



International Journal of Research in Agronomy

E-ISSN: 2618-0618
P-ISSN: 2618-060X
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NAAS Rating (2025): 5.20
www.agronomyjournals.com
2025; 8(12): 65-68
Received: 04-11-2025
Accepted: 06-12-2025

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Root-induced changes in rhizosphere pH and their effect on micronutrient availability in calcareous soils

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DOI: <https://www.doi.org/10.33545/2618060X.2025.v8.i12a.5022>

Abstract

Nearly 30% of the world's arable soils are calcareous, and micronutrient deficiency on these soils limits crop productivity across South and Southeast Asia. A pot experiment at Kasetsart University, Bangkok, Thailand assessed rhizosphere pH changes induced by five crop species—rice, maize, soybean, sunflower, and mung bean—and their effects on DTPA-extractable Fe, Mn, Zn, and Cu in a calcareous Vertisol (pH 7.83, free CaCO₃ 12.4%). Plants were grown for 45 days in a rhizobox system allowing separate collection of rhizosphere (0-2 mm from root surface) and bulk soil. All crops acidified the rhizosphere, but legumes caused the largest pH drops: mung bean lowered rhizosphere pH to 6.42 and soybean to 6.53. DTPA-extractable Zn rose by 47.3% in the mung bean rhizosphere and 39.8% in soybean compared to bulk soil. Extractable Fe and Mn increased similarly. These results indicate that including legumes in calcareous soil rotations may improve micronutrient availability to subsequent cereal crops through residual rhizosphere acidification effects.

Keywords: Rhizosphere pH, micronutrient availability, calcareous soils, root exudates, legumes, DTPA-extractable, rhizobox, iron deficiency, zinc bioavailability, crop rotation

Introduction

About 1.5 billion hectares of agricultural land worldwide contain free calcium carbonate, buffering soil pH above 7.5 and sharply reducing the solubility of iron, manganese, zinc, and copper ^[1, 2]. On such soils, total micronutrient content may be adequate, yet plant-available fractions remain below critical thresholds because high pH drives precipitation and adsorption reactions ^[3].

Plant roots actively modify the chemistry of the narrow soil zone they inhabit. Proton release during cation uptake, organic acid exudation, and CO₂ from root respiration can lower rhizosphere pH by 0.5-2.0 units relative to bulk soil ^[4, 5]. Legumes, which assimilate atmospheric N₂ and take up more cations than anions, tend to produce stronger acidification than cereals ^[6]. This localised pH drop increases the dissolution of carbonate-bound and oxide-bound micronutrients, raising their availability at the root surface ^[7].

Despite a considerable body of work in temperate calcareous soils, few controlled experiments have compared rhizosphere acidification intensity among tropical crops grown on the same calcareous material. This research used a rhizobox approach to quantify root-induced pH gradients and their consequences for DTPA-extractable Fe, Mn, Zn, and Cu across five crop species in a Thai calcareous Vertisol.

Research Area Description

The experiment was conducted in the net house and soil chemistry laboratory at the Faculty of Agriculture, Kasetsart University, Bangkhen Campus, Bangkok (13°50'N, 100°34'E). The calcareous soil was collected from Saraburi Province, approximately 110 km northeast of Bangkok, a region where limestone-derived Vertisols are common under sugarcane and upland crop cultivation.

Materials and Methods

Materials

Bulk soil (0-30 cm) was air-dried, sieved to 2 mm, and packed into rhizobox compartments.

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Five crops were tested: rice (KDML 105), maize (Suwan 5), soybean (Chiang Mai 60), sunflower (Pacific 77), and mung bean (Kamphaeng Saen 2). Seeds were surface-sterilised in 1% NaOCl for 10 min, rinsed, and pre-germinated for 48 h before transplanting. Each rhizobox (20 × 15 × 25 cm) had a root compartment separated from soil compartments by 30 µm nylon mesh, permitting water and solute exchange but restricting root penetration [8]. Plants received a basal solution of 40 mg N, 20 mg P, and 20 mg K per kg soil; no micronutrient fertiliser was added.

Methods

The trial followed a completely randomised design with five crop treatments and four replications, conducted between January and March 2024. After 45 days of growth, soil was sampled at four distances from the root surface: 0-2, 2-4, 4-6,

and 6-10 mm, plus bulk soil (>20 mm). Soil pH was measured in 1:2.5 soil-water suspension. DTPA-extractable Fe, Mn, Zn, and Cu were determined following the method of Lindsay and Norvell [9]. Organic acids in rhizosphere soil solution were analysed by HPLC [10]. Data were subjected to ANOVA and means separated by Duncan's multiple range test at $p \leq 0.05$.

