



Effect of Soil Application of Humic Acid on Nitrogen Availability and Nitrifying Bacteria in the Rhizosphere of Sorghum (*Sorghum bicolor* L.)

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ABSTRACT

Background: Humic acid improves soil fertility, nutrient availability and microbial activity. This study evaluated the effects of different humic acid levels on nitrogen availability and nitrifying bacteria in rhizosphere and outside of rhizosphere soils of sorghum.

Methods: A pot experiment was conducted at the College of Agriculture, University of Al-Qadisiyah, Iraq, during the autumn season of 2025. were planted with *Sorghum bicolor* (L.) Moench on 15 July 2025. Six levels of humic acid (0, 2, 4, 6, 8 and 10 ml L⁻¹) were applied using a completely randomized design (CRD) with three replicates. Sorghum plants were grown in plastic pots containing 20 kg of soil. Available ammonium (NH₄⁺), nitrate (NO₃⁻), numbers of nitrosomonas and nitrobacter, chlorophyll content and plant height were evaluated after 60 and 90 days of planting.

Result: The highest humic acid level (10 ml L⁻¹) significantly increased nitrogen availability and nitrifying bacterial numbers in both rhizosphere and outside of rhizosphere soils. The highest concentrations of ammonium and nitrate reached 49.81 and 55.13 mg kg⁻¹ soil, respectively, in the rhizosphere after 60 days of planting. The same treatment recorded the highest numbers of nitrosomonas and nitrobacter, reaching 19.03 × 10⁵ and 11.26 × 10⁵ cells g⁻¹ soil, respectively. Chlorophyll content and plant height were also significantly improved, reaching 55.7 SPAD units and 96.2 cm after 90 days of planting.

Key words: Humic acid, Nitrification, Nitrobacter, Nitrogen availability, Nitrosomonas, Rhizosphere, Soil fertility, *Sorghum bicolor*.

INTRODUCTION

Humic acid is an organic soil amendment high in organic matter that improves soil fertility through its effects on soil physical, chemical and biological properties. It stimulates root growth, nutrient uptake, enzymatic activity and chlorophyll formation, resulting in improved plant growth and productivity (Nardi *et al.*, 2002; Cimrin and Yilmaz, 2005; El-Ghamry *et al.*, 2009; Akinci, 2011; Ameri and Tehranifar, 2012; Yildiz *et al.*, 2023; Manna and Siddique, 2025).

Recent studies have shown that humic substances can be influence the activity of microorganisms responsible for nitrification and nitrogen mineralization, resulting in improved balance among forms of nitrogen in soil and increased nitrogen availability for plants (Jin *et al.*, 2024).

The rhizosphere is a biologically active zone in which nitrogen transformations are strongly influenced by root exudates and microbial activity. Root-derived carbon compounds stimulate microbial numbers and accelerate nitrogen cycling processes compared with soil outside the rhizosphere (Hu *et al.*, 2021; Miao *et al.*, 2026).

Although the beneficial effects of humic acid on soil fertility and plant growth have been widely reported, further studies are needed to better understand its effects on nitrogen availability and nitrifying bacteria populations in both rhizosphere and outside-rhizosphere soils under sorghum cultivation (Hu *et al.*, 2021; Jin *et al.*, 2024; Miao *et al.*, 2026). Therefore, this study aimed to evaluate the effects of different humic acid levels on ammonium, nitrate,

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nitrosomonas and nitrobacter numbers, as well as chlorophyll content and plant height of sorghum.

MATERIALS AND METHODS

The experiment was conducted in lathhouse of the Department of Soil Sciences, College of Agriculture, University of Al-Qadisiyah, Al-Diwaniyah, Iraq, during the autumn season of 2025. Plastic pots containing 20 kg of air-dried soil were planted with *Sorghum bicolor* (L.) Moench on 15 July 2025. Five seeds were planted per pot

