorrhizal dependency of barley. Mycorrhizal dependency is a measure of the extent to which plants depend on AMF-mediated foraging to attain maximum yield and growth. Each dot denotes one replicate. Red dot with error bars represents the average of five replicates ± standard deviation of the mean. Values that do not share an alphabet are significantly different ($p \leq 0.05$) in a Tukey's HSD post hoc test; and (B) Sensitivity of barley to *R. irregularis* in the four slag treatments. Mycorrhizal sensitivity is the variation in plant growth response to *R. irregularis* in the four tested concentrations of slag.

leachates collected on the 50th day compared to those collected on the 110th day (Fig. 6A–F). In both collections, leachates from pots containing steel slag had higher concentrations of PO_4, Fe, Ca, Mg and Cr than the control. NO_3 was an exception. Leachates collected on the 110th day from the control had higher NO_3 levels than those collected from pots with 2 and 5 t ha^{-1} steel slag (Fig. 6A). Concentrations of the six tested elements were lower in leachates of the mycorrhizal pots compared to the non-mycorrhizal pots. Although, there were a few exceptions. For instance, significant differences were not found in the concentrations of NO_3 in leachates collected on the 50th and 110th days from the mycorrhizal and non-mycorrhizal pots of the control and in leachates collected on the 50th day from pots with 2 t ha^{-1} steel slag (Fig. 6A). Likewise, significant differences in Cr concentrations were not found in the 50th day leachate collections from the mycorrhizal and non-mycorrhizal pots (Fig. 6F). Nevertheless, leachate concentrations of all six elements correlated negatively with the AMF extraradical hyphal length in the rhizosphere (Fig. 7A–F). The linear mixed effects model shows that both steel slag treatment and inoculation with AMF significantly influenced the concentrations of NO_3, PO_4, Fe, Ca, Mg and Cr in the leachates (Table 1).

4. Discussion

4.1. AMF colonisation and plant biomass

Here we simulated the impacts of manipulating agricultural soils with steel slag on AMF ecosystem multifunctionality and risks to human health. Slag addition increased the concentrations of N, P and metals: Fe, Ca, Mg and Cr in the soil (Supplementary S4). The effects of these increased concentrations were reflected in better grain yield and plant biomass but caused cutbacks in percent root mycorrhizal colonisation (Fig. 2A). Slag treatment and AMF inoculation influenced the plant biomass (Table 1). Consequently, mycorrhizal as well as non-mycorrhizal plants grown with steel slag had higher biomass than plants grown in the control.

A comparison of AMF colonisation in roots harvested on the 50th and 112th days shows that the rate of AMF colonisation was slower in pots containing higher concentrations of slag. For instance, percent AMF colonisation in roots of the control harvested on the 50th day did not differ significantly from roots of plants grown with 5 t ha^{-1} steel slag harvested on the 112th day. This indicates that barley grown with slag was more resistant to AMF colonisation compared to the control. Arbuscles are the sites of nutrient exchange between the plant and the AMF (Li et al., 2016; Begum et al., 2019), and a 17.4–11.2 % root arbuscle colonisation in pots containing 2–10 t ha^{-1} steel slag indicates that despite the drop in percent colonisation, nutrient exchange was taking place between the plant and the fungus.

The decline in root AMF colonisation in barley grown with slag must be seen in the context of its mycorrhizal dependency and sensitivity to *R. irregularis*. Mycorrhizal dependency is a measure of the extent to which plants depend on AMF-mediated foraging to attain maximum yield and growth at a particular level of nutrient availability (Kandhasamy et al., 2020). When N and P availabilities in the rhizosphere are not limiting, plants divert photosynthetic C to above ground tissues rather than to the AMF (Treseder et al., 2018; Phillips et al., 2019; Ven et al., 2019). The higher biomass of shoots compared to roots and the lower percent root colonisation and extraradical hyphal lengths in slag manipulated soils is ostensibly an outcome of barley diverting more photosynthetic resources to its biomass than to the AMF and indicates reduced allocation of resources to the fungal symbiont. The decrease in extraradical AMF hyphal lengths from 28.5 in the control to 11.7 m g^{-1} in pots with 10 t ha^{-1} steel slag is possibly a fallout of higher N and P availability.