Soil Quality Analysis

The Saraburi Vertisol had pH 7.83, EC 0.38 dS m⁻¹, organic matter 1.92%, free CaCO₃ 12.4%, CEC 34.7 cmol kg⁻¹, and clay content 52.3%. Initial DTPA-extractable concentrations were Fe 3.87, Mn 6.21, Zn 0.62, and Cu 1.14 mg kg⁻¹—Fe and Zn both below established critical levels of 4.5 and 0.8 mg kg⁻¹ respectively [9].

Results

Table 1: Rhizosphere pH (0-2 mm) and DTPA-extractable micronutrients as affected by crop species

Crop	pH (0-2 mm)	Fe (mg kg ⁻¹)	Mn (mg kg ⁻¹)	Zn (mg kg ⁻¹)	Cu (mg kg ⁻¹)
Rice	7.12	5.43	8.72	0.78	1.31
Maize	6.89	6.18	9.47	0.84	1.39
Soybean	6.53	7.91	11.83	1.02	1.58
Sunflower	6.71	7.14	10.38	0.93	1.47
Mung bean	6.42	8.37	12.41	1.09	1.63
Bulk soil	7.83	3.87	6.21	0.62	1.14
LSD (0.05)	0.18	0.74	1.13	0.11	0.14

All five crops significantly lowered rhizosphere pH compared with bulk soil (Table 1). Mung bean produced the strongest acidification (pH 6.42, a drop of 1.41 units), followed by

soybean (6.53). DTPA-extractable Zn in the mung bean rhizosphere (1.09 mg kg⁻¹) was 76% higher than bulk soil, while Fe rose by 116%.

Table 2: Organic acid concentration (µmol g⁻¹ soil) in rhizosphere (0-2 mm) of five crop species

Crop	Citric	Malic	Oxalic	Succinic	Total
Rice	1.23	0.87	0.64	0.31	3.05
Maize	1.78	1.14	0.82	0.43	4.17
Soybean	3.42	2.31	1.27	0.78	7.78
Sunflower	2.67	1.83	1.09	0.56	6.15
Mung bean	3.89	2.58	1.41	0.84	8.72
LSD (0.05)	0.48	0.32	0.21	0.12	0.89

Legumes exuded substantially more organic acids than cereals (Table 2). Mung bean had the highest total organic acid concentration (8.72 µmol g⁻¹), dominated by citric acid (3.89

µmol g⁻¹). Citric acid was the main organic acid across all species.

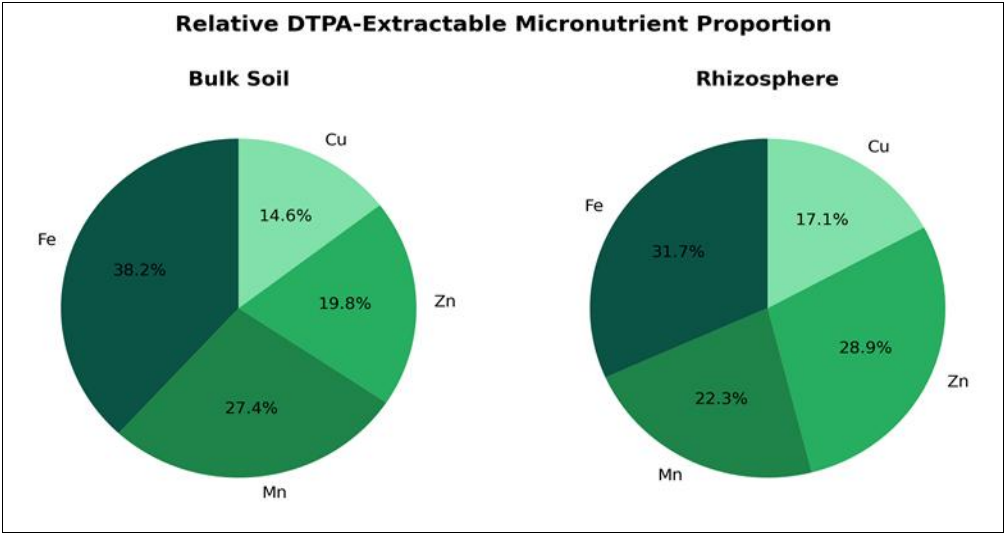


Fig 1: Relative proportion of DTPA-extractable micronutrients in bulk soil versus rhizosphere (averaged across crops).