and thinned to one plant after 15 days. Potassium sulfate ($100 \text{ kg K}_2\text{O ha}^{-1}$), triple superphosphate ($200 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$) and urea (320 kg N ha^{-1}) in two additions, the first 15 days after planting and the second 30 days after the first additions, according to the experimental design. Standard irrigation, weed control and pest management practices were carried out uniformly for all treatments. Soil analyses were conducted according to Jackson (1958); Black (1965a, 1965b) and Page *et al.* (1982). Soil samples were collected at 60 and 90 days after planting. Soil adhering to the roots was considered rhizosphere soil, whereas soil collected away from the roots was considered outside of rhizosphere soil. At each sampling period, soil samples were collected from each of the three replicates of every treatment (H_0 - H_5) for both rhizosphere and outside of rhizosphere soil. nitrosomonas and nitrobacter numbers were estimated using the MPN method, chlorophyll content was measured using a SPAD meter and plant height was measured at the flowering stage. Nutritional content of humic acid (according

to the manufacturer) 20% Humicacids and fulvic acid, 25% organic matter, 5% N, 0% P and 10% K. The main physical, chemical and biological characteristics of the soil before planting are presented in Table (1). The humic acid levels and their corresponding treatment symbols used in the experiment are presented in Table (2).

RESULTS AND DISCUSSION

Effect of humic acid on ammonium and nitrate concentration in soil

The results presented in Table (3) showed significant differences among humic acid levels in all rhizosphere and outside of rhizosphere soils during the two sampling periods. All humic acid treatments significantly increased ammonium and nitrate concentrations compared with the control treatment.

The highest ammonium concentration is recording in treatment H_5 during all study periods. After 60 days from

Table 1: Some chemical, physical and biological characteristics of the soil before planting.

Traits	Value	Unit	References
Soil pH (1:1)	7.7	-	Page <i>et al.</i> (1982)
Soil conductivity EC (1:1)	3.39	ds.m ⁻¹	Page <i>et al.</i> (1982)
Cation exchange capacity CEC	12.40	Cmol. charge. Kg ⁻¹ soil	Black (1965b)
Carbonate minerals CaCO ₃	265	g.kg ⁻¹	Page <i>et al.</i> (1982)
Organic matter O.M	13.13		Black (1965b)
Organic carbon	7.62		Black (1965b)
Total phosphorus	97	mg.kg ⁻¹	Page <i>et al.</i> (1982)
Available phosphorus	12.2		Page <i>et al.</i> (1982)
Dissolved phosphorus	0.36		Page <i>et al.</i> (1982)
Total nitrogen	375		Black (1965b)
Available nitrogen in	N-NH ₄	24.50	Black (1965b)
the 2 faces	N-NO ₃	21.40	Black (1965b)
Total potassium	1270		Page <i>et al.</i> (1982)
Available potassium	150		Page <i>et al.</i> (1982)
Available sulfates	260		Black (1965b)
Positive dissolved Ions	Ca	17	mol. charge. L ⁻¹
	Mg	10	Page <i>et al.</i> (1982)
	Na	33	Jacks0 (1958)
	K	2	Page <i>et al.</i> (1982)
Negative dissolved Ions	Cl	43	Jackson (1958)
	SO ₄	21	Black (1965b)
	CO ₃	Nil	Jackson (1958)
	HCO ₃	15	Jackson (1958)
Bulk density		1.27	Megagram. M ⁻³
Soil separators	Sand	260.00	g.kg ⁻¹
	Loam	530.00	
	Clay	210.00	
Soil texture	Silt loam		
Total bacteria	11.58×10^6	CFU.g ⁻¹ dry soil	Black (1965b)
Total fungi	3.10×10^4		
Nitrosomonas	0.11×10^5	Cell.g ⁻¹ soil	Alexander and
Nitrobacter	0.10×10^5		Francis (1965)

planting, ammonium concentrations reached 49.81 and 54.33 mg kg⁻¹ soil in rhizosphere and outside of rhizosphere soils, respectively, compared with 22.22 and 25.54 mg kg⁻¹ soil in treatment H₀, representing increases of 124.16% and 112.72%, respectively. After 90 days, H₅ recorded 41.77 and 45.56 mg kg⁻¹ soil compared with 17.49 and 19.32 mg kg⁻¹ soil in the control treatment, with increases of 138.82% and 135.81%, respectively.

