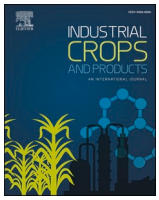
Industrial Crops & Products 226 (2025) 120761

Contents lists available at ScienceDirect

Industrial Crops & Products

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



Combined bamboo-derived biochar and DCD improves the catalytic potentials of ureases in forest and agricultural soils

Qiyuan Zheng [a], Weijin Wang [b], Pengcheng Zhang [a], Meng Zhang [c], Fen Yu [a], Lin Yu [d], Weiyi Liu [e], Wenyuan Zhang [a,*], Manyun Zhang [a,f,g,**]

[a] Jiangxi Provincial Key Laboratory of Silviculture, College of Forestry, Jiangxi Agricultural University, Nanchang 330045, China
[b] School of Agriculture and Food Sciences, The University of Queensland, St Lucia, QLD 4072, Australia
[c] Jilin Agricultural University, Changchun 130118, China
[d] Jiangxi Academy of Forestry, Nanchang 330032, China
[e] Southwest Forestry University, Kunming 650224, China
[f] College of Resources and Environment, Hunan Agricultural University, Changsha 410128, China
[g] School of Environment and Science, Griffith University, Brisbane, Queensland 4111, Australia

ARTICLE INFO

Keywords:
Bamboo-derived biochar
Dicyandiamide
Urease kinetics
Mineral nitrogen
Microbial community diversity

ABSTRACT

Soil urease kinetics reflects the affinity and catalytic reaction rate of urease. However, an incomplete understanding on soil urease kinetic hampered the optimisation of soil N management practices for agricultural and forestry ecosystems. In the present study, different soils from farmland and forest land were subjected to the following four treatments: (1) blank control (CK), (2) bamboo-derived biochar application (BC), (3) DCD application (DC), and (4) combined application of bamboo-derived biochar and DCD (CBAD). In the forest soil, the urease kinetic half-saturation constant (K_m) values decreased significantly by 28 %, 73 % and 45 % in the BC, DC and CBAD treatments, respectively. Similarly, in the agricultural soil, these values decreased by 46 %, 29 %, and 46 %, respectively. However, no significant difference in urease decomposition rates at saturated substrate concentrations (V_{max}) was observed among the four treatments. The contribution percentages of soil abiotic and biotic (the composition of bacterial and fungal community) properties to the variability in K_m were 58.6 % and 41.4 %, respectively. Biochar and DCD application improved the catalytic potential of urease in the agricultural and forest soils, which would not be conducive to the retention of nitrogen in soils. The increases in the catalytic potential of urease were associated with the increased content of mineral N and fungal community diversity under the application of biochar or DCD. Abiotic factors outweighed the microbial community composition in altering urease kinetics in various soils. These findings highlighted the necessity of nitrogen-fixing measures in soils amended with biochar or DCD and advanced our comprehension of how abiotic and biotic factors influence the urease kinetics in agricultural and forest soils.

1. Introduction

Soil enzymes originate from the activities of various soil microorganisms, animals and plants. Enzymes from different species (isoenzyme) exhibited different kinetics properties (e.g., substrate affinity, catalytic efficiency) due to they have different protein structure (Purich, 2010). Soil enzyme kinetics reflects the affinity and catalytic reaction rate of soil enzyme (Song et al., 2019), which conforms with Michaelis-Menten model (Marangoni, 2002; Tan et al., 2021), in which the half-saturation constant (K_m) and urease decomposition rates at saturating substrate concentration (V_{max}) are important kinetic parameters (Marangoni, 2002). K_m indicates the substrate affinity of the enzyme and represents the enzyme performance (Sinsabaugh et al., 2014), and V_{max} indicates the maximum catalytic velocity of enzyme at the saturated substrate concentration (Wang et al., 2020b). An isoenzyme system with a higher substrate affinity is normally essential to maintain nutrient flow efficiently in the agricultural and forest soil ecosystems. However, this is not necessarily true for urease. The rapid decomposition of urea accelerates ammonia (NH_3) volatilisation into the atmosphere, and cause water pollution through NH_3 deposition (Sun

* Corresponding author.
** Corresponding author at: Jiangxi Provincial Key Laboratory of Silviculture, College of Forestry, Jiangxi Agricultural University, Nanchang 330045, China.
E-mail addresses: zhangwenyuan@jxau.edu.cn (W. Zhang), manyunzhang@126.com (M. Zhang).

https://doi.org/10.1016/j.indcrop.2025.120761
Received 19 December 2024; Received in revised form 11 February 2025; Accepted 21 February 2025
Available online 27 February 2025

et al., 2023). Therefore, the urease with high affinity urease affinity for urea can rapidly decompose urea in soil, which would be disadvantage in the retention of nitrogen (N) in both agricultural and forestry ecosystems and may accelerate environmental risks.