We did not find any correlation between mycorrhizal dependency and the sensitivity of barley to *R. irregularis* colonisation (Fig. 4D). Diminishing cost-benefit ratio of maintaining AMF symbiosis in nutrient rich soils might result in a low or even negative plant sensitivity to AMF colonisation (Reynolds et al., 2005; Raven et al., 2018; Romero et al., 2023). Barley generally has low sensitivity to mycorrhizal colonisation (Thirkell et al., 2021). This declines further in nutrient rich substrates (Goswami et al., 2023a). Mycorrhizal sensitivity in the four treatments was <1 % (Fig. 3B). This low range of mycorrhizal sensitivity could be a reason for the lack of correlation with mycorrhizal dependency (Romero

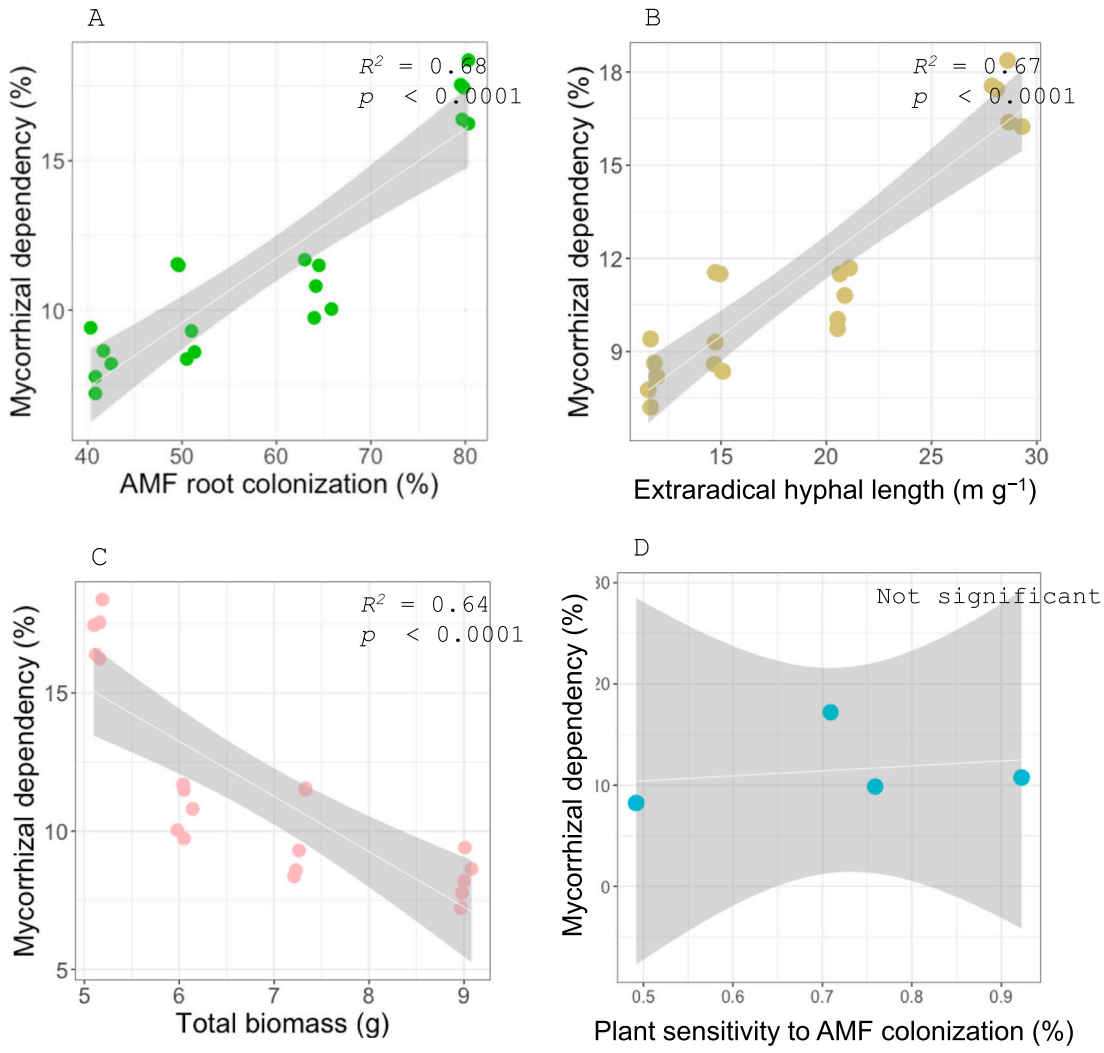


Fig. 4. Relationship between mycorrhizal dependency and (A) AMF root colonisation; (B) extraradical hyphal length; (C) total biomass of barley; and (D) plant sensitivity to AMF colonisation in the four slag treatments. Mycorrhizal dependency is a measure of the extent to which plants depend on AMF-mediated foraging to attain maximum yield and growth. Plant sensitivity to AMF is the variation in growth response to *R. irregularis* in the four tested concentrations of slag.

et al., 2023).

The mycorrhizal dependency of barley was only 17.2 % even in pots with no slag. Being sourced from an active agricultural field that had received fertilisation prior to crop sowing, the soils used here had high concentrations of both N and P even without slag addition (Supplementary S4). Plant mycorrhizal dependency is a function of nutrient availability and root architecture (Zhang et al., 2021; Goswami et al., 2023a; Romero et al., 2023). Barley has fibrous roots. In nutrient rich soils, the large surface area to volume ratio that fibrous roots provide makes the C cost of nutrient acquisition directly by the roots lower