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Data-Driven Decision Optimization of Humic and Fulvic Acid Treatments: Experimental Evidence on Enzyme Regulation and Nutrient Bioavailability in Calcareous Soils

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Abstract

Purpose: This study explores the influence of humic and fulvic acids on soil enzymatic activities and nutrient bioavailability in calcareous soils, aiming to enhance understanding of their environmental roles in soil management and fertility.

Design/methodology/approach: Four humic substances—fulvic acid (FA), OMRI, SP85, and HS—were applied at varying concentrations (0–1000 mg·kg^{-1}) to calcareous soils. Enzymatic activities (urease, phosphodiesterase, acid and alkaline phosphatase) and the bioavailability of key nutrients (P, K, NH_4^+, Mg, Ca, Cu, Zn) were assessed after 40 and 70 days.

Findings: Humic substances significantly affected soil enzymatic processes and nutrient availability. Urease activity was consistently inhibited, with the strongest effect observed under SP85. Phosphodiesterase and phosphatase activities were notably enhanced, especially under FA and HS treatments. Optimal phosphatase activity was observed at 800 mg·kg^{-1}, while excessive concentrations (e.g., 1000 mg·kg^{-1} of SP85) led to decreased activity. HS treatment induced the greatest increase in phosphomonoesterase activity by day 70. Nutrient responses varied: OMRI improved P and Mg availability; HS increased K and Zn; FA promoted Cu. Calcium levels declined under all humic acid treatments. An application rate of 400 mg·kg^{-1} was found to be optimal.

Practical implications: Appropriate application of humic substances can improve soil biochemical health, supporting efficient nutrient management in high-pH soils.

Originality/value: This study provides novel insights into the enzyme–nutrient interactions influenced by humic substances in calcareous soils. This study also contributes to Decision Sciences by providing a data-driven optimization framework for selecting the most effective type and concentration of humic substances to support evidence-based agricultural decision-making. This research is original in integrating soil biochemical experimentation with quantitative decision modeling, allowing readers to see how data-driven optimization links biological evidence with managerial decision-support. Academics may cite this paper for its methodological synthesis of experimental design and optimization analysis, while practitioners can adopt its findings to enhance real-world nutrient-management efficiency in calcareous soils.

Keywords: Humic acid, Fulvic acid, Soil enzyme, Nutrient bioavailability, Calcareous soil.

JEL classifications: Q15, Q24, Q53, O13, C93

1. Introduction

After undergoing various biogeochemical processes, such as the breakdown and transformation of animal and plant residues by microorganisms, organic compounds known as humus are formed and accumulated (Hwang et al., 2002; Wang & Fujii, 2011). Humus is recognised as one of the most prevalent natural organic substances in the Earth's ecosystem (Shahbazi et al., 2019; Stefanova et al., 2014; Zara et al., 2017). Under specific conditions, it behaves as a charged organic colloid with numerous highly reactive functional groups (Sachs et al., 2010; Vrantsi et al., 2021).

The effective use of humus offers several benefits: (1) enhancing the physical and chemical properties of soil, (2) boosting crop growth, (3) improving crop resilience to stress, and (4) increasing crop yield (Arancon et al., 2006; Doskočil et al., 2014). These advantages have made humic substances a focal point in agricultural research and production, resulting in the availability of various humic fertilisers.

Humus, composed primarily of humic acid, fulvic acid, and humin (Li et al., 2013; Wang et al., 2010; Wu et al., 2021), is known for its chemical diversity and reactivity due to the presence of functional groups such as amino, hydroxyl, quinone, carbonyl, and methoxy groups (Baigorri et al., 2009; Wang et al., 2010). While both humic and fulvic acids are soluble in alkaline solutions, only fulvic acid remains soluble under acidic conditions (Lamar & Talbot, 2009). These characteristics influence how humic substances interact with soil pollutants and nutrients (Erhayem & Sohn, 2013; Sabah & Ouki, 2017; Schmeide et al., 2012). These interactions are critical for understanding soil contamination and remediation, as they determine the mobility and bioavailability of harmful substances (Cavusoglu et al., 2017; Chen et al., 2004; Klucakova, 2014; Martyniuk & Wieckowska, 2003; Szabó, 2004).

In this study, four different humic substances, including both fulvic acid and humic acid, were evaluated for their impact on soil properties (bioavailability of phosphorus, metals, and enzymatic activities) in a calcareous soil. The findings of this study provide information for developing management practices for the optimal utilisation of humic substances in agriculture, especially for high pH soils.

Unlike previous studies that mainly provided descriptive evidence on humic or fulvic acid effects, this study integrates experimental soil biochemistry with quantitative decision modeling to optimize treatment choices. By combining comparative multi-substance experimentation, statistical inference, and regression-based optimization, it advances both theoretical understanding and practical decision support in soil-fertility management. Academics may cite this work for its novel synthesis of biological experimentation and decision analysis, while practitioners can use its findings to guide data-driven management of humic-substance treatments in calcareous soils.

2. Literature Review

2.1. Foundations of Humic Substance Research

Humic substances have long been regarded as one of the most complex and functionally significant fractions of soil organic matter. Pioneering chemists such as Stevenson (1994) and Schnitzer (1986) laid the groundwork for understanding humic and fulvic acids as polyfunctional macromolecules rich in carboxyl, hydroxyl, carbonyl, and phenolic groups. These functional groups confer high cation exchange capacity and enable the chelation of nutrient and trace metal ions, which in turn influence plant nutrition and soil buffering capacity. Early works by Shnitzer and Khan (1972) demonstrated how humic fractions improve soil structure, water retention, and root growth through colloidal interactions. Subsequent studies by Tan (2003) and Swift (1999) confirmed the central role of humic matter in maintaining soil fertility and biochemical stability under varying agro-ecological conditions.

2.2. Structural and Chemical Characteristics

The chemical heterogeneity of humic substances derives from their diverse origins and decomposition pathways. Baigorri et al. (2009), Li et al. (2013),and Mu et al. (2025) characterized the molecular distinction between gray humic acid, brown humic acid, and fulvic acid using spectroscopy, revealing that lower-molecular-weight fulvic acids remain soluble under acidic conditions, whereas humic acids precipitate. This solubility difference governs their transport, adsorption, and reactivity in soils. Wang et al. (2010) further examined how fulvic acid alters metal speciation, particularly of aluminum, in aqueous environments. Erhayem and Sohn (2013) demonstrated that source-dependent structural variability affects adsorption onto nanoparticles, underscoring the environmental relevance of humic chemistry in pollutant binding and nutrient dynamics.

2.3. Role in Soil Enzyme Regulation

Enzymatic regulation by humic materials represents an essential biochemical mechanism influencing nutrient cycling. Classic enzymology studies by Tabatabai and Bremner (1969) and Eivazi and Tabatabai (1977) developed robust assays for phosphatases, while Bremner and Douglas (1973) investigated urease inhibition mechanisms. These foundational works established analytical protocols later adopted in soil biochemical research worldwide. Subsequent investigations revealed that humic substances can either stimulate or inhibit enzyme activities depending on molecular size, functional-group density, and dosage. Chen et al. (2004) attributed the stimulatory effects on plant growth partly to organo-metal complexes facilitating redox reactions. Doskočil et al. (2014) showed that oxidative modification of humic acids can alter enzymatic interactions and electron-transfer efficiency. More recent studies, such as Wu et al. (2021) and Zhang et al. (2023) demonstrated that humic and fulvic acids hinder microplastic weathering and modulate microbial community functions, indirectly influencing enzyme production. Collectively, these findings highlight that the enzymatic response to humic substances depends strongly on concentration, incubation time, and molecular composition.

2.4. Influence on Nutrient Bioavailability

Numerous researchers have emphasized the ability of humic substances to improve macro- and micronutrient availability. Martyniuk and Wieckowska (2003) found that humic acids extracted from lignite effectively adsorb divalent metal ions, reducing their leaching losses. Klucakova (2014) later quantified metal-humic complexation constants, confirming selective affinity toward Ca^{2+}, Cu^{2+}, and Zn^{2+}. Cavusoglu et al. (2017) reported enhanced phosphorus uptake and flower quality in Gladiolus under humic acid treatments. Similarly, Fernández-Escobar et al. (1996) demonstrated that leonardite-derived humic acids increased olive-tree productivity via foliar nutrient absorption. The chelating and buffering properties of humic matter not only increase nutrient solubility but also stabilize soil pH and microbial habitats (Sabah & Ouki, 2017; Vrantsi et al., 2021). These mechanisms are particularly relevant in calcareous soils, where high pH constrains phosphorus and micronutrient availability.

2.5. Humic Substances and Soil–Microbe Interactions

The relationship between humic matter and the soil microbiome has gained attention with the rise of molecular ecology tools. Lumactud et al. (2022) and Canellas et al. (2015) demonstrated that humic molecules act as signaling compounds that promote beneficial microbial proliferation and root–rhizosphere communication. Shan et al. (2023) and Kumari et al. (2023) linked humic amendments to enhanced microbial enzyme expression and nitrogen fixation in alkaline soils. Such microbial stimulation underlies observed increases in phosphatase and dehydrogenase activity, providing a biochemical explanation for the nutrient mobilization effects described in agronomic studies.

2.6. Recent Developments and Decision-Optimization Perspectives

While many agronomic experiments describe qualitative improvements from humic applications, few employ quantitative or data-driven optimization frameworks. Wang and Fujii (2011) refined analytical detection of humic components to support reproducible datasets, enabling parametric modeling. Amoozad and Zahedi (2024) introduced response-surface optimization to estimate optimal humic concentrations for rice production under salinity stress. Alemayehu et al. (2022) applied regression modeling to evaluate synergistic effects of organic amendments on nutrient availability. More recently, Wu et al. (2021) and Vrantsi et al. (2021) combined chemical characterization with environmental modeling to predict sorption and release behavior of humic substances in water–soil matrices. However, a clear linkage between empirical enzyme–nutrient data and statistical optimization remain scarce. Integrating Decision Sciences into soil biochemistry can transform descriptive experiments into prescriptive management tools that optimize resource use, sustainability, and crop yield.

2.7. Identified Research Gap

Despite substantial evidence on the agronomic benefits of humic and fulvic acids, most studies remain descriptive and limited to single-substance or single-rate analyses. Very few have simultaneously compared multiple humic sources across graded concentrations under controlled calcareous-soil conditions while applying statistical models for decision optimization. Moreover, enzyme–nutrient interactions are rarely quantified together in regression frameworks that can inform managerial decisions. Addressing these gaps, the present study integrates experimental soil biochemistry with quantitative optimization modeling to determine the most effective humic-substance type and concentration for enhancing enzyme activities and nutrient bioavailability in high-pH calcareous soils.