Similarly, nitrate concentrations increased significantly with increasing humic acid levels. Treatment H₅ recorded the highest nitrate concentrations, reaching 55.13 and 57.42 mg kg⁻¹ soil after 60 days and 44.33 and 49.36 mg kg⁻¹ soil after 90 days in rhizosphere and outside of rhizosphere soils, respectively. These values were significantly higher than these of the control treatment, with increases ranging from 112.35% to 131.44%.

The increase in ammonium and nitrate concentrations with increasing humic acid levels indicates improved nitrogen availability and enhanced nitrogen transformation processes in soil. Humic acid promotes microbial activity and improves soil fertility by stimulating nitrogen mineralization and nitrification processes. In addition, humic acid improves soil structure, increases cation exchange capacity (CEC) and enhances nutrient retention, thereby reducing ammonium losses and maintaining a continuous supply of substrate for nitrification thereby increasing nitrogen availability for plant uptake.

These results agree with those reported by Nelson and Huber (2001), who indicated that humic substances improve nitrogen availability by enhancing soil fertility and nutrient retention. Tan (2003) also reported that humic acid stimulates nitrogen transformation processes and improves nutrient uptake by plants. Similarly, Patra *et al.* (2009) observed increased ammonium and nitrate concentrations following humic acid application due to enhanced microbial activity. Al-Taweel and Abo-Tabikh (2019, 2020) found that humic acid stimulated nitrogen mineralization and increased nitrogen availability in soil, while Jarallah and Rahi (2020) reported that enhanced nitrification activity promoted nitrate accumulation in agricultural soils.

Furthermore, Nitrogen concentrations generally decreased at 90 days compared with 60 days, which may be attributed to increasey plant uptake and nitrogen losses through biological and chemical transformation processes. Nitrate concentrations were generally higher than ammonium concentrations, reflecting the nitrifying of activity

Table 2: Humic acid levels used in the study and their symbols.

Symbol	Concentration
H ₀ (Cont.) without addition	0 ml L ⁻¹
H ₁	2 ml L ⁻¹
H ₂	4 ml L ⁻¹
H ₃	6 ml L ⁻¹
H ₄	8 ml L ⁻¹
H ₅	10 ml L ⁻¹

Table 3: Effect of humic acid on N-NH₄⁺ mg kg⁻¹ and on N-NO₃⁻ mg kg⁻¹ ion concentration of rhizosphere soil and outside it for periods 60 and 90 days of planting.

Treatments	NH ₄ ⁺		NO ₃ ⁻		NO ₃ ⁻	
	60 days from planting		90 days from planting		90 days from planting	
	Sampling location		Sampling location		Sampling location	
	Rhizosphere	Outside of rhizosphere	Rhizosphere	Outside of rhizosphere	Rhizosphere	Outside of rhizosphere
H ₀	22.22	25.54	17.49	19.32	23.82	27.04
H ₁	32.66	36.93	24.51	27.83	34.09	38.22
H ₂	37.67	40.55	28.72	33.64	37.73	42.61
H ₃	41.28	45.76	32.88	36.07	44.85	47.53
H ₄	46.74	51.68	37.61	43.25	48.21	54.11
H ₅	49.81	54.33	41.77	45.56	55.13	57.42
LSD (0.05)	2.726	2.334	4.647	10.96	14.08	8.53

microorganisms responsible for the conversion of ammonium to nitrate (Okabe *et al.*, 2010; Jarallah and Rahi, 2020). The simultaneous increase in ammonium and nitrate concentrations suggests that humic acid enhanced the overall nitrogen cycle by stimulating both nitrogen mineralization and nitrification processes.

Table (3) shows that ammonium and nitrate concentrations increased with increasing humic acid levels during all study periods, with the highest values recorded in treatment H5. Nitrogen concentrations decreased at 90 days compared with 60 days and were generally lower in the rhizosphere than outside of rhizosphere soil, which may be due to greater biological activity and nitrogen utilization in the root zone. Nitrate concentrations were generally higher than ammonium concentrations due to nitrification processes mediated by nitrosomonas and nitrobacter.