than through AMF (Raven et al., 2018; Ven et al., 2019; Treseder, 2013). Indeed, AMF become a parasitic carbon drain for plant hosts in soils with high nutrient availability (Reynolds et al., 2005). This reduces the symbiotic growth benefits from AMF and results in lowered mycorrhizal dependencies. The drop in mycorrhizal dependency of barley in pots containing slag, from 10.7 % in 2 t ha^{-1} to 8.2 % in 10 t ha^{-1} respectively, is possibly a fallout of the increased availability of N and P in these pots (Supplementary S4). The negative correlation of mycorrhizal dependency with total biomass (Fig. 4C) is a further indictment of the lowered percent growth benefit incurred by mycorrhizal compared to the non-mycorrhizal barley.

4.2. Nutrient uptake

Slag treatment as well as AMF inoculation contributed to nutrient transfer to above, and below ground tissues of barley (Table 1). Steel slag addition to soils increased uptake of N, P and metals in grains and plant tissues in both mycorrhizal as well as the non-mycorrhizal plants.

Transfer of nutrients and metals to edible grains in higher concentrations than in the non-mycorrhizal plants implicate a role for AMF in the human food chain (Goswami et al., 2023a). The grain concentrations of Cr, which we used here as a representative heavy metal, were respective 0.0027 and 0.0018 mg g^{-1} in mycorrhizal and non-mycorrhizal plants grown at 10 t ha^{-1} slag treatment. This is within the WHO recommended limits (FAO/WHO, 2001; WHO/FAO, 2007). Fe, Ca and Mg which are important micronutrients in low concentrations but toxic at higher concentrations were also within WHO permissible limits. However, depending on the impurities present in the ore, slag addition could introduce other toxic pollutants to the soil. AMF have been shown to amplify transfer of such pollutants to the human food chain (Goswami et al., 2023a).