Enzyme kinetic parameters (K_m and V_{max}) are inherent properties that depend on protein structure (Srinivasan, 2022). Abiotic factors, such as soil nutrient conditions, have a strong effect on enzyme kinetics (Wu et al., 2022). N addition resulted in lower K_m and higher V_{max} values for N-cycling enzymes (Stone et al., 2012). Tischer et al. (2015) indicated a significant correlation between the substrate affinity of the carbon (C)-cycling enzymes and dissolved organic carbon (DOC). Biotic factors, such as soil microbial community composition, could also affect the kinetic parameters of soil enzymes. Enzymes from different species exhibit different kinetics properties (Purich, 2010). The composition of isoenzyme is associated with soil microbial community composition (Tan et al., 2020). Changes in the abundance of aerobic heterotrophic microorganisms could alter the kinetic properties of soil invertase and alkaline phosphomonoesterase (Tan et al., 2021). Soil microbial biomass is also regulating factors of the enzyme kinetics (Wang et al., 2023a). Therefore, the kinetics properties of soil enzymes are determined by multiple isoenzymes and affected by multiple factors, including abiotic and biotic factors (Aguilar-Pontes et al., 2018). Studies on the influence of soil properties on enzymes from an enzyme kinetics perspective have attracted considerable attention recently, similar studies in soil urease research have rarely been reported (Srinivasan, 2022). The urease catalyzes the hydrolysis of urea and plays a crucial role in N mineralization. Clarifying the dominant factors of urease kinetic properties and exploring their regulatory mechanisms is important for regulating the catalytic process of soil urease and improving soil nitrogen utilization efficiency. Examining the determinants of urease kinetics is also critical for minimizing environmental impacts of soil N management practices in agricultural and forestry ecosystems. However, an incomplete understanding of these determinants on enzyme kinetic hampers the optimization of soil N management practices for agricultural and forestry ecosystems. In this study, we developed the hypothesis: Abiotic factors outweighed the microbial community composition in driving urease kinetics in different soils.

Fertiliser efficiency enhancers and soil conditioners have been used with urea to reduce N loss and avoid the environmental risks of excessive fertilisers, such as dicyandiamide (DCD) and biochar (Luo et al., 2016; Weiske et al., 2001). Nonetheless, the application of biochar or DCD has a risk of causing soil ammonium nitrogen (NH_4^+-N) accumulation (Nelissen et al., 2012; Yang et al., 2022). Changes in soil NH_4^+-N concentrations may influence soil microbial community composition and then affect the secretion of urease isoenzymes (Liu et al., 2020). Therefore, we hypothesised that biochar and DCD could affect soil urease kinetics by changing soil mineral N content.

In the present study, bamboo-derived biochar and DCD were applied to various soil types (forest and agricultural soils), and soil urease kinetic parameters, abiotic properties and microbial communities in various treatments were examined. The study aims to (1) quantify the influences of biotic and abiotic factors on soil urease kinetics, (2) reveal the most significant factors influencing urease kinetics in different soils, and (3) examine how the applications of biochar and DCD modify the substrate affinity of soil urease. This study could facilitate accurately regulating soil urease activity and provide a theoretical basis for optimising biochar and DCD usage in different soils.

2. Materials and methods

2.1. Experimental materials

The forest and agricultural soils were taken from Meiling National Forest Park (28°45'48"N, 115°50'6"E), and educational base of Jiangxi Agricultural University (28°41'24"N, 115°51'36"E) in Nanchang City, China, respectively. The main plants grown at these sites were artificial Masson's pine and oilseed rapeseed. The soil used in the experiment were gathered randomly from five representative points in each site, then mixed and sieved to < 2 mm, respectively. The DCD was sourced from Macklin Biochemical Co., Ltd., Shanghai, China, while the bamboo-derived biochar was processed from *Phyllostachys edulis* through pyrolysed at 300°C. The chemical properties of the soils and the biochar were listed in Table S1. For the scanning electron microscope (SEM) morphologies of the biochar, see our previously published article (Zheng et al., 2023).