Nitrosomonas and Nitrobacter bacteria count $\times 10^5$ cells g^{-1} soil

The results presented in Table (4) showed significant increases in nitrosomonas and nitrobacter numbers with increasing humic acid levels in all rhizosphere and outside of rhizosphere soils during the two sampling periods (60 and 90 days after planting). In general, bacterial populations increased progressively with increasing humic acid application and remained consistently higher in the rhizosphere than in the non-rhizosphere throughout the study period, indicating the favorable microbial environment associated with root activity.

Treatment H5 recorded the highest nitrosomonas numbers, reaching 19.03 and 5.94×10^5 cells g^{-1} soil in rhizosphere and outside of rhizosphere soils, respectively, after 60 days, compared with 1.50 and 1.22×10^5 cells g^{-1} soil in the control treatment. After 90 days, the corresponding values reached 12.63 and 1.85×10^5 cells g^{-1} soil compared with 0.43 and 0.20×10^5 cells g^{-1} soil in the control treatment.

A similar response was observed for *nitrobacter*. Treatment H5 produced the highest populations, reaching 11.26 and 5.46×10^5 cells g^{-1} soil in the rhizosphere and non-rhizosphere, respectively, after 60 days. After 90 days, the corresponding values decreased to 1.88 and 1.65×10^5 cells g^{-1} soil, while the control treatment consistently recorded the lowest bacterial numbers throughout the experiment.

The gradual increase in nitrifying bacterial numbers with increasing humic acid levels demonstrates the beneficial role of humic substances in stimulating soil microbial activity. This effect can be due to improved organic carbon availability, enhanced nutrient retention, better soil structure and more favorable physicochemical conditions, all of which promote microbial growth and metabolic activity. In addition, humic acid enhances root development and root exudation, providing a continuous supply of readily available carbon compounds that stimulate the proliferation and activity of beneficial microorganisms,

Table 4: Effect of humic acid on the numbers of *nitrosomonas* and *nitrobacter* ($\times 10^5$ cells g^{-1} soil) in rhizosphere and outside of rhizosphere soils during the study periods (60 and 90 days after planting).

Treatments	Nitrosomonas		Nitrosomonas		Nitrobacter		Nitrobacter	
	60 days from planting		90 days from planting		60 days from planting		90 days from planting	
	Sampling location		Sampling location		Sampling location		Sampling location	
	Rhizosphere	Outside of rhizosphere	Rhizosphere	Outside of rhizosphere	Rhizosphere	Outside of rhizosphere	Rhizosphere	Outside of rhizosphere
H ₀	1.50	1.22	0.43	0.20	0.72	0.23	0.18	0.15
H ₁	5.16	1.88	2.76	0.56	1.66	1.54	0.40	0.26
H ₂	8.33	2.54	3.90	0.92	2.35	1.96	0.58	0.32
H ₃	11.76	3.36	7.72	1.23	5.82	2.68	1.33	1.21
H ₄	16.18	4.71	9.58	1.67	8.43	4.54	1.67	1.43
H ₅	19.03	5.94	12.63	1.85	11.26	5.46	1.88	1.65
LSD (0.05)	0.243	0.193	0.271	0.115	0.190	0.226	0.088	0.107

Table 5: Effect of humic acid levels on chlorophyll content and plant height during the study periods (60 and 90 days after planting).

Treatments	Chlorophyll		Plant height	
	60 days from planting	90 days from planting	60 days from planting	90 days from planting
H ₀	35.4	37.2	48.7	70.2
H ₁	38.2	40.5	53.1	76.4
H ₂	41.7	44.2	57.6	82.3
H ₃	45.6	47.8	61.8	87.9
H ₄	49.3	52.1	66.4	93.5
H ₅	52.4	55.7	71.1	96.2
LSD (0.05)	1.808	2.136	2.621	26.99

particularly within the rhizosphere. humic acid also acts as a source of biologically active organic compounds that provide energy and nutrients for soil microorganisms, thereby stimulating microbial proliferation and enhancing the abundance of nitrifying bacteria in the rhizosphere.