Importantly, concentrations of these elements were higher in the below ground tissues than in the shoots (Supplementary S3). We have shown previously that in barley the concentrations of nutrients and metals localised in the roots are higher than in shoots or grains

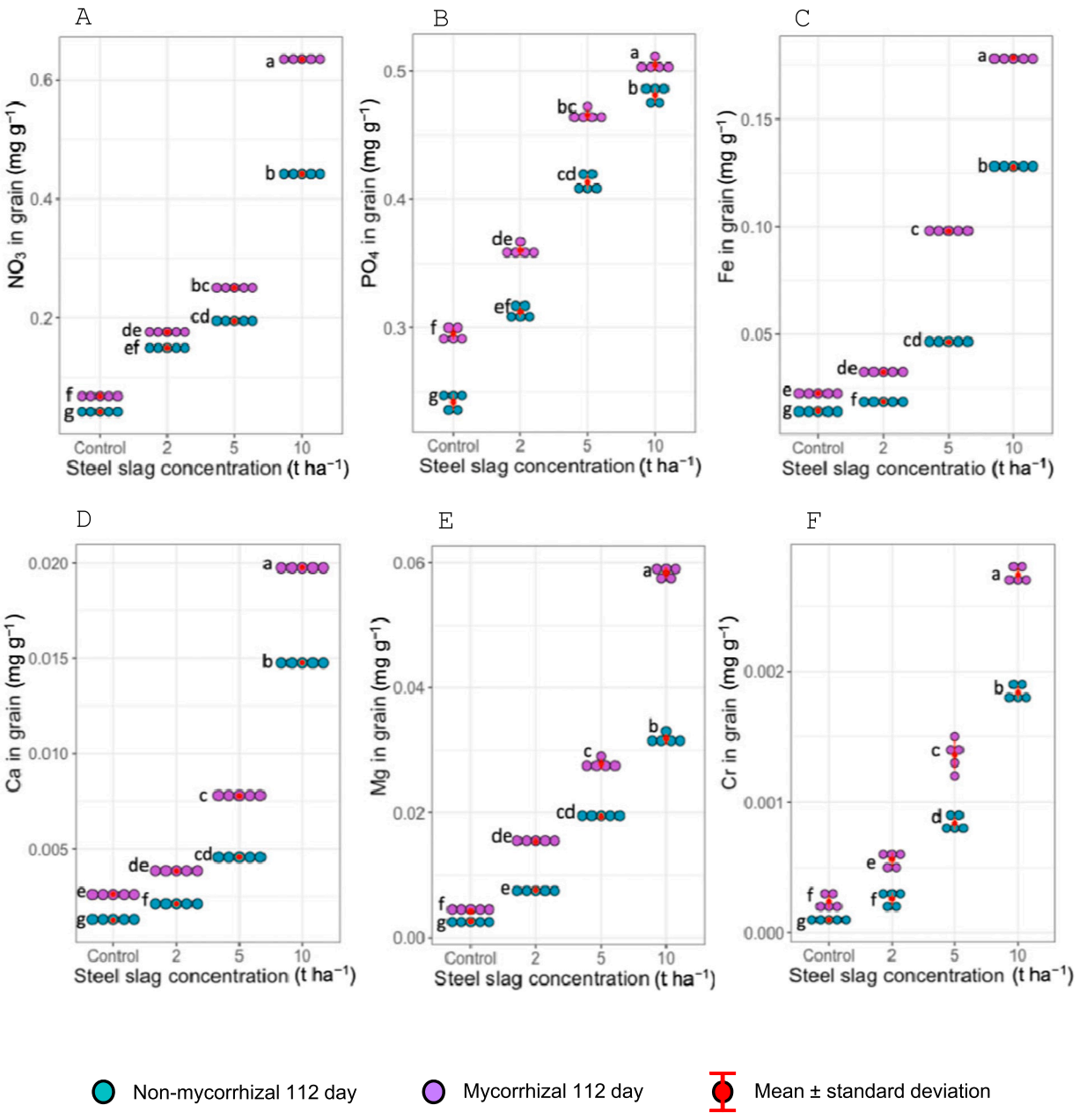


Fig. 5. Nutrient concentrations in grains of barley grown with steel slag. (A) $NO_3 - N$; (B) $PO_4 - P$; (C) Fe; (D) Ca; (E) Mg; and (F) Cr. Each dot denotes one replicate. Red dot with error bars represents the average of five replicates ± standard deviation of the mean. Values that do not share an alphabet are significantly different ($p \leq 0.05$) in a Tukey's HSD post hoc test.