2.2. Experimental design

Four treatments were used for each soil type (forest and agricultural soils), with four replicates for each treatment: (1) blank control (CK), (2) bamboo-derived biochar application (BC), (3) DCD application (DC), and (4) combined application of bamboo-derived biochar and DCD (CBAD). There were thirty-two soil subsamples, distributed across four treatments. Each treatment was replicated four times, and encompassing two distinct soil types.

The sieved soil samples (< 2 mm) were sprayed with urea (analytically pure) solution at the rate of 100 mg N kg^{-1} dry soil. 80 g of dry soil was mixed with biochar at a 97 %:3 % (w/w) and placed into each 250 mL flask for the BC and CBAD treatments. 80 g of dry soil of the soil was directly placed into each 250 mL flask for CK and DC treatments. DCD solution was added at a rate of 1 % (w/w) of the applied nitrogen in DC and CBAD treatments. Each flask was covered with plastic film punctured with ventilation holes and incubated at 25°C. Maintained the soil moisture in each flask at 60 % of its water-holding capacity during incubation. After 35 d of incubation, soil samples were collected from each flask and divided into three parts for soil chemical analyses, urease activity analyses and soil DNA extraction.

2.3. Urease kinetic parameter analyses

To determine soil urease activity, 1 g of soil was treated with toluene for 15 min in a 50 mL conical centrifuge tube. Subsequently, 2 mL urea solutions at different concentrations (0.005, 0.01, 0.05, 0.1, 0.2, 0.3, 0.5, 1, 1.5, 2 and 2.5 mol L^{-1}) and 4 mL citrate buffer solution were introduced (Bhaduri et al., 2016). The NH_4^+-N content of the mixed soil suspensions were determined after incubation. The K_m and V_{max} of urease were determined using Eq. (1) (Marangoni, 2002) as follows:

$$\frac{1}{t} \times \ln\left(\frac{[S_0]}{[S_t]}\right) = -\frac{1}{K_m} \times \frac{[S_0] - [S_t]}{t} + \frac{V_{\max}}{K_m} \quad (1)$$

where t is the reaction time (h) of urease enzyme, and S_0 (mmol L^{-1}) and S_t (mmol L^{-1}) represent the initial and point-in-time substrate concentration, respectively. V_{max} and K_m are the decomposition rates at saturating substrate concentration of the enzyme and the affinity to substrates, respectively (Tan et al., 2021).

2.4. Soil chemical property analyses

Soil pH was determined using a pH meter in a 1:2.5 (w/v) soil-water solution. DOC and dissolved organic nitrogen (DON) were determined using the Multi N/C 2100 Analyser (Analytik Jena, Germany) in a 1:12.5 (w/v) soil-water suspension. The assay methods for NH_4^+-N and nitrate-nitrogen (NO_3^--N) were based on O'Callaghan et al. (2010).

2.5. Amplicon sequencing and sequenced data analysis

The soil DNA was extracted with soil DNA Extraction Kit (Omega Bio-tek, Norcross, GA, U.S.). 338F_806R and ITS1F_ITS2R were used to amplify the 16S and ITS genes, respectively (Xia et al., 2024). The amplified products were sequenced on the *MiSeq* PE300 platform (Illumina, San Diego, USA). Sequencing data have been uploaded to the

NCBI for Biotechnology Information database (accession number PRJNA1009014). The high-quality 16S rRNA and ITS reads were classified with the SILVA and UNITE databases, respectively (Quast et al., 2013). Table S2 presents essential details regarding the effective sequences of the different samples.

The ureolytic microorganisms were identified using PICRUSt2 (Douglas et al., 2020). The sum of the ureA (K01430), ureB (K01429) and ureC (K01428) abundances were selected to represent the abundance of soil ureolytic microorganisms.