The consistently higher numbers of *nitrosomonas* and *nitrobacter* in the rhizosphere compared with the outside of rhizosphere may be attributed to the greater availability of root exudates, amino acids, sugars and other organic compounds released by plant roots. These compounds serve as important energy sources for soil microorganisms and create a biologically active environment that favors nitrifying bacteria responsible for nitrogen transformations (Innes *et al.*, 2004; Meyhew, 2004).

The increase in *nitrosomonas* and *nitrobacter* populations indicates enhanced nitrification activity and improved nitrogen cycling efficiency in soil. As *nitrosomonas* oxidizes ammonium to nitrite and *nitrobacter* subsequently converts nitrite to nitrate, the increased abundance of these bacteria contributes to greater nitrogen availability for plant uptake. Furthermore, improved soil aeration and aggregation following humic acid application may have enhanced oxygen availability within soil pores. Since nitrification is an aerobic process, these conditions favor the activity of nitrifying bacteria and improve nitrogen transformation efficiency. Similar findings have been reported by Rui *et al.* (2024); Chen *et al.* (2024); Jin *et al.* (2024); Ma *et al.* (2024) and Qin *et al.* (2025). Guo *et al.* (2025); Han *et al.* (2025).

The reduction in bacterial numbers after 90 days compared with 60 days may be associated with the gradual depletion of readily available nutrients, increased competition among soil microorganisms and plant roots and age-related changes in root exudation during plant development, as well as the reduced availability of easily degradable carbon substrates as plant growth progressed, resulting in reduced microbial activity during the later growth stage. Similar explanations have been reported by Smalla *et al.* (2001); Bezbaruah and Zhang (2004) and Saito *et al.* (2007). Overall, the present findings are consistent with those of Tsuchiya *et al.* (2011); Okabe *et al.* (2012); Wu *et al.* (2017), confirming the positive role of humic acid in

enhancing nitrifying bacterial populations and improving biological nitrogen transformations in soil.

Effect of humic acid on chlorophyll content and plant height of sorghum

The results presented in Table (5) showed significant differences among treatments, with chlorophyll content and plant height increasing progressively with increasing humic acid levels. Treatment H₅ recorded the highest chlorophyll content, reaching 52.4 and 55.7 SPAD units after 60 and 90 days from planting, respectively, compared with 35.4 and 37.2 SPAD units in the control treatment, represented increased of 48.02% and 49.73%, respectively.

Similarly, treatment H₅ produced the greatest plant height, reaching 71.1 and 96.2 cm after 60 and 90 days from planting, respectively, compared with 48.7 and 70.2 cm in the control treatment, with increases of 45.99% and 37.03%, respectively.

The improvement in chlorophyll content and plant height may be attributed to the role of humic acid in enhancing nutrient availability and uptake, particularly nitrogen, magnesium and iron, which are directly associated with chlorophyll synthesis and plant growth. Increased chlorophyll content improves photosynthetic efficiency and promotes biomass accumulation, while the growth-regulating effects humic substances stimulate root development and stem elongation (Helaly, 2021; Chen *et al.*, 2022; Zandonadi *et al.*, 2025; Nabi *et al.*, 2025; Al-Musawi *et al.*, 2025). Similar findings were reported by Abu-Ria *et al.* (2024); Abdulmajeed *et al.* (2025) and Al-Bayaty and Raheem (2026).

CONCLUSION

Humic acid application significantly increased ammonium and nitrate availability, enhanced *nitrosomonas* and *nitrobacter* populations and improved chlorophyll content and plant height of sorghum. The highest application level (10 ml L⁻¹) consistently produced the greatest values for all measured parameters in both rhizosphere and outside of rhizosphere soils. These results demonstrate the effectiveness of humic acid in improving nitrogen transformation, microbial activity and soil fertility, thereby supporting sorghum growth under the conditions of this study.

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Disclaimers

The views expressed in this article are those of the authors. The authors are responsible for the accuracy of the information presented.