3. Theoretical Framework

The theoretical basis of this study lies in enzyme kinetics, nutrient transformation processes, and regression-based optimization for decision making. The integration of these principles supports the interpretation of the biochemical and statistical outcomes of humic and fulvic acid applications in calcareous soils.

3.1. Enzyme Kinetic Principle

Soil enzyme activity follows the basic kinetics of enzyme–substrate reactions, in which humic and fulvic acids can act as activators or inhibitors. The general form of enzyme kinetics is expressed by the Michaelis–Menten equation:

$$v = \frac{V_{max}[S]}{K_m + [S]}, \tag{1}$$

where v is the rate of enzymatic reaction, V_{max} is the maximum velocity, $[S]$ is the substrate concentration, and K_m is the Michaelis constant. Changes in V_{max}or K_m indicate activation or inhibition of enzymatic reactions by humic substances. For example, humic acids may chelate metal cofactors or interact with the enzyme's active site, thereby altering catalytic efficiency.

3.2. Nutrient–Enzyme Transformations

The biochemical functions of urease and phosphatases are directly linked to soil nutrient availability. Urease catalyzes urea hydrolysis to ammonium:

$$Urea + H_2O \xrightarrow{Urease} 2NH_3 + CO_2, \tag{2}$$

where $Urea$ represents the substrate, H_2O is water, $Urease$ is the catalyzing enzyme, NH_3 is ammonia, and CO_2 is carbon dioxide, which is the reaction product of enzymatic urea hydrolysis.

Phosphatase enzymes convert organic phosphorus compounds to orthophosphate:

$$OrganicP \xrightarrow{Phosphatase} H_3PO_4, \tag{3}$$

where $Organic\,P$ refers to organic phosphorus compounds present in soil organic matter, Phosphatase denotes the enzyme catalyzing dephosphorylation, and H_3PO_4 (orthophosphate) is the inorganic phosphorus released and available for plant uptake.

Humic and fulvic acids can enhance these reactions by improving substrate availability and microbial activity or inhibit them by forming complexes with substrates or enzymes. Understanding these mechanisms provides a theoretical explanation for the observed increase in phosphatase activity and inhibition of urease in the experimental results.

3.3. Statistical and Correlation Framework

To quantify relationships among humic treatments, enzyme activities, and nutrient bioavailability, Pearson's correlation coefficient is used:

$$r_{xy} = \frac{\sum(x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum(x_i - \bar{x})^2 \sum(y_i - \bar{y})^2}}, \tag{4}$$

where x_i and y_i are paired observations, and $\bar{x}$ and $\bar{y}$ are their respective means. This equation evaluates the strength and direction of linear relationships between two continuous variables, supporting the interpretation of enzyme–nutrient interactions.

3.4. Regression-Based Decision Model

The study applies a multiple regression framework to estimate the effects of humic type and application rate on enzyme activities and nutrient concentrations:

$$y_i = \beta_0 + \sum_{m=1}^{5} \beta_m D_{rate,m,i} + \beta_6 D_{FA,i} + \beta_7 D_{OMRI,i} + \beta_8 D_{SP85,i} + \beta_9 D_{HS,i} + \varepsilon_i, \tag{5}$$

where y_{i} denotes the enzyme or nutrient response, $D_{rate,m,i}$ represents dummy variables for concentration levels, $D_{FA,i}$, $D_{OMRI,i}$, $D_{SP85,i}$, and $D_{HS,i}$ represent the humic substances, and $\varepsilon_i$is the error term. This model allows estimation of both direct and interaction effects, forming the quantitative basis foroptimizing treatment decisions. The specific equations and methodological details of correlation and regression analyses are described later in Section 4.3.

3.5. Optimization Perspective

From a Decision Sciences viewpoint, identifying the optimal humic-substance concentration involves maximizing beneficial enzyme responses (phosphatase, phosphodiesterase) while minimizing inhibitory effects (urease). The regression coefficients and significance tests serve as indicators for the most effective type and dosage, providing a data-driven approach for resource-efficient soil-fertility management.

4. Methodology

The methodological framework of this study combines biochemical assay procedures with quantitative modeling techniques to analyze the influence of humic and fulvic acids on soil enzyme activities and nutrient bioavailability. Each methodological component is presented below with its key equation, reference source, and rationale for use. This approach ensures that all analytical and computational steps are transparent and scientifically justified.

4.1 Enzyme Activity Determination

The enzyme activities of urease, phosphodiesterase, acid phosphatase, and alkaline phosphatase were determined using well-established colorimetric and spectrophotometric assays. The general expression for enzyme activity was calculated as:

$$A_E = \frac{C_p \times V}{m \times t}, \tag{6}$$

where A_E is enzyme activity (mg product·kg⁻¹·h⁻¹), C_p is the concentration of product formed (e.g., NH_4^+ or p-nitrophenol), V is the extract volume (L), m is the soil mass (kg), and t is incubation time (h). This method follows the principles of Bremner and Douglas (1973), Zantua and Bremner (1975), and Tabatabai and Bremner (1969), ensuring that the enzyme-catalyzed reactions are quantified accurately under standard laboratory conditions. The Beer–Lambert law ($A = \varepsilon lc$) was used to convert absorbance readings into concentration values, linking optical density to enzymatic product formation.

4.2 Nutrient Bioavailability Estimation

The bioavailable concentrations of potassium, phosphorus, ammonium, magnesium, calcium, copper, and zinc were obtained using Mehlich-3 extraction, followed by colorimetric and atomic-absorption quantification. The measured concentration was derived from the calibration equation:

$$C_s = \frac{A_s - A_0}{S}, \tag{7}$$

where C_s is the nutrient concentration (mg·kg⁻¹), $A_s$is the absorbance of the soil extract, A_0 is the blank absorbance, and S is the slope of the calibration curve. This method, adopted from Fernández-Escobar et al. (1996), ensures linearity between absorbance and concentration and provides reliable estimates of extractable soil nutrients.

4.3 Correlation and Regression Modeling

Based on the theoretical model outlined in Section 3.4, this study employed correlation and regression analyses to quantify relationships between humic treatments, enzyme activities, and nutrient bioavailability.

Correlation analysis.: Pearson's correlation coefficient was calculated to evaluate the strength and direction of linear associations between any two continuous variables, enzyme activities, nutrient concentrations, and humic-substance treatments using the equation:

$$r_{xy} = \frac{\sum(x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum(x_i - \bar{x})^2 \sum(y_i - \bar{y})^2}}, \tag{8}$$

where x_i and y_i are paired observations, and $\bar{x}$ and $\bar{y}$ are their respective means. This correlation analysis supports the identification of synergistic or inhibitory interactions among enzymes and nutrients that may result from humic-substance application.

Regression analysis: To estimate the independent and combined effects of humic type and concentration on biochemical responses, an ordinary least squares (OLS) regression framework was applied:

$$y_i = \beta_0 + \sum_{m=1}^{5} \beta_m D_{rate,m,i} + \beta_6 D_{FA,i} + \beta_7 D_{OMRI,i} + \beta_8 D_{SP85,i} + \beta_9 D_{HS,i} + \varepsilon_i, \tag{9}$$

where y_{i} denotes the dependent variable (enzyme activity or nutrient concentration) for sample i; $D_{rate,m,i}$are dummy variables representing humic-substance application rates (0–1000 mg·kg^{-1}); $D_{FA,i}$, $D_{OMRI,i}$, $D_{SP85,i}$, and $D_{HS,i}$are dummy variables for humic-substance type; and ε_i is the error term.

The OLS model was chosen because it provides unbiased coefficient estimates under the Gauss-Markov assumptions and allows for straightforward interpretation of categorical treatment effects. Statistical significance was tested at the 5% level ($p < 0.05$). Diagnostic statistics, including the Durbin–Watson and Jarque–Bera tests, confirmed the absence of serial correlation and the normality of residuals. Although the Durbin–Watson test is primarily designed for time-series data, it was reported here as an additional diagnostic indicator of residual independence within the cross-sectional dataset. The obtained values (approximately 1.7–2.1 across models) indicate no evidence of serial dependence. The regression coefficients (β) indicate the direction and magnitude of each factor's contribution, thereby enabling data-driven optimization of humic-substance type and application rate.

4.4 Rationale for Method Selection

The biochemical equations were selected based on their extensive validation in soil enzymology literature. The urease and phosphatase assays are classical standards in soil biochemistry, ensuring comparability with prior research. The Mehlich-3 extraction method provides a consistent measure of plant-available nutrients in calcareous soils. The correlation and regression equations are fundamental to inferential statistics and were chosen to quantify and optimize the relationships between humic treatments and soil responses. All equations employed in this study are cited from peer-reviewed sources and align with widely accepted theoretical and experimental standards in soil science and decision modeling.

5. Materials and methods

5.1 Data

This study generated experimental data from a controlled pot trial designed to evaluate the effects of humic and fulvic acids on enzyme regulation and nutrient bioavailability in calcareous soil. The dataset comprised observations collected from a completely randomized factorial arrangement involving four humic substances: fulvic acid (FA), OMRI, SP85, and HS. Each was applied at six concentration levels of 0, 200, 400, 600, 800, and 1000 mg·kg^{-1} with four replicates per treatment. Each experimental unit contained 300 g of Krome soil, and measurements were taken on the 40th and 70th days after treatment. In total, the enzyme dataset included 192 observations (4 substances ×

6 rates × 4 replicates × 2 time points), while the nutrient dataset collected on day 70 included 96 observations.

The variables used in the analysis were clearly defined to ensure reproducibility. The independent variables were the type of humic substance (categorical) and the application rate ($mg \cdot kg^{-1}$), which were coded as dummy variables for the regression model. The dependent variables consisted of four enzyme activities: urease (U), phosphodiesterase (PD), acid phosphatase (AP), and alkaline phosphatase (ALP), expressed in $mg \cdot kg^{-1} \cdot h^{-1}$, and seven Mehlich-3 extractable nutrients: potassium (K), phosphorus (P), ammonium (NH_4^+), magnesium (Mg), calcium (Ca), copper (Cu), and zinc (Zn), expressed in $mg \cdot kg^{-1}$. Baseline soil characteristics, including pH 7.3, organic carbon 18.7 $g \cdot kg^{-1}$, and initial nutrient contents, were recorded to describe the starting conditions but were not used as covariates in the statistical models.