2.6. Statistical analysis

A one-way analysis, followed by Duncan's test, was performed with the SPSS (IBM, USA). Principal component analysis (PCA) followed by the analysis of similarity (ANOSIM) was performed with the "*vegan*" package. The relationship between the first principal components of PCA and soil chemical properties, soil microbial community structures, and diversity were showed in supplement (Table S4 and S5). Pathway analysis was performed with the SPSS Amos 26.0 (IBM, USA). Use R software for redundancy analysis (RDA) to detect the relationships between the kinetic parameters, soil biotic and abiotic properties. Variation partitioning analysis (VPA) were utilized to analyse the contributions of soil biotic and abiotic properties on urease kinetics.

3. Results

3.1. Soil urease kinetic parameters

The K_m values of urease in the BC, DC and CBAD treatments showed significant ($P < 0.05$) reduction compared to those in the CK treatment in both forest and agricultural soils (Fig. 1a). The average K_m value of urease in the CK treatment for forest soil was 0.11 mol L^{-1} (Fig. 1a). Compared to the CK treatment, the K_m values of urease in the BC, DC and CBAD treatments for forest soil significantly ($P < 0.05$) decreased by 28 %, 73 % and 45 %, respectively (Fig. 1a). The average K_m value of urease in the CK treatment for the agricultural soil was 0.24 mol l^{-1}