Informed consent

Not applicable. This study did not involve human participants or experimental animals.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

- Abdulmajeed, A.H., Madhan H.S., Al-Fahdawy M.I.K. and Al-Fahdawi, O.I. (2025). Improving growth and productivity of two maize (*Zea mays* L.) cultivars through foliar application of humic acid under saline soil conditions. *Iraqi Journal of Desert Studies*. **15(2)**: 41-47. <https://doi.org/10.36531/ijds.2025.167372.1108>.
- Abu-Ria, M.E., Elghareeb, E.M., Shukry, W.M., Abo-Hamed, S.A. and Ibraheem, F. (2024). Mitigation of drought stress in maize and sorghum by humic acid: Differential growth and physiological responses. *BMC Plant Biology*. **24**: 514. <https://doi.org/10.1186/s12870-024-05184-4>.
- Akıncı, Ş. (2011). Humic acids, plant growth and nutrient uptake. *Marmara Journal of Pure and Applied Sciences*. **23(1)**: 46-56.
- Al-Bayaty, A.J., Abdl-Rhmaan and Ali, H.R. (2026). Physiological effect of adding concentrations of humic acid on growth and protein percentage of *Zea mays* L. plant genotypes. *Indian Journal of Agricultural Research*. **60(4)**: 539-544. doi: 10.18805/IJARE.AF-1026.
- Alexander, M. and Francis, E.C. (1965). C.I. Nitrifying Bacteria In: Methods of Soil Analysis Part 2. Chemical and Microbiological Properties. [(Eds) Black, C.A. et al.]. P: 1477-1488.
- Al-Musawi, Z.K., Khalaf, H.S.M., Hassouni, A.A., Shakir, R.R., Vona, V. and Istvan, M.K. (2025). Preliminary study on synergistic effects of humic acid and seaweed extract on cereal crop yield and competitiveness with wild weed beets (*Beta vulgaris* L.). *Plants*. **14(24)**: 3770. <https://doi.org/10.3390/plants14243770>.
- Al-Taweel, L.S. and Abo-Tabikh, M.M. (2019). Effect of humic acid, urea and Ammonium Sulphate on Ammonium and *Nitrosomonas* Bacteria and Outside the Rhizosphere of *Zea mays* L. 9th International BHYU al Conference for Sustainable Agricultural Development 4-6 March 2019 Fayoum J. *Agric. Res. and Dev*. **33**. **1(B)**.
- Al-Taweel, L.S. and Abo-Tabikh, M.M. (2020). Effect of humic acid, urea and ammonium sulphate fertilizers on nitrite, nitrate and *Nitrobacter* in the rhizosphere and bulk soils of *Zea mays* L. 10th International Conference for Sustainable Agricultural Development 2-4 March 2020 Fayoum J. *Agric. Res. and Dev*. **34**: 1(B) March, 2020.
- Ameri, A. and Tehranifar A. (2012). Effect of humic acid on nutrient uptake and physiological characteristic *Fragaria ananassa* var. Camarosa. *Journal of Biological and Environmental Sciences*. **6**: 77-79.
- Bezbaruah, A.N. and Zhang, T.C. (2004). pH, redox and oxygen microprofiles in rhizosphere of bulrush (*Scirpus validus*) in a constructed wetland treating municipal wastewater. *Biotechnol. Bioeng*. **88**: 60-70.