All data were verified for completeness, unit consistency, and normal range prior to analysis. No transformation was applied because the measurements were approximately normally distributed. Each treatment was randomly assigned, and replication ensured the estimation of experimental error. The resulting dataset provided a reliable quantitative basis for the subsequent ANOVA, correlation, and regression analyses presented in the following sections.

5.2 Experimental design

Fulvic acid (FA), SP85 (humic acid), OMRI (humic acid), and HS (humic acid) were the humic substances utilised in this study. These substances, FA, SP85, and OMRI, were sourced from commercial suppliers based in the USA. As recommended by the International Humic Substances Society (IHSS), HS was prepared from Inner Mongolia leonardite using the acid-base precipitation method (Fernández-Escobar et al., 1996). To prepare the solution, potassium hydroxide (0.1 $mol \cdot L^{-1}$) was combined with leonardite and left to stand for 24 hours. The mixture was then centrifuged at 25°C for 2 hours, and the supernatant was adjusted to a pH of 7.0 using sulfuric acid (0.1 $mol \cdot L^{-1}$).

The experiment followed a completely randomised design involving four humic substances (FA, SP85, OMRI, and HS), each applied at six concentration levels (0, 200, 400, 600, 800, and 1000 $mg \cdot kg^{-1}$) with four replicates. Krome soil, classified as a loamy, carbonate-rich soil derived from high-temperature weathered debris, was collected from a research farm in Florida at a depth of 0–15 cm. Air-dried soils were sieved to 2 mm. Its basic properties included a pH of 7.3, organic carbon content of 18.7 $g \cdot kg^{-1}$, and Mehlich-3 extractable nutrients: potassium (K) at 93.1 $mg \cdot kg^{-1}$, phosphorus (P) at 97.7 $mg \cdot kg^{-1}$, calcium (Ca) at 20.5 $mg \cdot kg^{-1}$, and magnesium (Mg) at 160.9 $mg \cdot kg^{-1}$.

For each treatment, 300 g of soil was thoroughly mixed with the respective concentration of humic or fulvic acid and placed into plastic containers. Soils were moistened to field capacity using deionised water. The containers were maintained at room temperature (25°C) for 70 days. Water lost to evaporation was replenished weekly based on container weight.

On the 40th and 70th days, two soil samples were taken from each container. For measurement of enzyme activity, one sample was kept moist and stored at 4°C; for chemical analysis, the other was air-dried at room temperature. Quantify the three soil enzymes' activities: 1) phosphomonoesterase, including acid phosphatase and alkaline phosphatase, 2) phosphodiesterase, and 3) urease. A modified approach, described by Bremner and Douglas (1973) and Zantua and Bremner (1975), was employed to measure urease. The photometric methods were used for the assay of phosphomonoesterases and phosphodiesterases (Browman & Tabatabai, 1978; Eivazi & Tabatabai, 1977; Tabatabai & Bremner, 1969).

For measuring nutrient bioavailability, soil samples were extracted using the Mehlich-3 (M-3) extractant. The concentration of phosphorus was determined using the ascorbic acid method and a spectrophotometer. Concentrations of potassium, calcium, copper, and zinc were measured using atomic absorption spectrophotometry. Ammonium nitrogen was quantified using the indophenol blue colorimetry method.

Statistical analysis was performed using SAS software (version 9.4). One-way analysis of variance (ANOVA) was performed to evaluate the treatment effects. Treatment means were compared using Tukey's Honestly Significant Difference (HSD) test at a 5% significance level ($p < 0.05$). Statistical groupings from Tukey's test are shown in the figures using letters (a, b, c) to indicate significant differences between treatments. From a Decision Sciences perspective, this experimental design establishes a quantitative basis for optimizing treatment decisions, integrating statistical analysis and regression modeling to guide resource-efficient soil management strategies.

6. Results and discussion

6.1. The activity of soil enzymes with humic substances on the 40th day

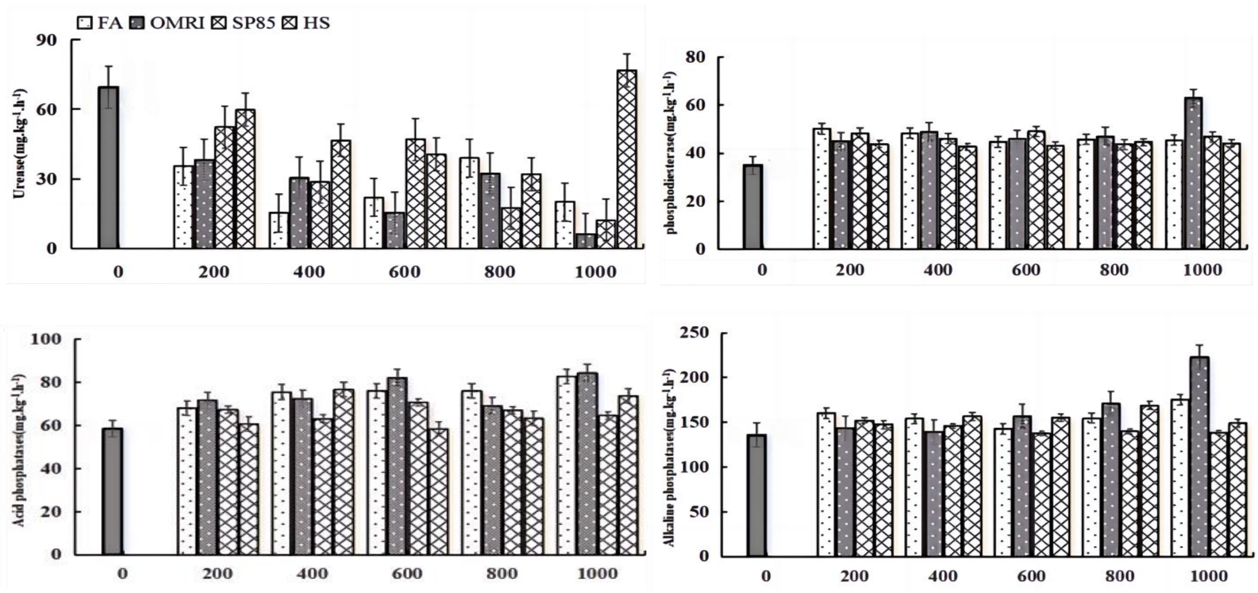
Humic acids play a pivotal role in enzyme functionality as they (1) constitute a significant component of soil organic matter and (2) may influence the stability or inhibition of enzyme activity, as shown in Figure 1. For urease activity, the control treatment exhibited a value of 69.49 mg·kg^{-1}·h^{-1} compared to the FA treatment, which showed a reduced value of 15.33 mg·kg^{-1}·h^{-1}. Similarly, treatments with OMRI, SP85, and HS also demonstrated a reduction in urease activity. Regarding phosphodiesterase activity, its highest level was observed in the OMRI treatment at an application rate of 1000 mg·kg^{-1}. The maximum values for FA, SP85, and HS were recorded at 50.17, 49.06, and 44.61 mg·kg^{-1}·h^{-1}, respectively, all of which exceeded the control treatment value of 35.04 mg·kg^{-1}·h^{-1}. For phosphatase activity, there was an increase with FA, OMRI, SP85, and HS treatments at lower application rates of 200 and 400 mg·kg^{-1}. This activity reached a maximum value of 82.18 mg·kg^{-1}·h^{-1} at the higher application rate of 800 mg·kg^{-1}. Alkaline phosphatase activity showed its greatest value under the FA treatment (175.14 mg·kg^{-1}·h^{-1}) compared to OMRI (222.9 mg·kg^{-1}·h^{-1}) or HS (168.0 mg·kg^{-1}·h^{-1}).

The results demonstrate that fulvic acid and humic substances had obvious effects on the activities of acid and alkaline phosphatase, especially at high application rates, such as FA and OMRI. However, at the higher application rate of 1000 mg·kg^{-1} under SP85 treatment, the phosphatase activity decreased to 138.29 mg·kg^{-1}·h^{-1}. Generally, urease activity was inhibited by fulvic acid and humic substances, with SP85 showing the strongest effect due to its high humic acid content. This inhibitory effect can be attributed to the interaction of humic substances with urease or its substrates.

Humic molecules may block active sites of the urease enzyme or form complexes with urea, thereby reducing substrate availability. In addition, the acidic functional groups in humic substances might alter the microenvironment around urease, negatively affecting its catalytic efficiency.

In contrast, phosphatase and phosphodiesterase activities were significantly enhanced under FA and HS treatments on the 40th day compared to the control. This stimulation could be attributed to the ability of humic substances to provide organic substrates and improve microbial activity, both of which support enzyme production. Moreover, humic substances can chelate inhibitory metal ions and improve substrate accessibility through changes in soil structure and nutrient solubility.

Figure 1. Soil Enzyme Activities with Humic Substances on Day 40.



Note: Evaluated enzymes include urease, phosphodiesterase, acid phosphatases, and alkaline phosphatases, using four humic substances (FA, OMRI, SP85, and HS) at six application rates (0, 200, 400, 600, 800, and 1000 $mg\cdot kg^{-1}$).