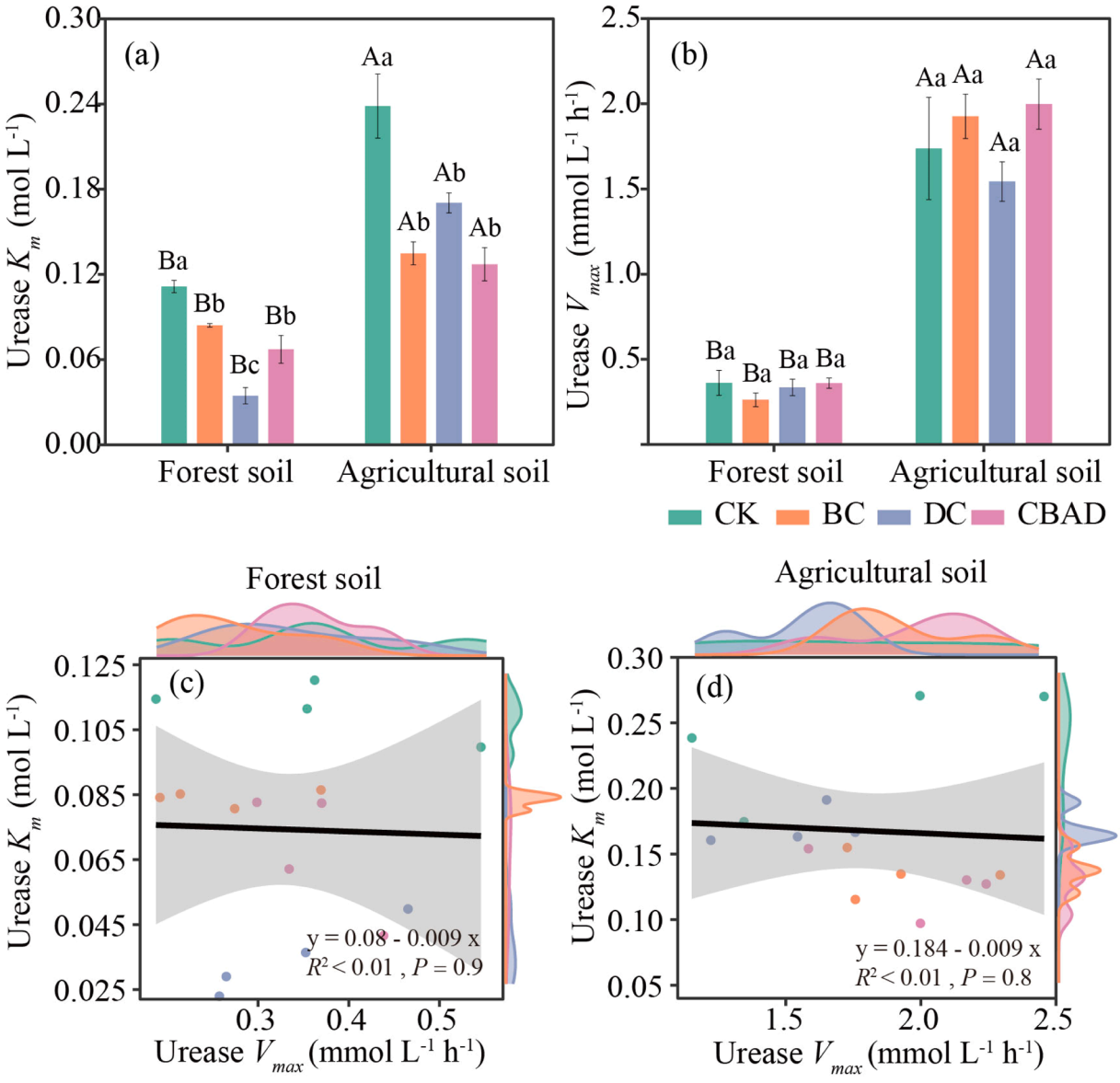


Fig. 1. Effects of biochar and DCD applications on the (a) K_m and (b) V_{max} values of urease kinetics of the forest and agricultural soils, and the regressions between K_m and V_{max} of urease of the (c) forest and (d) agricultural soils. CK: blank control; BC: biochar application; DC: DCD application; CBAD: combined biochar and DCD application. Different capital letters represent significant differences between forest and agricultural soils, and different lower-case letters represent significant differences among the treatments, at $P < 0.05$.

(Fig. 1a). In the agricultural soil, the K_m values of urease in the BC, DC and CBAD treatments were 0.13, 0.17 and 0.13 mol L^{-1}, respectively, demonstrated a significant ($P < 0.05$) decrease compared to those in the CK treatment (Fig. 1a). There was no significant difference in V_{max} of urease between the CK, BC, DC and CBAD treatments in forest and agricultural soils (Fig. 1b). There was no significant correlation between K_m and V_{max} of urease in either soil type (Fig. 1c, d). Regardless of the treatment, the average K_m and V_{max} values of urease were higher in agricultural soil than in forest soil (Fig. 1a, b).

3.2. Soil chemical properties

The average DOC content was 0.50 g kg^{-1} in the CBAD treatment, which was the lowest in the forest soil ($P < 0.05$, Fig. 2a). The DOC content of the agricultural soil in the four treatments was in the order of CBAD > BC > DC > CK (Fig. 2a). Regardless of the treatment, the DOC content was significantly ($P < 0.05$) lower in forest soils than in agricultural soils (Fig. 2a). The DON contents in the CK treatment for the forest and the agricultural soils were 60.6 and 21.5 mg kg^{-1} dry soil, respectively. The BC and CBAD treatments exhibited significantly ($P < 0.05$) higher levels of DON than the CK treatment in the forest soils (Fig. 2b). The NH_4^+-N in CK treatment for forest and agricultural soils were 74.5 and 13.2 mg kg^{-1}, respectively (Fig. 2c). Relative to the CK treatment of forest soil, the NH_4^+-N content decreased by 62 % and 32 % in the BC and CBAD treatments, respectively ($P < 0.05$, Fig. 2c). The NH_4^+-N content in the DC treatment was the highest in both the forest and agricultural soils. In the forest soils, the lowest NO_3^--N content was consistently observed in the DC treatment ($P < 0.05$, Fig. 2d). The NO_3^--N content in the BC treatment was the highest in the forest and agricultural soils ($P < 0.05$, Fig. 2d). The NH_4^+-N and NO_3^--N contents in forest soils under CK, BC, DC and CBAD treatments were significantly greater compared to those in agricultural soils for the corresponding treatments ($P < 0.05$, Fig. 2c, d). The CBAD treatment resulted in the highest pH levels regardless of soil type ($P < 0.05$, Fig. 2e).

3.3. Soil microbial community compositions and diversities

The combination of biochar and DCD significantly changed the bacterial and fungal community structure in agricultural soils (Fig. 3, Table S3). The abundance of soil ureolytic fungi was higher than that of ureolytic bacteria, regardless of soil type and treatment (Fig. 3e). Compared to the CK treatment, the BC and CBAD treatments showed higher levels of the ACE and Chao1 richness estimators for bacterial community in the forest soils, and resulted in lower levels of the ACE and Chao1 richness estimators in the agricultural soils ($P < 0.05$, Table 1). Compared with the CK treatment, the ACE and Chao1 of the bacterial community in the DC treatment of the agricultural soil were reduced by 2.5 % and 2.6 %, respectively ($P < 0.05$, Table 1). For the forest soil, lowest fungal community Shannon indices (Table 2) were observed in the BC treatment (2.66 ± 0.11) and the CBAD treatment (2.7 ± 0.09). In the forest soils, the Simpson indices of fungal community increased in the BC or CBAD treatment ($P < 0.05$, Table 2). In the agricultural soil, the ACE richness estimator of the fungal community of the CBAD treatment was significantly ($P < 0.05$) lower than that of the CK treatment (Table 2).