- Black C.A. (1965a). Methods of Soil Analysis. Part-1. Physical and Mineralogical Properties. Am. Soc. Agron. Inc. Publisher, Madison Wisconsin., USA.
- Black C.A. (1965b). Methods of Soil Analysis. Part-2. Chemical and Microbiological Properties. Am. Soc. Agron. Inc. Publisher, Madison Wisconsin., USA.
- Chen, J., Zhang, B., Wang, C., Wang, P., Cui, G., Gao, H., Feng, B. and Zhang, J. (2024). Insight into the enhancement effect of humic acid on microbial degradation of triclosan in anaerobic sediments. *Journal of Hazardous Materials*. **461(5)**: 132549.
- Chen, Q., Qu, Z., Ma, G., Wang, W., Dai, J., Zhang, M., Wei, Z. and Liu, Z. (2022). Humic acid modulates growth, photosynthesis, hormone and osmolytes system of maize under drought conditions. *Agricultural Water Management, Elsevier*. **263**: 107447. doi: 10.1016/j.agwat.2021.107447.
- Cimrin, K.M. and Yilmaz I. (2005). Humic acid applications to lettuce do not improve yield but do improve phosphorus availability. *Acta Agriculturae Scandinavica, Section B-Soil and Plant Science*. **55**: 58-63. doi: 10.1080/09064710510008559.
- El-Ghamry, A.M., El-Hai K.M.A. and Ghoneem, K.M. (2009). Amino and humic acids promote growth, yield and disease resistance of faba bean cultivated in clayey soil. *Australian Journal of Basic and Applied Sciences*. **3**: 731-739.
- Guo, Z., Gao, J., Lv, T., Zheng, Y., Deng, C., Sun, X., Sun, Y., Wang, C. and Deng, Y. (2025). Humic acid enhances soil fertility and microbial diversity under optimized nitrogen fertilization in quinoa the rhizosphere. *Plants*. **14(24)**: 3850. <https://doi.org/10.3390/plants14243850>.
- Han, X., Yang, J., Li, Q., Zhang, L., Li, Y., Gai, Y., Sang, Y. and Zhang, Z. (2025). Organic fertilizer application rates affect rhizosphere microbial communities and yield optimization in potato (*Solanum tuberosum* L. V7). *Front. Microbiol.* 06 August 2025 Sec. Microbe and Virus Interactions with Plants. **16**. <https://doi.org/10.3389/fmicb.2025.1651178>.
- Helaly, A.A. (2021). Enhancing the productivity and quality of tomato using magnetized water and humic acid as bio-stimulant agents. *Indian Journal of Agricultural Research*. **55(6)**: 645-655. doi: 10.18805/IJARE.A-616.
- Hu, J., Zhao, Y., Yao, X., Wang, J., Zheng, P. and Xi, C. (2021). Dominance of comammox *Nitrospira* in soil nitrification. *Science of The Total Environment*. **780(1)**: 146558.
- Innes, L., Hobbs, P.J. and Bardgett, R.D. (2004). The impacts of individual plant species on rhizosphere microbial communities in soils of different fertility. *Biol Fertil Soils*. **40**: 7-13.
- Jackson, M.L. (1958). Soil Chemical Analysis. Prentice-Hall. Inc. Englewood. Cliffs, N.J.
- Jarallah, R.S. and Rahi, F.M. (2020). Effect of fertilization type on the concentration of ammonium ion and nitrate in the rhizosphere of *Zea mays* L. *Plant Archives*. **20(1)**: 3216-3220.