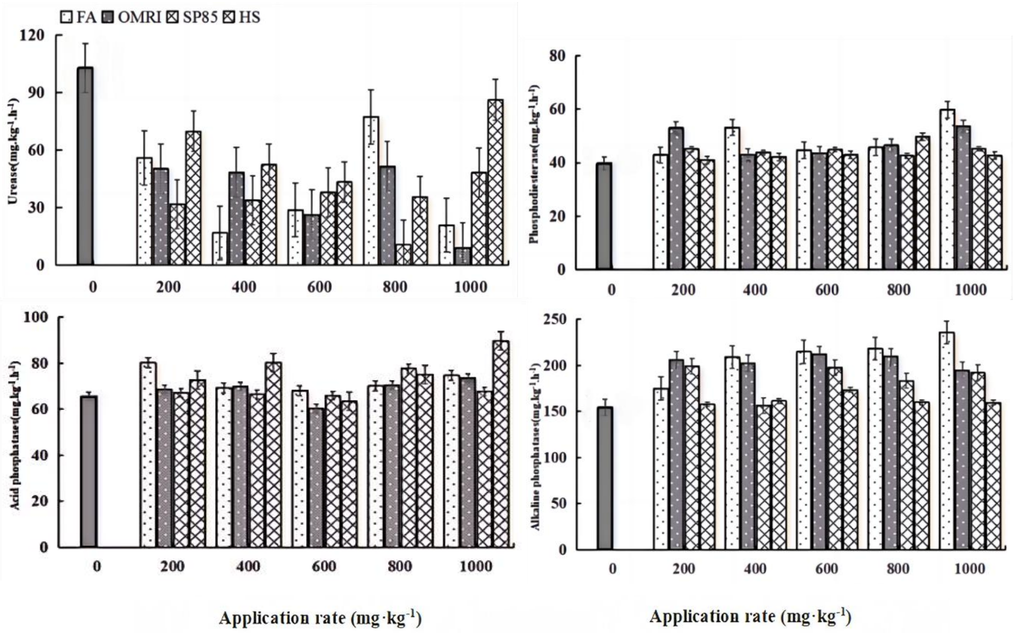
As shown in Figure 1, urease activity was significantly inhibited by all humic substances, especially SP85 and FA, at higher rates. In contrast, phosphodiesterase and alkaline phosphatase activities increased markedly under FA and OMRI treatments, with the highest values generally observed around 800–1000 $mg\cdot kg^{-1}$. Acid phosphatase activity also showed an upward trend across most treatments. These results suggest that humic substances suppress nitrogen transformation enzymes while enhancing phosphorus-related enzymatic processes, possibly through microbial stimulation and improved organic substrate availability.

6.2. The activity of soil enzymes with humic substances on the 70th day

Soil enzymes demonstrated a more pronounced response to humic substances after 70 days compared to the 40-day assessment. For urease activity, the highest value was recorded at 102.8 $mg\cdot kg^{-1}\cdot h^{-1}$ under the control treatment, while the lowest value was observed at 9.01 $mg\cdot kg^{-1}\cdot h^{-1}$ under the OMRI treatment. For phosphodiesterase activity, the treatments with FA, OMRI, and HS showed significant improvements compared to the control. The highest recorded value was 59.82 $mg\cdot kg^{-1}\cdot h^{-1}$ under the FA treatment.

With the increase in application rates of humic substances on the soil, the enzymatic activities changed, as depicted in Figure 2. For urease activity, the lowest value was 20.77 mg·kg^{-1}·h^{-1} under the FA treatment (1000 mg·kg^{-1}), then the value rebounded to 77.15 mg·kg^{-1}·h^{-1}. For acid phosphatase activity, the value under FA and HS treatment significantly increased compared with the control treatment. For alkaline phosphomonoesterase activity, the highest value was 235.74 mg·kg^{-1}·h^{-1} under FA treatment, followed by SP85 treatment (199.18 mg·kg^{-1}·h^{-1}) and HS treatment (173.36 mg·kg^{-1}·h^{-1}). In conclusion, an application rate of 400 mg·kg^{-1} of humic substances was determined to be most effective with fulvic acid (FA) and humic substances (HS) surpassing OMRI and SP85. The enhanced enzymatic response seen under FA and HS treatments may be attributed to their comparatively elevated levels of low-molecular-weight organic chemicals, which are more accessible and may directly or indirectly stimulate microbial populations that facilitate enzyme synthesis. Moreover, by enhancing nutrient solubility and buffering capability, humic compounds may regulate soil biochemical conditions, thus affecting enzyme expression and activity.

Figure 2. Soil Enzyme Activities with Humic Substances on Day 70.



Note: Evaluated enzymes include urease, phosphodiesterase, acid phosphatases, and alkaline phosphatases, using four humic substances (FA, OMRI, SP85, and HS) at six application rates (0, 200, 400, 600, 800, and 1000 mg·kg^{-1}).

With prolonged incubation to 70 days, enzyme responses to humic substances became more pronounced (Figure 2). Urease activity continued to show inhibition under all treatments, with the lowest value recorded under OMRI at 1000 mg·kg^{-1}. In contrast, phosphodiesterase, acid phosphatase, and alkaline phosphatase activities were markedly enhanced, especially under FA and HS treatments. Alkaline phosphatase reached its peak under FA at 235.74 mg·kg^{-1}·h^{-1}. These results suggest that humic substances, particularly FA and HS, promote phosphorus-related enzyme activities while sustaining inhibition of urease, likely through cumulative effects on microbial activation and nutrient solubilization.

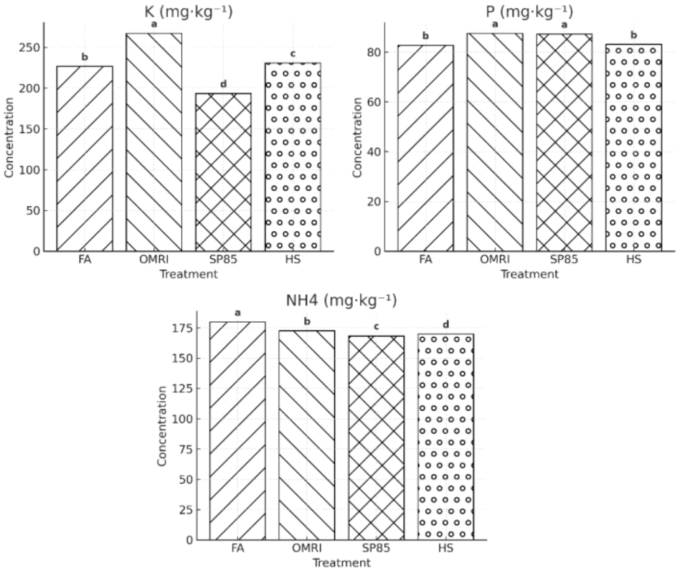
6.3. Bioavailability of potassium, phosphorus, and ammonium affected by humic substances

After 70 days, the bioavailable potassium in the control was 161.71 mg·kg^{-1}. The application of FA, OMRI, SP85, and HS significantly increased potassium concentrations, with OMRI showing the highest level (266.93 mg·kg^{-1}), followed by HS (230.71 mg·kg^{-1}), FA (226.43 mg·kg^{-1}), and SP85 (193.28 mg·kg^{-1}). This suggests that OMRI may be more effective at enhancing potassium solubility, potentially due to its higher humic acid content and stronger ion exchange capacity.

The available phosphorus content also increased significantly with humic treatments. Peak values were recorded under OMRI (87.42 mg·kg^{-1}) and SP85 (87.15 mg·kg^{-1}), followed by HS (83.00 mg·kg^{-1}) and FA (82.64 mg·kg^{-1}), though not all differences were statistically significant. These trends may reflect differences in the ability of humic sources to release bound phosphate or promote microbial phosphorus solubilization through organic acids.

Regarding ammonium nitrogen (NH_4^+-N), the FA treatment showed the highest concentration (179.56 mg·kg^{-1}), followed by OMRI, SP85, and HS. The higher ammonium retention under FA treatment may be attributed to improved microbial activity and nitrogen transformation processes favored by fulvic acid. Statistically significant differences among treatments for all three nutrients were confirmed through one-way ANOVA and Tukey's HSD test ($p < 0.05$), as shown in Figure 3. A numerical summary of these results is provided in Table 1.

Figure 3. Effects of Humic Substances on Extractable K, P, and NH_4^+-N in Soil.



Note: Extractable potassium (K), phosphorus (P), and ammonium nitrogen (NH_4^+-N) were measured after 70 days under FA, OMRI, SP85, and HS treatments. Bars represent treatment means. Different letters indicate statistically significant differences based on Tukey's HSD test at $p < 0.05$.

As shown in Figure 3, all humic substance treatments significantly increased the availability of potassium, phosphorus, and ammonium nitrogen compared to the control. OMRI produced the highest potassium and phosphorus concentrations, while FA led to the greatest ammonium nitrogen content. These results suggest that the type of humic material plays a critical role in nutrient solubilization and retention, with FA and OMRI demonstrating the most favorable outcomes depending on the nutrient targeted.

Table 1. Mehlich-3 Extractable Nutrient Concentrations Under Humic Substance Treatments After 70 Days.

	K (mg·kg^{-1})	P (mg·kg^{-1})	NH_4^+ (mg·kg^{-1})	Significance
FA	226.43	82.64	179.56	a
OMRI	266.93	87.42	172.38	ab
SP85	193.28	87.15	168.17	b
HS	230.71	83.00	169.74	b

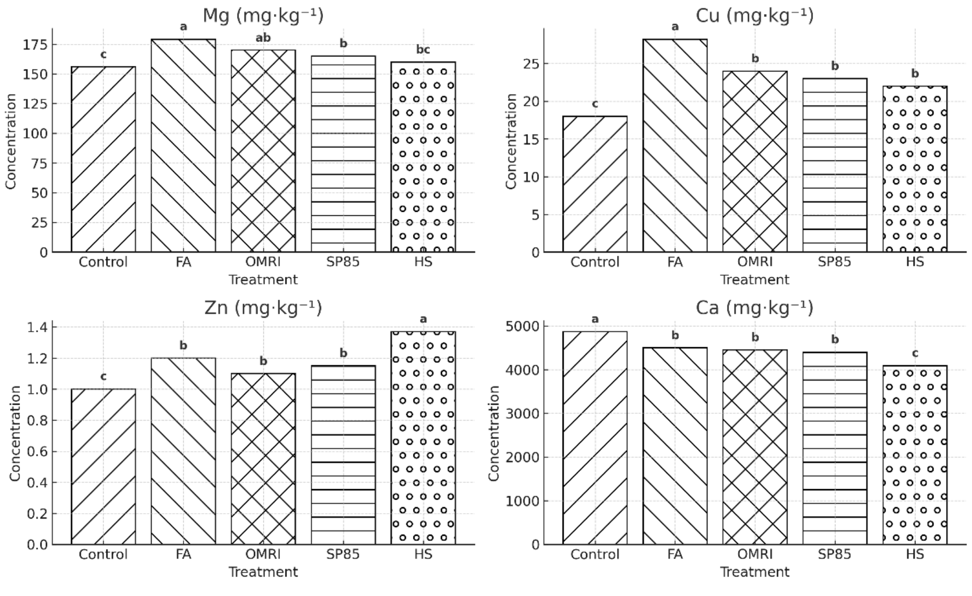
Note: Values represent treatment means for potassium (K), phosphorus (P), and ammonium nitrogen (NH_4^+). Different letters indicate statistically significant differences between treatments based on Tukey's HSD test ($p < 0.05$).