(Goswami et al., 2023a). An important fallout of this was that N, P, and the metals were being extracted from the soil solution. However, higher elemental concentrations in the mycorrhizal plants, particularly those grown with 10 t ha^{-1} steel slag implies, as mentioned, that AMF were contributing to nutrient uptake of barley.

Even though the AMF symbiosis is fundamentally nutritional, where P and other nutrients are exchanged for photosynthetic C with plants, interactions of the extraradical hyphae with the abiotic environment impact ecosystem function (Powell and Rillig, 2018). The spread of extraradical hyphae changes the soil physical properties by forming macroaggregates and increasing the surface absorption capabilities (Smith and Read, 2008; Simard et al., 2012; Leifheit et al., 2014). This benefits the plants by expanding the nutrient interception areas (Bender et al., 2015; Xiao and Chen, 2022). The extraradical hyphae are particularly relevant in reducing nutrient loss as well as pollutant movement to underground aquifers via leaching (Cavagnaro et al., 2015; Martínez-García et al., 2017; Goswami et al., 2023b). The AMF extraradical hyphae were extracting N, P, Fe, Ca, Mg and Cr from the leachates in higher amounts than the plant roots (Fig. 6A–F). Indeed, the concentrations of these elements in the leachates correlated negatively with lengths of the AMF extraradical hyphae (Fig. 7A–F). Nutrient and pollutant transfer from soils to aquifers threaten ecosystem health by making soils unproductive and has adverse environmental consequences. Plants and associated mycorrhizas reduce losses through leaching by retaining dissolved minerals in their biomass (Köhl and van der Heijden, 2016; Goswami et al., 2023b). Moreover, AMF can extract harmful pollutants such as Cr, Ni, Co and Pb from the substrate (Goswami et al., 2023a). The significantly lower concentrations of N, P and the metals Fe, Ca, Mg and Cr in leachates collected from the mycorrhizal pots compared to the non-mycorrhizal ones are indicative of the vital role of AMF in maintaining the quality and health of agricultural soils.