- Jin, Y., Yuan, Y., Liu, Z., Gai, S., Cheng, K. and Fan, Y. (2024). Effect of humic substances on nitrogen cycling in soil-plant ecosystems: Advances, issues and future perspectives. *Journal of Environmental Management*. **351**: 119738.
- Ma, Y., Cheng, X. and Zhang, Y. (2024). Impact of humic acid fertilizers on crop yield and nitrogen use efficiency. A meta-analysis. *Agronomy*. **14(12)**: 2763. <https://doi.org/10.3390/agronomy14122763>.
- Manna, T. and Anaytullah, S. (2025). Humic acid and sulphur modulating phenology, membrane integrity, lipid peroxidation and oil yield in *Brassica juncea* under water regimes. *Agricultural Science Digest*. **45(4)**: 613-619. doi: 10.18805/ag.D-6222.
- Meyhew, L. (2004). Humic Substances in Biological Agriculture (online) available at www.Acresusa.com./too/box/reprints/Janou.Humic%20substances.Pdf. (2004).
- Miao, C., Zhu, Y., Wu, X., Yan, Y., Yuan, Q., Hu, Z., Zhong, W., Chen, C., Zhang, T., et al. (2026). Effects of different nitrogen levels on growth and rhizosphere soil microorganisms of *Idesia polycarpa* maxim. *Front. Plant Sci*. **17**: 1743337. <https://doi.org/10.3389/fpls.2026.1743337>.
- Nabi, F., Sarfaraz, A., Kama, R., Kanwal, R. and Huashou, L. (2025). Structure-based function of humic acid in abiotic stress alleviation in plant: A review. *Plants*. **14**: 1916. <https://doi.org/10.3390/plants14131916>.
- Nardi, S., Pizzeghello, D., Muscolo, A. and Vianello, A. (2002). Physiological effects of humic substances on higher plants. *Soil Biology and Biochemistry*. **34**: 1527-1536. doi: 10.1016/S0038-0717(02)00174-8.
- Nelson, D.W. and Huber, D. (2001). Nitrification Inhibitors for Corn Production, Iowa State University, National Corn Hand Book. Neit-55 Electronic July 2001.
- Okabe, S., Oshiki, M. and Kamagata, Y. (2010). A great leap forward in microbial ecology. *Microbes Environ*. **25**: 230-240.
- Okabe, S., Nakamura, Y. and Satoh, H. (2012). Community structure and *in situ* activity of nitrifying bacteria in phragmites root-associated biofilms. *Microbes Environ*. **27(3)**: 242-249.
- Page, A.L., Miller, R.H. and Keeny, D.R. (1982). Methods of Soil Analysis. In: Chemical and Microbiological Properties. Part 2. 2nd Edition. Am. Sco. of Ag. inc., Madison, Wisconsin, USA.
- Patra, D., Usha, D., Chand, S. and Anoar, M. (2009). Use of urea coated with natural products to inhibit urea hydrolysis and nitrification in soil. *Biol. Fertil. Soils*. **45**: 617-621.
- Qin, T., Yang, Z., Dou, Y., Wang, J., Wang, L., Tang, L., Wu, Z. and Rongliang, Q. (2025). Effects of artificial humic acid on rhizosphere ecology and microbial regulation in a ryegrass-*Bacillus cereus* symbiotic system for remediating Cr(VI)-contaminated soil. *Journal of Hazardous Materials*. **5(497)**: 139566. doi: 10.1016/j.jhazmat.2025.139566. Epub 2025 Aug 15.
- Rui, R., Hei, J., Li, Y., Al Farraj, D.A., Noor, F., Wang, S. and Xiahong, H. (2024). Effects of humic acid fertilizer on the growth and microbial network stability of *Panax notoginseng* from the forest understorey. *Scientific Reports*. **14**: 17816. <https://doi.org/10.1038/s41598-024-68949-9>.
- Saito, A., Ikeda, S., Ezura, H. and Minamisawa, K. (2007). Microbial community analysis of the phytosphere using culture-independent methodologies. *Microbes. Environ*. **22**: 93-105.
- Smalla, K., Wieland, G. and Buchner, A. (2001). Bulk and rhizosphere soil bacterial communities studied by denaturing gradient gel electrophoresis: Plant-dependent enrichment and seasonal shifts revealed. *Appl. Environ. Microbiol*. **67**: 4742-4751.
- Tan, K.H. (2003). Humic Matter in Soil and the Environment: Principles and Controversies. New York, Marcel Dekker, Inc., pp: 127-250.
- Tsuchiya, Y., Hiraki, A., Kiriya, C., Arakawa, T., Kusakabe, E. and Morisaki, H. (2011). Seasonal change of bacterial community structure in a biofilm formed on the surface of the aquatic macrophyte *Phragmites australis*. *Microbes. Environ*. **26**: 113-119.
- Wu, H., Wang, X. and He, X. (2017). Effects of selected root exudate components on nitrogen removal and development of denitrifying bacteria in constructed wetlands. *Water*. **9(430)**: <https://doi.org/10.3390/w9060430>.
- Yildiz, B., Tutuncu, A.Ç., Demirkaya, S., Özer, H. and Ugur, A. (2023). The Effects of different growing media and humic acid applications on the growth of tomato plants. *Horticultural Studies*. **40(2)**: 43-48. <http://doi.org/10.16882/HortiS.1279147>.
- Zandonadi, D.B., Monda, H., Gralian, J., James, A., Lamar, R.T. and Santos, M.P. (2025). Humic acids as drivers of plant growth: Regulating root development and photobiology through redox modulation. *Chem. Biol. Technol. Agric*. **12**: 71. <https://doi.org/10.1186/s40538-025-00789-9>.