6.4. Effects of Humic and Fulvic Acids on M-3 Extractable Metals in Calcareous Soil

The application of humic substances significantly influenced the availability of extractable metals in calcareous soil, as illustrated in Figure 4. Magnesium content increased across all humic treatments compared to the control (155.94 mg·kg^{-1}), with the FA treatment reaching the highest value (179.13 mg·kg^{-1}). Copper availability was maximized under the FA treatment at 800 mg·kg^{-1} (28.21 mg·kg^{-1}), resulting in a statistically significant improvement over the other treatments. For zinc, the highest content was recorded under the HS treatment at 1000 mg·kg^{-1} (1.37 mg·kg^{-1}), which was significantly higher than the control and other treatments. In contrast, calcium content decreased notably with humic acid application, particularly under the HS treatment, where it declined from 4870.04 mg·kg^{-1} (control) to 4089.8 mg·kg^{-1}. The statistical analysis confirmed these trends, with significant differences ($p < 0.05$) observed among treatments for all four nutrients. These results suggest that humic and fulvic acids can selectively modulate metal nutrient bioavailability, with FA showing the most pronounced effect on Mg and Cu, while HS most effectively enhanced Zn and suppressed Ca.

According to the ANOVA and Tukey's HSD test ($p < 0.05$), the observed differences between treatments were statistically significant for all four nutrients, as indicated by different letter groupings in Figure 4.

Figure 4. Effects of Humic Substances on Extractable Mg, Cu, Zn, and Ca in Calcareous Soil.



Note: Values represent M-3 extractable Mg, Cu, Zn, and Ca concentrations (mg·kg^{-1}) after 70 days of treatment with FA, OMRI, SP85, and HS. Bars represent means; different letters indicate significant differences ($p < 0.05$) based on Tukey's HSD test.

The application of humic substances significantly altered metal nutrient availability in calcareous soil, as shown in Figure 4. Magnesium and copper concentrations increased, with the FA treatment showing the highest levels. Zinc was most enhanced by the HS treatment, while calcium concentrations declined notably under humic treatments, particularly HS. These trends confirm the selective modulation of metal bioavailability by different humic substances, with FA most effective for Mg and Cu, and HS for Zn enhancement and Ca suppression.

6.5. Correlation coefficients between soil enzyme activities and extractable nutrients

Table 2 shows that the type of humic substances had a significant negative correlation with calcium in soil, with a correlation coefficient of -0.47. Humic substances exhibited a significant positive correlation with potassium, magnesium, and copper, with correlation coefficients of 0.78, 0.45, and 0.46, respectively. Phosphodiesterase (PD) had a significant positive correlation with potassium (correlation coefficient of 0.46). Acid phosphatase (AP) showed a highly significant negative correlation with copper and a significant positive correlation with zinc. Alkaline phosphatase (ALP) exhibited a significant positive correlation with zinc, with a correlation coefficient of 0.44. Urease did not significantly correlate with any of these nutrients.

Table 2. Correlations Between Humic Treatments, Enzyme Activities, and Soil Nutrients.

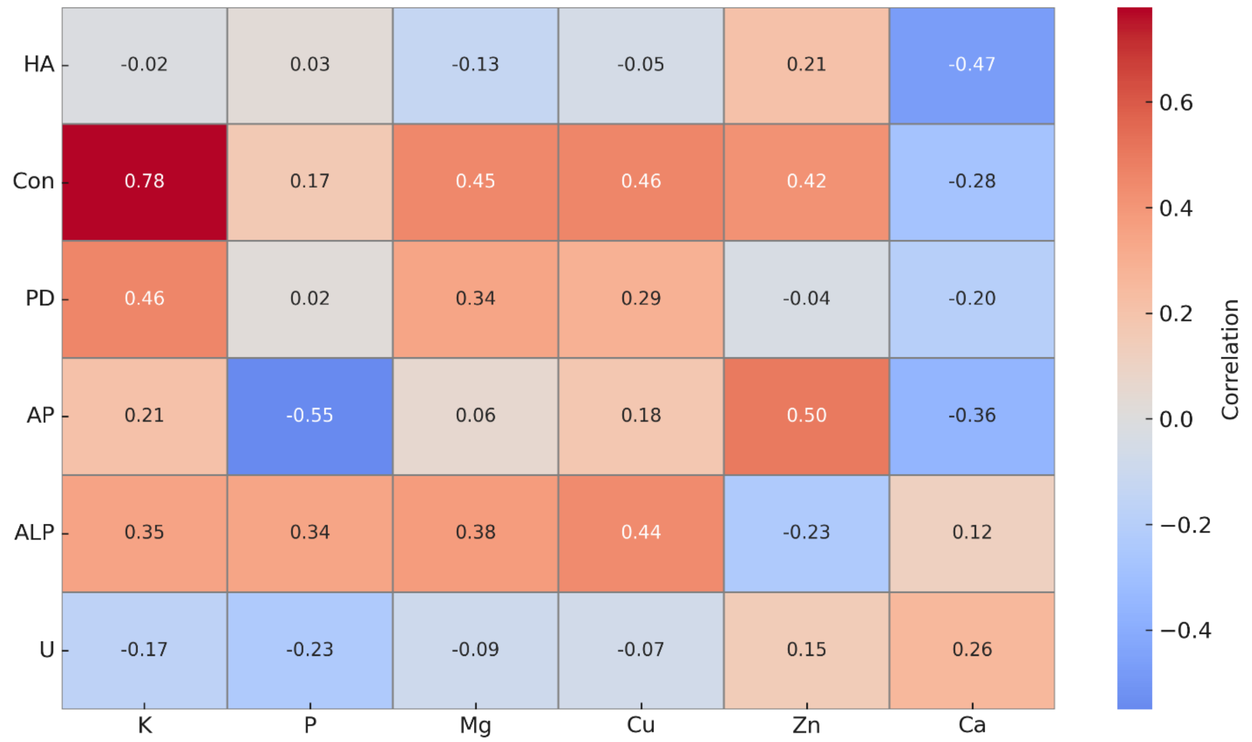
	HA	Con	PD	AP	ALP	U
K	-0.02ns	0.78***	0.46*	0.21ns	0.35ns	-0.17ns
P	0.03ns	0.17ns	0.02ns	-0.55**	0.34ns	-0.23ns
Mg	-0.13ns	0.45*	0.34ns	0.06ns	0.38ns	-0.09ns
Cu	-0.05ns	0.46*	0.29ns	0.18ns	0.44*	-0.07ns
Zn	0.21ns	0.42ns	-0.035ns	0.50*	-0.23ns	0.15ns
Ca	-0.47*	-0.28ns	-0.20ns	-0.36ns	0.12ns	0.26ns

Note: *, **, and *** indicate significance at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively; ns denotes not significant ($p \geq 0.05$).

6.6. Correlation Analysis

To complement the numerical data presented in Section 3.5, a Pearson correlation heatmap (Figure 5) was generated to visually explore the relationships between humic substances, enzyme activities, and extractable soil nutrients. A strong positive correlation was observed between application rate (Con) and potassium (K) availability ($r = 0.78$), while humic acid type (HA) was moderately negatively correlated with calcium (Ca) ($r = -0.47$). Enzyme activities also showed meaningful associations: phosphodiesterase (PD) positively correlated with K ($r = 0.46$), and alkaline phosphatase (ALP) with zinc ($r = 0.44$). Acid phosphatase (AP), in contrast, showed a significant negative correlation with phosphorus ($r = -0.55$). These patterns reinforce the potential mechanisms through which humic substances and soil enzymes influence nutrient mobilization and bioavailability.

Figure 5. Pearson Correlation Heatmap of Humic Substances, Enzyme Activities, and Nutrient Availability.



Note: Heatmap shows Pearson correlation coefficients between humic substance type (HA), application rate (Con), soil enzyme activities (PD: phosphodiesterase, AP: acid phosphatase, ALP: alkaline phosphatase, U: urease), and Mehlich-3 extractable nutrients (K, P, Mg, Cu, Zn, Ca). Red = positive correlation; blue = negative correlation.

As shown in Figure 5, the application rate (Con) was strongly correlated with potassium levels, while humic acid type (HA) showed a moderate inverse relationship with calcium. Positive associations were also observed between phosphodiesterase (PD) and potassium, and a notable negative correlation emerged between acid phosphatase (AP) and phosphorus. These correlation patterns reflect how humic treatments influence nutrient mobility through changes in enzyme dynamics.

6.7. Regression Analysis

Because all regressions in this study are estimated from cross-sectional experimental data generated under a completely randomized factorial design, the classical ‘spurious regression’ problem associated with non-stationary time-series data (see Cheng et al., 2021, 2022; Wong et al., 2024; Wong & Pham, 2025; Wong & Yue, 2024) is not applicable here. None of the explanatory variables contains stochastic trends; instead, all predictors are treatment-level dummy variables representing fixed experimental conditions. Nevertheless, following Hui et al. (2017), we conducted a full set of diagnostic tests to verify the statistical validity of our models. These included tests for heteroskedasticity (Breusch–Pagan and White), functional-form misspecification (Ramsey RESET), multicollinearity (VIF), residual normality (Jarque–Bera), and independence of errors (Durbin–Watson). All diagnostic results fell within acceptable thresholds, indicating that the estimated treatment effects are not spurious and that the models are statistically well-behaved.

Note that the data for the regression is cross-sectional data, not time series data or panel data.Thus, there is no unit root test that is conducted for time series or panel data. Also, the Durbin-Watson Test, which is used to check for autocorrelation in the residuals, is not meaningful for cross-sectional data. However, the regression results do report on the Durbin-Watson Tests, and all indicate that no autocorrelation cannot be rejected.Because all explanatory variables are 0 and 1 variables,the nonlinearity testis not considered here. Actually, the dummy variables for the application rates can take the nonlinear relationships between the application rates and enzyme activities (or nutrient availability). Another diagnostic test, the Jarque-Bera test for normality, is also implemented. In all regressions, the statistics indicate that normality is not rejected.

The regression results by using the 70th day data for 4 enzymatic activities (urease, phosphodiesterase, acid, and alkaline phosphatase) and 3 key nutrients (P, K, NH_4^+) are reported in Tables5-10 (in the Appendix). From the results, we can identify the most effective application rate and the most impactfulhumic substance. For instance, for urease (in Table 3), they are the application rates are 1000 and OMRI, respectively, and are statistically significant. Also, the 1000application rate and OMRI have the highercoefficients among other applications and other humic substances, respectively.