The relative share of Zn among extracted micronutrients increased from 19.8% in bulk soil to 28.9% in the rhizosphere

(Figure 1), indicating that Zn availability responded more strongly to pH reduction than Fe or Mn on a proportional basis.

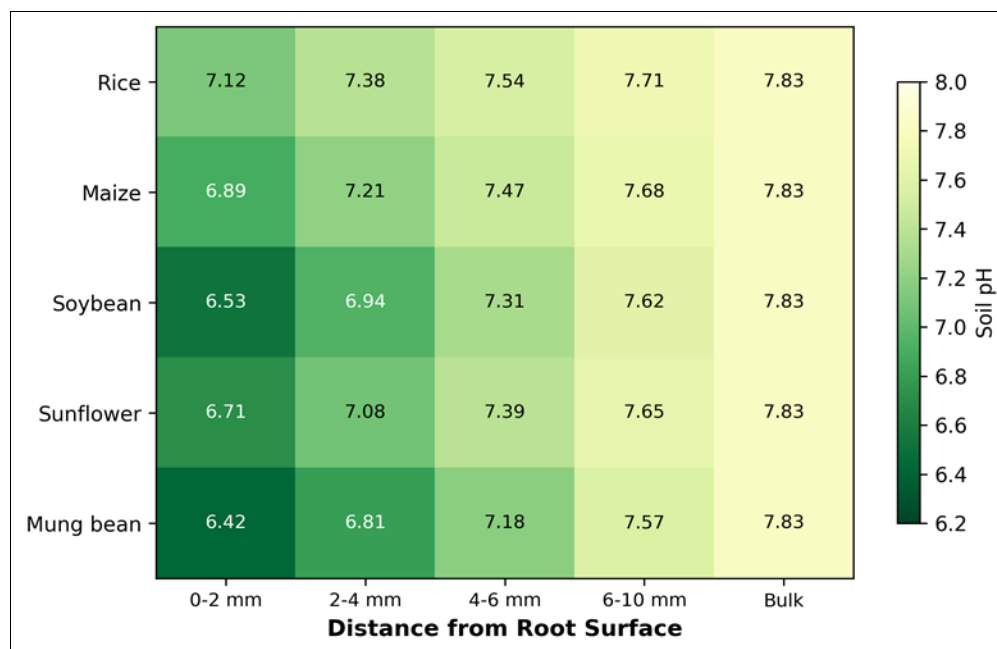


Fig 2: Soil pH gradient from root surface to bulk soil for five crop species in calcareous Vertisol.

The pH gradient was steepest within the first 2 mm and flattened beyond 6 mm (Figure 2). Mung bean and soybean maintained acidified zones extending further from the root surface than rice or maize.

Comprehensive Interpretation

Pearson correlation between rhizosphere pH (0-2 mm) and DTPA-Zn yielded $r = -0.91$ ($p < 0.01$), confirming the strong inverse relationship. Total organic acid concentration correlated with the pH drop ($r = 0.87$, $p < 0.01$), supporting organic acid exudation as a primary acidification mechanism on this calcareous soil.

Discussion

The magnitude of rhizosphere acidification observed here—up to 1.41 pH units for mung bean—falls at the upper end of values reported in the literature. Hinsinger *et al.* [4] noted that legumes commonly lower rhizosphere pH by 0.5-1.5 units in calcareous soils due to excess cation uptake during N_2 fixation. The strong correlation between total organic acids and pH change supports the dual mechanism of proton extrusion plus organic acid chelation [11, 12].

From a practical standpoint, these results reinforce the agronomic value of including legumes in rotations on calcareous soils. Residual acidification from a legume crop may persist long enough to benefit a succeeding cereal. Singh *et al.* [13] showed that rhizosphere pH effects lasted 15-20 days after legume harvest in a calcareous Indian soil, sufficient to overlap with early growth of a following crop.

The disproportionately large response of Zn to pH reduction (47% increase for mung bean versus 38% for Mn) is consistent with the steep solubility curve of zinc carbonate between pH 7.0 and 8.0 [14]. This suggests that even modest pH reductions on calcareous soils can substantially improve Zn nutrition.

Conclusion

Root-induced acidification significantly lowered rhizosphere pH and improved DTPA-extractable Fe, Mn, Zn, and Cu in a

calcareous Vertisol. Legumes, particularly mung bean and soybean, caused the greatest pH reductions through organic acid exudation and proton release. DTPA-extractable Zn increased by up to 76% in the mung bean rhizosphere relative to bulk soil. These findings support the inclusion of legumes in crop rotations on calcareous soils as a low-cost strategy to improve micronutrient availability for subsequent cereal crops.

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