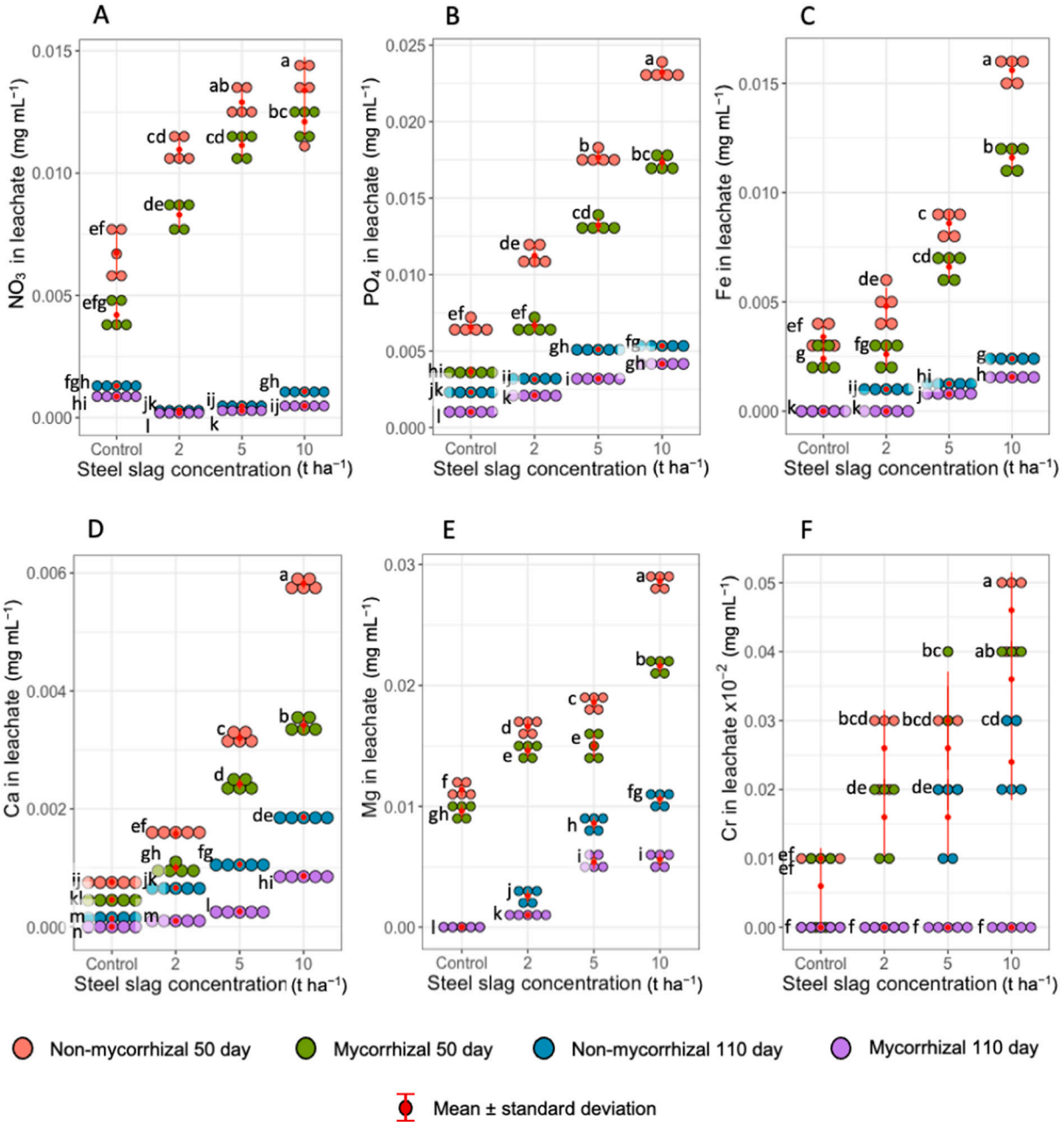


Fig. 6. Loss of nutrients in leachates. (A) NO_3 − N; (B) PO_4 − P; (C) Fe; (D) Ca; (E) Mg; and (F) Cr. Each dot denotes one replicate. Red dot with error bars represents the average of five replicates ± standard deviation of the mean. Values that do not share an alphabet are significantly different ($p \leq 0.05$) in a Tukey's HSD post hoc test.

Therefore, ecosystem and human health implications need thorough evaluation prior to application of industrial by-products as fertiliser alternatives to agricultural soils. The principal concern of amending soils with slag is the potential for accumulation of heavy metals. Trace concentrations of heavy metals in slag do not pose immediate environmental risks (Gwon et al., 2018) but long-term application may proffer human health risks due to heavy metal accumulation in soil (Das et al., 2019).

Our observations show that unlike other industrial wastes applied to agriculture like fly ash which completely inhibited AMF function (Goswami et al., 2023a), slag addition reduced plant dependence on AMF but did not block AMF functionality. Moreover, the concentrations of slag used in this study were higher than the 2–8 t ha^{-1} tested in agriculture (Deus et al., 2019). Whether AMF can maintain their function at even higher concentrations of slag, and whether steel slag addition influences AMF diversity needs further investigating with long-term experimentation.

CRediT authorship contribution statement

Vikrant Goswami: Validation, Methodology, Investigation, Data curation. **Sharma Deepika:** Writing – review & editing, Writing – original draft, Formal analysis, Conceptualization. **Pulkit Sharma:** Methodology, Investigation. **David Kothamasi:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial

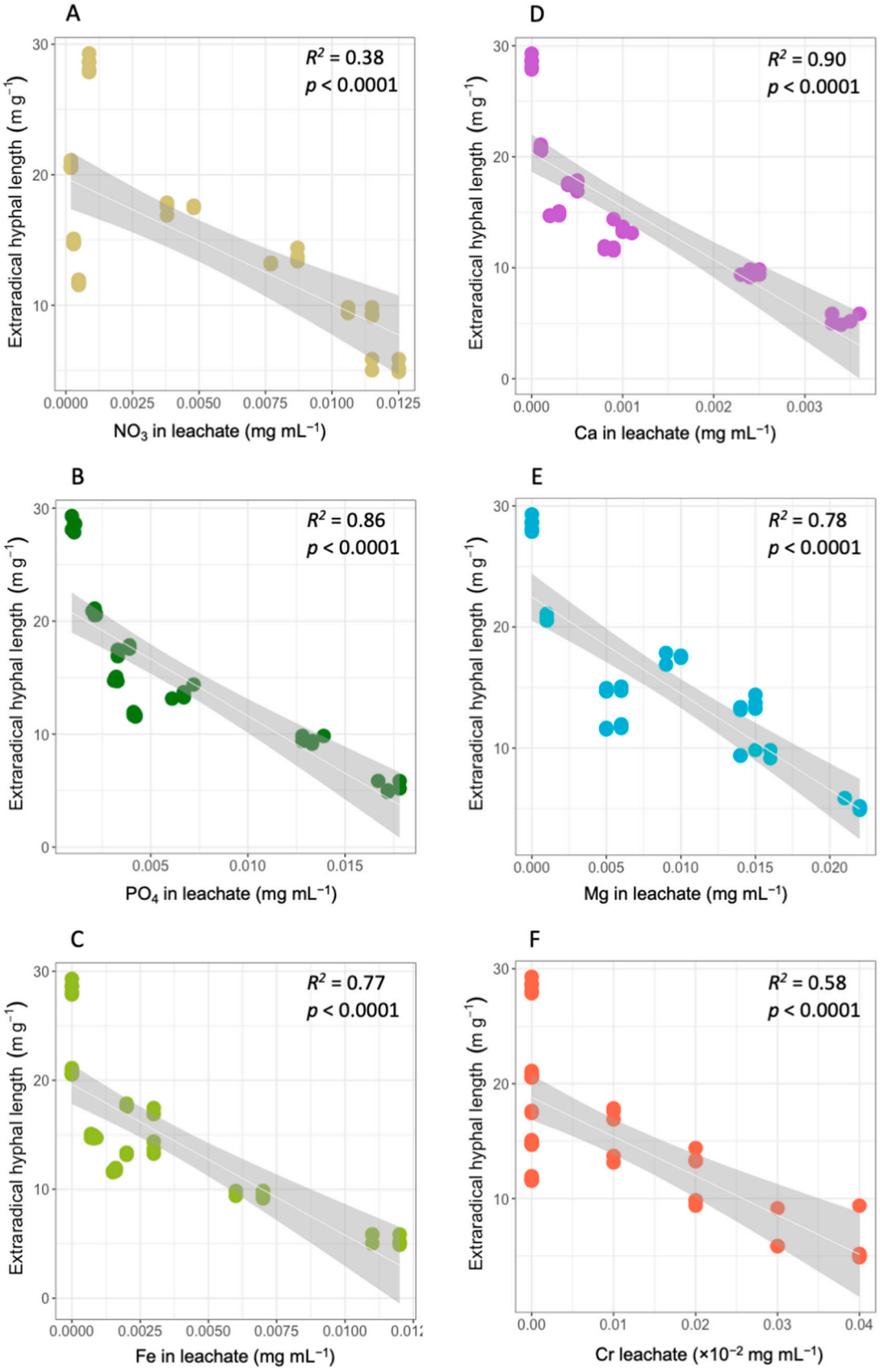


Fig. 7. Relationship between AMF extraradical hyphal length and loss of (A) NO_3 − N; (B) PO_4 − P; (C) Fe; (D) Ca; (E) Mg; and (F) Cr from the substrate via leaching.

interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

Authors thank Drs. G. Abraham, Livleen Shukla and Bhupender Singh of the Indian Agricultural Research Institute, New Delhi for providing industrial grade steel slag used in this study; Prof. Stephane Declerck for *R. irregularis* MUCL 41833; and the anonymous Referees for comments that improved the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.176750.

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