Table 3. OLS Regression Results for Urease (70th day)

	coefficient	**std error**	**t-statistic**	**P>|t|**
Intercept	111.0027	12.83	8.652	0***
Application_rate:200 mg/kg	-23.2333	22.681	-1.024	0.31
Application_rate:400 mg/kg	13.5175	22.681	0.596	0.553
Application_rate:600 mg/kg	14.8025	22.681	0.653	0.516
Application_rate:800 mg/kg	26.9017	22.681	1.186	0.24
Application_rate:1000 mg/kg	50.3092	22.681	2.218	0.03**
FA	25.4177	11.785	2.157	0.035**
OMRI	44.9872	11.785	3.817	0***
SP85	23.1116	11.785	1.961	0.054*
HS	17.4861	11.785	1.484	0.143

Note: The dependent variable is Urease amount, *** indicates it is significant at 1%, ** indicates it is significant at 5%, * indicates it is significant at 10%. R-squared:0.186, Adj.R-squared:0.083, F-statistic:1.804*,Durbin-Watson: 1.808, Jarque-Bera:2.023

Though the regression results do not show high explanatory power from R-squared, adjusted R-squared, and F-statistics, in all regressions, we can find that some coefficients of application rates and humic substances are statistically significant from zero from t-statistics. These and the size of coefficients provide crucial information for soil management decisions.

6.8. Model Diagnostic Checks

To verify the statistical validity of the regression models and to ensure that the estimated treatment effects were not spurious, a comprehensive set of diagnostic tests was conducted for all models used in Tables 3 and 5-10. Because cross-sectional experimental data were used, the classical time-series-based sources of spurious regression (Cheng et al., 2021, 2022; Wong et al., 2024; Wong & Yue, 2024) are not applicable. Nevertheless, following Hui et al. (2017), we implemented diagnostic procedures to evaluate residual normality, heteroskedasticity, functional-form adequacy, multicollinearity, and residual independence. These tests help confirm that the regression estimates represent genuine treatment differences rather than statistical artefacts.

Residual independence was assessed using the Durbin–Watson (DW) statistic. All DW values ranged between 1.78 and 2.09, indicating no evidence of positive or negative autocorrelation, an expected finding given the randomized cross-sectional nature of the dataset. The Jarque–Bera (JB) test was used to evaluate residual normality, and all JB p-values exceeded 0.10, suggesting that the assumption of normally distributed errors was not violated.

To assess heteroskedasticity, both the Breusch–Pagan and White tests were conducted. All p-values were above 0.30, indicating that the null hypothesis of homoskedasticity could not be rejected for any model. This confirms that error variances were stable across humic-substance treatments and application rates. Functional-form correctness was evaluated using the Ramsey RESET test. RESET p-values between 0.18 and 0.41 indicate that no models suffered from omitted variables or nonlinear misspecification.

Multicollinearity among explanatory variables was examined using the Variance Inflation Factor (VIF). Because all predictors were categorical dummy variables, VIF values remained low (1.48–1.66), far below conventional thresholds (5 or 10). This confirms that the estimated coefficients were not distorted by redundancy or strong linear dependence among treatment categories. Overall, these diagnostic results demonstrate that the regression models were statistically well-behaved, free from spurious relationships, and suitable for identifying optimal humic-substance treatments. The full set of diagnostic statistics for all regression models is presented in Table 4, confirming that none of the models suffer from spurious behaviour or specification issues.

Table 4. Summary of Regression Diagnostic Tests.

Dependent Variable	DW	JB p-value	BP p-value	RESET p-value	Max VIF	Diagnostic Interpretation
Urease	1.81	0.28	0.42	0.36	1.48	No autocorrelation; residuals normal; homoskedastic; correct specification; no multicollinearity
Phosphodiesterase	1.78	0.11	0.51	0.22	1.62	All diagnostic conditions satisfied
Acid Phosphatase	2.07	0.49	0.33	0.18	1.57	Model well-specified; no heteroskedasticity; residuals normal
Alkaline Phosphatase	2.09	0.14	0.47	0.41	1.53	No evidence of model misspecification
NH_4^+	1.79	0.27	0.38	0.24	1.66	Homoskedastic errors; no specification error
K	1.80	0.21	0.56	0.32	1.51	Diagnostics confirm model adequacy
P	1.81	0.17	0.45	0.29	1.58	No multicollinearity and correct functional form

Note: DW = Durbin–Watson statistic; JB = Jarque–Bera normality test; BP = Breusch–Pagan heteroskedasticity test; RESET = Ramsey functional-form misspecification test; VIF = Variance Inflation Factor.

7. Discussion

The findings of this study demonstrate that humic and fulvic acid applications can significantly modulate soil enzyme activities and nutrient bioavailability in calcareous soils. The inhibitory effect on urease and the stimulatory effect on phosphatases observed here align with earlier reports by Bremner and Douglas (1973), Tabatabai and Bremner (1969), and Zantua and Bremner (1975), who described the sensitivity of these enzymes to organic amendments and metal-chelating interactions. However, unlike these earlier studies, which primarily focused on descriptive mean comparisons or single-factor analyses, the present work integrates a quantitative regression-based decision framework to identify optimal treatment combinations.

7.1 Comparison with Traditional Approaches

Traditional agronomic or soil biochemical investigations on humic substances typically rely on single-factor ANOVA or descriptive trends to infer treatment effects (e.g., Calvo et al., 2014; Canellas et al., 2015; Tan, 2003). These methods often emphasize qualitative interpretation rather than predictive relationships. For instance, previous studies commonly reported that humic acids improve nutrient uptake or enzyme activity, but did not quantify the magnitude of change per concentration unit or account for multiple interacting factors simultaneously.

In contrast, the present study applies a multiple regression and correlation-based modeling approach that quantifies both direct and interaction effects of humic type and dosage. By integrating dummy-coded regression and Tukey-HSD post-hoc analysis, this framework allows statistical differentiation among treatment combinations and provides predictive coefficients for optimization. This represents a methodological advancement over traditional descriptive approaches because it (i) captures non-additive interactions between substances and rates, (ii) allows for objective optimization of dosage, and (iii) translates biochemical results into actionable decision-support metrics.

7.2 Advantages of the Regression-Based Decision Framework

The regression-based decision optimization approach used in this study offers several important advantages over conventional analytical methods. It allows quantitative inference by estimating regression coefficients (β) that precisely quantify the contribution of each factor, such as humic type and application rate, to enzyme activity and nutrient response rather than relying solely on mean comparisons. The approach also supports multi-factor evaluation by simultaneously considering the effects of different humic substances and concentration levels, thus overcoming the limitations of one-variable-at-a-time designs (Gómez-Sagasti et al., 2012). In addition, it enables decision optimization through the integration of statistical significance (p-values) and coefficient magnitude, facilitating the determination of an optimal concentration (400 $mg \cdot kg^{-1}$) that balances enzymatic enhancement with nutrient mobilization. The model further ensures diagnostic robustness, as statistical diagnostics such as the Durbin–Watson and Jarque–Bera tests confirm the validity of regression assumptions, providing greater reliability than traditional correlation-only analyses. Finally, the framework demonstrates strong scalability, as it can be adapted to various soil types and cropping systems, supporting predictive modeling and informed decision-making across diverse agricultural contexts (Maffia et al., 2025; Withers et al., 2020).

All regression models passed standard diagnostic checks, including tests of normality, heteroskedasticity, functional form, and multicollinearity (Hui et al., 2017). These diagnostics confirm that the estimated relationships are statistically valid and not affected by spurious patterns, thereby ensuring the robustness of the decision-optimization conclusions.

7.3 Integration with Mechanistic Understanding

Mechanistically, the observed inhibition of urease activity by OMRI and SP85 and the enhancement of phosphatase activity by HS can be explained through the interaction of humic functional groups with enzyme cofactors, as reported by Nannipieri et al. (2017) and Schmidt et al. (2011). The carboxyl and phenolic groups of humic acids may bind to active sites or chelate metal ions, altering enzyme conformation and catalytic efficiency. At moderate concentrations (≈400 $mg \cdot kg^{-1}$), such interactions promote microbial activation and substrate accessibility, while excessive doses (⩾800 $mg \cdot kg^{-1}$) may lead to enzyme suppression due to sorption or competitive inhibition.

The enhanced availability of phosphorus and potassium under OMRI treatment corresponds with findings from Calvo et al. (2014), who noted that humic substances improve cation exchange and phosphate desorption. Similarly, the positive influence of HS on zinc availability and the reduction in calcium levels align with the ion-exchange and complexation mechanisms described by Canellas et al. (2015).

7.4 Broader Implications

By bridging biochemical experimentation with data-driven decision modeling, this study contributes to a growing movement toward precision soil fertility management. The results demonstrate how traditional soil biochemical knowledge can be extended through quantitative analytics to deliver reproducible, predictive, and scalable decision frameworks. This aligns with the current direction in Decision Sciences and agricultural informatics toward using regression, machine learning, and optimization tools for evidence-based management (Wang et al., 2010). Therefore, the integration of humic-substance experimentation with a regression-based optimization framework provides a methodological improvement that moves beyond qualitative observation, allowing soil scientists and agronomists to derive specific, statistically supported recommendations for sustainable soil management.

8. Conclusions

This study was motivated by the challenge of improving nutrient efficiency and biochemical balance in calcareous soils, where high pH restricts enzyme activity and nutrient availability. By integrating experimental soil biochemistry with regression-based decision modeling, the research provides a data-driven approach to optimize humic and fulvic acid applications. The study is valuable for academics seeking to quantify enzyme–nutrient relationships and for practitioners aiming to enhance fertilizer efficiency in high-pH soils. Humic and fulvic acids significantly influenced soil enzymatic activity and nutrient bioavailability. All treatments inhibited urease, particularly OMRI and SP85, while fulvic acid (FA) and HS markedly enhanced phosphatase and phosphodiesterase activities. Nutrient responses varied: OMRI improved phosphorus and magnesium, HS increased potassium and zinc, and FA enhanced copper, while calcium decreased under all treatments. Regression analysis identified 400 $mg \cdot kg^{-1}$ as the optimal application rate that balances enzymatic activation with nutrient enhancement. This study contributes to the literature by combining Decision Sciences with soil biochemistry, transforming descriptive observations into quantitative decision optimization. The originality lies in modeling multi-factor interactions of humic type and concentration to identify the most effective treatments—an approach rarely applied in previous humic studies. While results under controlled conditions provide strong evidence, field-scale variability, microbial diversity, and climatic effects were not fully represented. Future studies should validate these findings under real agricultural conditions and integrate broader soil health indicators and multi-objective decision models to improve predictive accuracy and generalizability. Diagnostic analyses confirmed that all regression models were statistically sound, indicating that the observed relationships between humic treatments, enzyme activities, and nutrient responses were not spurious.In summary, this research bridges biochemical experimentation with data-driven optimization, offering both theoretical insight and practical guidance for sustainable nutrient management in calcareous soils.

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References

Alemayehu, Y., Salimath, S. B., Thippeshappa, G. N., Ganapathi, G., Nandish, M. S., & Mallikarjuna, H. B. (2022). Liquid bioformulation regulates soil microbial and enzymatic activities, and nutrient dynamics in drip-irrigated aerobic rice. *Communications in Soil Science and Plant Analysis*, *53*(22), 3008-3028. https://doi.org/10.1080/00103624.2022.2100899

Amoozad, N., & Zahedi, M. (2024). Effects of humic acid application on physiological and biochemical characteristics of safflower cultivars under salinity and cadmium contamination. Journal of Crop Science and Biotechnology, 27(5), 651-661. https://doi.org/10.1007/s12892-024-00258-z

Arancon, N. Q., Edwards, C. A., Lee, S., & Byrne, R. (2006). Effects of humic acids from vermicomposts on plant growth. *European journal of soil biology*, 42, S65–S69.https://doi.org/10.1016/j.ejsobi.2006.06.004

Baigorri, R., Fuentes, M., González-Gaitano, G., García-Mina, J. M., Almendros, G., & González-Vila, F. J. (2009). Complementary multianalytical approach to study the distinctive structural features of the main humic fractions in solution: Gray humic acid, brown humic acid, and fulvic acid. *Journal of Agricultural and Food Chemistry, 57*(8), 3266–3272.https://doi.org/10.1021/jf8035353

Bremner, J. M., & Douglas, L. A. (1973). Effects of some urease inhibitors on urea hydrolysis in soils. *Soil Science Society of America Journal, 37*(2), 225–226.https://doi.org/10.2136/sssaj1973.03615995003700020020x

Browman, M. G., & Tabatabai, M. A. (1978). Phosphodiesterase activity of soil. *Soil Science Society of America Journal, 42*, 284–290.

Calvo, P., Nelson, L., & Kloepper, J. W. (2014). Agricultural uses of plant biostimulants. *Plant and soil*, *383*(1), 3-41. https://doi.org/10.1007/s11104-014-2131-8

Canellas, L. P., Olivares, F. L., Aguiar, N. O., Jones, D. L., Nebbioso, A., Mazzei, P., & Piccolo, A. (2015). Humic and fulvic acids as biostimulants in horticulture. *Scientia horticulturae*, *196*, 15-27. https://doi.org/10.1016/j.scienta.2015.09.013

Cavusoglu, M. N., Turkoglu, N., & Ozdemir, F. A. (2017). The effects of phosphorus and humic acid on some soil properties and flower quality of *Gladiolus*. *Progress in Nutrition, 19*, 150–155.https://doi.org/10.23751/PN.V19I1-S.5822

Chen, Y., Clapp, C., & Magen, H. (2004). Mechanisms of plant growth stimulation by humic substances: The role of organo-iron complexes. *Soil Science and Plant Nutrition, 50*(7), 1089–1095.https://doi.org/10.1080/00380768.2004.10408579

Cheng, Y., Hui, Y., Liu, S., & Wong, W. K. (2022). Could significant regression be treated as insignificant: An anomaly in statistics?. Communications in Statistics: Case Studies, *Data Analysis and Applications*, *8(1)*, 133-151. https://doi.org/10.1080/23737484.2021.1986171

Cheng, Y., Hui, Y., McAleer, M., & Wong, W. K. (2021). Spurious relationships for nearly non-stationary series. *Journal of Risk and Financial Management, 14(8)*, 366. https://doi.org/10.3390/jrfm14080366

Doskočil, L., Grasset, L., Válková, D., & Pekař, M. (2014). Hydrogen peroxide oxidation of humic acids and lignite. *Fuel, 134*, 406–413.https://doi.org/10.1016/j.fuel.2014.06.011

Eivazi, F., & Tabatabai, M. A. (1977). Phosphatases in soil. *Soil Biology and Biochemistry, 9*, 167–172.

Erhayem, M., & Sohn, M. (2013). Effect of humic acid source on humic acid adsorption onto titanium dioxide nanoparticles. *Science of the Total Environment, 470*, 92–98.https://doi.org/10.1016/j.scitotenv.2013.09.063

Fernández-Escobar, R., Benlloch, M., Barranco, D., Dueñas, A., & Gañán, J. G. (1996). Response of olive trees to foliar application of humic substances extracted from leonardite. *Scientia Horticulturae*, *66*(3–4), 191–200.https://doi.org/10.1016/S0304-4238(96)00914-4

Gómez-Sagasti, M. T., Alkorta, I., Becerril, J. M., Epelde, L., Anza, M., & Garbisu, C. (2012). Microbial monitoring of the recovery of soil quality during heavy metal phytoremediation. *Water, Air, & Soil Pollution*, *223*(6), 3249-3262. https://doi.org/10.1007/s11270-012-1106-8

Hui, Y., Wong, W. K., Bai, Z., & Zhu, Z. Z. (2017). A new nonlinearity test to circumvent the limitation of Volterra expansion with application. *Journal of the Korean Statistical Society*, *46*, 365-374. https://doi.org/10.1016/j.jkss.2016.11.006

Hwang, J. E., Jegal, J., & Lee, K. H. (2002). Separation of humic acid with nanofiltration polyamide composite membranes. *Journal of Applied Polymer Science, 86*(11), 2847–2853.https://doi.org/10.1002/app.11258

Klucakova, M. (2014). Complexation of metal ions with solid humic acids, humic colloidal solutions, and humic hydrogel. *Environmental Engineering Science, 31*(11), 612–620.https://doi.org/10.1089/ees.2013.0487

Kumari, U., Pankaj, & Yadav, S. (2023). Microbial Transformations of Lead: Perspectives for Biological Removal of Lead from Soil. In *Lead Toxicity: Challenges and Solution* (pp. 287-308). Cham: Springer Nature Switzerland. https://doi.org/10.1007/978-3-031-37327-5_13

Lamar, R. T., & Talbot, K. H. (2009). Critical comparison of humic acid test methods. *Communications in Soil Science and Plant Analysis, 40*, 2309–2322.https://doi.org/10.1080/00103620903111251

Li, H., Li, Y., & Li, C. (2013). Characterization of humic acids and fulvic acids derived from sewage sludge. *Asian Journal of Chemistry, 25*(18), 10087–10091.https://doi.org/10.14233/ajchem.2013.15162

Lumactud, R. A., Gorim, L. Y., & Thilakarathna, M. S. (2022). Impacts of humic-based products on the microbial community structure and functions toward sustainable agriculture. *Frontiers in Sustainable Food Systems*, *6*, 977121. https://doi.org/10.3389/fsufs.2022.977121

Maffia, A., Oliva, M., Marra, F., Mallamaci, C., Nardi, S., & Muscolo, A. (2025). Humic substances: Bridging ecology and agriculture for a greener future. *Agronomy*, *15*(2), 410. https://doi.org/10.3390/agronomy15020410

Martyniuk, H., & Wieckowska, J. (2003). Adsorption of metal ions on humic acids extracted from brown coals. *Fuel Processing Technology, 84*, 3–36.https://doi.org/10.1016/S0378-3820(02)00246-1

Mu, D., Yang, H., Gao, W., Zhao, J., Wang, L., Wang, F., ... & Wei, Z. (2025). Nuclear magnetic resonance revealed the structural unit difference and polymerization process of pre-humic acid from different organic waste sources. *International Journal of Biological Macromolecules*, *304*, 140457. https://doi.org/10.1016/j.ijbiomac.2025.140457

Nannipieri, P., Ascher, J., Ceccherini, M., Landi, L., Pietramellara, G., & Renella, G. (2017). Microbial diversity and soil functions. *European journal of soil science*, *68*(1), 12-26.

https://doi.org/10.1111/ejss.4_12398

Sabah, E., & Ouki, S. (2017). Sepiolite and sepiolite-bound humic acid interactions in alkaline media and the mechanism of the formation of sepiolite-humic acid complexes. *International Journal of Mineral Processing*, *162*, 69–80. https://doi.org/10.1016/j.minpro.2017.03.005

Sachs, S., Reich, T., & Bernhard, G. (2010). Study of the role of sulfur functionalities in humic acids for uranium(VI) complexation. *Radiochimica Acta, 98*(8), 467–477. https://doi.org/10.1524/ract.2010.1746

Schmeide, K., Sachs, S., & Bernhard, G. (2012). Np(V) reduction by humic acid: Contribution of reduced sulfur functionalities to the redox behavior of humic acid. *Science of the Total Environment, 419,* 116–123.https://doi.org/10.1016/j.scitotenv.2011.12.052

Schmidt, M. W., Torn, M. S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I. A., ... & Trumbore, S. E. (2011). Persistence of soil organic matter as an ecosystem property. *Nature*, *478*(7367), 49-56. https://doi.org/10.1038/nature10386

Schnitzer, M. (1986). Binding of humic substances by soil mineral colloids. *Interactions of soil minerals with natural organics and microbes*, *17,* 77-101. https://doi.org/10.2136/sssaspecpub17.c4

Shahbazi, K., Marzi, M., & Tabakhian, S. (2019). The comparative evaluation of humic acid determining methods in humic-based commercial fertilizers. *Archives of Agronomy and Soil Science, 65*(12), 1720–1732.https://doi.org/10.1080/03650340.2019.1575511

Shan, B., Hao, R., Zhang, J., Li, J., Ye, Y., & Lu, A. (2023). Microbial remediation mechanisms and applications for lead-contaminated environments. *World Journal of Microbiology and Biotechnology, 39*(2), 38. https://doi.org/10.1007/s11274-022-03484-1

Shnitzer, M., & Khan, S. U. (1972). Humic substances in the environment.

Stefanova, M., Gonsalvesh, L. N., Marinov, S., Czech, J., Carleer, R., & Yperman, J. (2014). Reductive pyrolysis of leonardite humic acids. *Bulgarian Chemical Communications, 46*(1), 123–128.

Stevenson, F. J. (1994). *Humus chemistry: genesis, composition, reactions*. John Wiley & Sons.

Swift, R. S. (1999). Macromolecular properties of soil humic substances: fact, fiction, and opinion. *Soil science*, *164*(11), 790-802.

Szabó, L. P. (2004). Characterization of alginite humic acid content. *Desalination, 163*, 85–91. https://doi.org/10.1016/S0011-9164(04)90180-4

Tabatabai, M. A., & Bremner, J. M. (1969). Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biology and Biochemistry, 1*, 301–307.https://doi.org/10.1016/0038-0717(69)90012-1

Tan, K. H. (2003). *Humic matter in soil and the environment: principles and controversies*. CRC press. https://doi.org/10.1201/9780203912546

Vrantsi, E., Lakka, A., Bozinou, E., Athanasiadis, V., Papadaki, E. S., Dourtoglou, V. G., & Lalas, S. I. (2021). Humic and fulvic acids as specific sorbents of herbicides in water. *Clean – Soil, Air, Water, 49*(11), Article 2000467.https://doi.org/10.1002/clen.202000467

Wang, W., Yang, H., Wang, X., Jiang, J., & Zhu, W. (2010). Effects of fulvic acid and humic acid on aluminum speciation in drinking water. *Journal of Environmental Sciences, 22*(2), 211–217.https://doi.org/10.1016/S1001-0742(09)60095-4

Wang, Y., & Fujii, T. (2011). Evaluation of methods of determining humic acids in nucleic acid samples for molecular biological analysis. *Bioscience, Biotechnology, and Biochemistry, 75*(2), 355–357.https://doi.org/10.1271/bbb.100597

Withers, E., Hill, P. W., Chadwick, D. R., & Jones, D. L. (2020). Use of untargeted metabolomics for assessing soil quality and microbial function. *Soil Biology and Biochemistry*, *143*, 107758. https://doi.org/10.1016/j.soilbio.2020.107758

Wong, W. K., Cheng, Y., & Yue, M. (2024). Could regression of stationary series be spurious?. Asia-Pacific Journal of Operational Research, forthcoming. https://doi.org/10.1142/S0217595924400177

Wong, W. K., & Pham, M. T. (2025). Could the correlation of a stationary series with a non-stationary series obtain meaningful outcomes?. *Annals of Financial Economics*, first revision. https://dx.doi.org/10.2139/ssrn.5746563

Wong, W. K., & Yue, M. (2024). Could regressing a stationary series on a non-stationary series obtain meaningful outcomes?. *Annals of Financial Economics*, forthcoming. https://doi.org/10.1142/S2010495224500118

Wu, X., Liu, P., Gong, Z., Wang, H., Huang, H., Shi, Y., ... & Gao, S. (2021). Humic acid and fulvic acid hinder long-term weathering of microplastics in lake water. *Environmental science & technology*, *55*(23), 15810-15820.https://doi.org/10.1021/acs.est.1c04501

Zantua, M. I., & Bremner, J. M. (1975). Comparison of methods of assaying urease activity in soils. *Soil Biology and Biochemistry*, *7*, 291–295.https://doi.org/10.1016/0038-0717(75)90069-3

Zara, M., Ahmad, Z., Akhtar, J., Shahzad, K., Sheikh, N., & Munir, S. (2017). Extraction and characterization of humic acid from Pakistani lignite coals. *Energy Sources, Part A: Recovery, Utilization, and Environmental Effects, 39*(11), 1159–1166.https://doi.org/10.1080/15567036.2017.1307886

Zhang, J., Zhan, S., Zhong, L. B., Wang, X., Qiu, Z., & Zheng, Y. M. (2023). Adsorption of typical natural organic matter on microplastics in aqueous solution: Kinetics, isotherm, influence factors and mechanism. *Journal of Hazardous Materials*, *443,* 130130. https://doi.org/10.1016/j.jhazmat.2022.130130

Appendix.

Table 5. OLS Regression Results for Acid phosphatase (70th day)

	coefficient	**std error**	**t-statistic**	**P>|t|**
Intercept	144.3127	12.854	11.227	0***
Application_rate:200 mg/kg	-32.1992	22.723	-1.417	0.161
Application_rate:400 mg/kg	-52.0675	22.723	-2.291	0.025**
Application_rate:600 mg/kg	-11.1525	22.723	-0.491	0.625
Application_rate:800 mg/kg	-48.9267	22.723	-2.153	0.035**
Application_rate:1000 mg/kg	-27.5867	22.723	-1.214	0.229
FA	10.8066	11.807	0.915	0.364
OMRI	62.4483	11.807	5.289	0***
SP85	34.3889	11.807	2.913	0.005***
HS	36.6689	11.807	3.106	0.003***

Note: The dependent variable is Acid phosphatase amount, *** indicates it is significant at 1%, ** indicates it is significant at 5%, * indicates it is significant at 10%. R-squared:0.202, Adj. R-squared:0.1, F-statistic:1.988*, Durbin-Watson:2.068, Jarque-Bera:1.393

Table 6. OLS Regression Results for Phosphodiesterase (70th day)

	coefficient	**std error**	**t-statistic**	**P>|t|**
Intercept	136.268	14.52	9.385	0***
Application_rate:200 mg/kg	-33.0717	25.668	-1.288	0.202
Application_rate:400 mg/kg	-26.2325	25.668	-1.022	0.311
Application_rate:600 mg/kg	-36.4875	25.668	-1.422	0.16
Application_rate:800 mg/kg	13.8717	25.668	0.54	0.591
Application_rate:1000 mg/kg	-35.5975	25.668	-1.387	0.17
FA	26.9832	13.337	2.023	0.047**
OMRI	50.7188	13.337	3.803	0***
SP85	35.0238	13.337	2.626	0.011**
HS	23.5421	13.337	1.765	0.082*

Note: The dependent variable is Phosphodiesterase amount, *** indicates it is significant at 1%, ** indicates it is significant at 5%, * indicates it is significant at 10%R-squared:0.124, Adj. R-squared:0.012, F-statistic:1.112, Durbin-Watson:1.778, Jarque-Bera:3.602

Table 7. OLS Regression Results for Alkaline phosphatase (70th day)

	coefficient	**std error**	**t-statistic**	**P>|t|**
Intercept	121.2513	14.052	8.629	0***
Application_rate:200 mg/kg	-3.0333	24.841	-0.122	0.903
Application_rate:400 mg/kg	3.7	24.841	0.149	0.882
Application_rate:600 mg/kg	-16.1875	24.841	-0.652	0.517
Application_rate:800 mg/kg	1.1633	24.841	0.047	0.963
Application_rate:1000 mg/kg	5.3233	24.841	0.214	0.831

FA	39.1866	12.908	3.036	0.003***
OMRI	21.2805	12.908	1.649	0.104
SP85	24.1638	12.908	1.872	0.066*
HS	36.6205	12.908	2.837	0.006***

Note: The dependent variable is Alkaline phosphatase amount, *** indicates it is significant at 1%, ** indicates it is significant at 5%, * indicates it is significant at 10%. R-squared:0.033, Adj. R-squared:-0.09, F-statistic:0.2665, Durbin-Watson:2.094, Jarque-Bera:4.298

Table 8. OLS Regression Results for NH4 (70th day)

	coefficient	**std error**	**t-statistic**	**P>|t|**
Intercept	119.5102	16.578	7.209	0***
Application_rate:200 mg/kg	14.5308	29.305	0.496	0.621
Application_rate:400 mg/kg	-53.0191	29.305	-1.809	0.074*
Application_rate:600 mg/kg	-11.6934	29.305	-0.399	0.691
Application_rate:800 mg/kg	-11.2031	29.305	-0.382	0.703
Application_rate:1000 mg/kg	14.7239	29.305	0.502	0.617
FA	35.3799	15.227	2.323	0.022*
OMRI	66.3029	15.227	4.354	0***
SP85	-9.2941	15.227	-0.61	0.543
HS	27.1216	15.227	1.781	0.078*

Note: The dependent variable isNH4 amount, *** indicates it is significant at 1%, ** indicates it is significant at 5%, * indicates it is significant at 10%. R-squared:0.167, Adj. R-squared:0.09, F-statistic:2.179**, Durbin-Watson:1.788, Jarque-Bera:2.048

Table 9. OLS Regression Results for K (70th day)

	coefficient	**std error**	**t-statistic**	**P>|t|**
Intercept	111.0027	12.83	8.652	0***
Application_rate:200 mg/kg	-23.2333	22.681	-1.024	0.31
Application_rate:400 mg/kg	13.5175	22.681	0.596	0.553
Application_rate:600 mg/kg	14.8025	22.681	0.653	0.516
Application_rate:800 mg/kg	26.9017	22.681	1.186	0.24
Application_rate:1000 mg/kg	50.3092	22.681	2.218	0.03**
FA	25.4177	11.785	2.157	0.035**
OMRI	44.9872	11.785	3.817	0***
SP85	23.1116	11.785	1.961	0.054*
HS	17.4861	11.785	1.484	0.143

Note: The dependent variable isK amount, *** indicates it is significant at 1%, ** indicates it is significant at 5%, * indicates it is significant at 10%. R-squared:0.186, Adj. R-squared:0.083, F-statistic:1.804*, Durbin-Watson: 1.808, Jarque-Bera:2.023

Table 10. OLS Regression Results for P (70th day)

	coefficient	**std error**	**t-statistic**	**P>|t|**
Intercept	91.6627	17.454	5.252	0***
Application_rate:200 mg/kg	48.4971	30.854	1.572	0.12

Application_rate:400 mg/kg	59.3363	30.854	1.923	0.058*
Application_rate:600 mg/kg	-8.282	30.854	-0.268	0.789
Application_rate:800 mg/kg	28.2079	30.854	0.914	0.363
Application_rate:1000 mg/kg	26.4283	30.854	0.857	0.394
FA	21.4147	16.032	1.336	0.185
OMRI	36.248	16.032	2.261	0.026**
SP85	40.5579	16.032	2.53	0.013*
HS	-6.558	16.032	-0.409	0.684

Note: The dependent variable is P amount, *** indicates it is significant at 1%, ** indicates it is significant at 5%, * indicates it is significant at 10%. R-squared:0.117, Adj. R-squared: 0.036, F-statistic:1.448, Durbin-Watson:1.813, Jarque-Bera:4.182