The Shannon, ACE, and Chao1 indices of the bacterial and fungal community in forest soils were lower than those in agricultural soils for the respective treatments, irrespective of treatment type ($P < 0.05$, Tables 1 and 2). The Shannon and Chao1 indices of CBAD treatment were lower in forest soils than those in agricultural soils ($P < 0.05$, Table 3).

3.4. The comprehensive analyses of biotic and abiotic factors affecting urease kinetic parameters

There were negative correlations between the urease kinetic K_m and DON, NO_3^--N and NH_4^+-N contents, but a positive correlation between urease kinetic K_m and DOC content ($P < 0.05$, Fig. 4). The urease kinetic V_{max} was negatively correlated with NH_4^+-N and NO_3^--N contents but was positively correlated with DOC content and pH ($P < 0.05$, Fig. 4). The RDA results emphasised the significant ($P < 0.05$) effects of NH_4^+-N, NO_3^--N, DON and Shannon indices on soil urease K_m (Fig. 5b). The VPA results revealed that soil chemical properties were the most significant factors affecting the forest soil urease K_m ($R^2 = 0.15$; Fig. 5c). The contribution percentages of abiotic and biotic factors to the variability of K_m were 58.6 % and 41.37 %, respectively (Table 3). Overall, the chemical properties outweighed the microbial community structures and diversities driving urease kinetics in various soils.

4. Discussion

4.1. The relationships of urease kinetics to soil biotic and abiotic properties

The Pathway analysis showed a direct relationship between urease kinetics and soil chemical properties (DON, NO_3^--N, NH_4^+-N and pH) in both forest and agricultural soils ($P < 0.05$, Fig. 5a). Available N can limit urease secretion in the soil and change the K_m value of urease because N is the key component of urease (Proshlyakov et al., 2021). The resources allocation theory tells us microbes assign more resources to produce enzymes for energy or other nutrients when N availability increases (Stone et al., 2012). As resource allocation tends to favour the acquisition of other nutrients, the enzymes involved in N acquisition become more efficient, leading to an increase in the substrate affinity of urease. However, generating more efficient isoenzymes requires more energy and resources, because efficient isoenzymes have more complex chemical structures (Min and Suseela, 2020). This might explain why the substrate affinity of urease increased as the soil mineral nitrogen contents ($P < 0.05$, Fig. 4).

Effects of abiotic factors (contribution percentage: 58.6 %) on urease kinetics were higher than those of the microbial community (contribution percentage: 41.37 %) (Table 3). Tan et al. (2021) found the same trend in agricultural soils where winter wheat was planted. This confirmed the hypothesis that abiotic factors outweighed the microbial community composition in driving urease kinetics. The mineral N contents determined urease synthesis as a key component for urease synthesis and mediate urease synthesis through controlling the soil microbial metabolism (Wang et al., 2023a; Yang et al., 2016). Additionally, the mineral N content can affect the urease-catalysed hydrolysis of urea (Proshlyakov et al., 2021). Thus, mineral N content may have played a dominant role in influencing urease kinetics. Microorganisms secrete soil urease (Alizadeh et al., 2017; Krajewska, 2009), while also regulate the kinetic properties of soil enzymes (Hamer et al., 2021). Fungal community diversity was positively associated with urease kinetics K_m ($P < 0.05$, Fig. 5). The relationship between the soil microorganism communities and urease K_m was also observed by Tan et al. (2021). This may be because fungal groups are the dominant urease producers in soils treated with urea (Fig. 3e) (Alizadeh et al., 2017).

4.2. Soil chemical and microbial properties

The DON and NO_3^--N contents increased with the application of biochar (Fig. 2). This may be attributed to the mineralization of soil organic N following biochar application (Ameloot et al., 2014). However, in certain scenarios, the biochar application could also decrease the NO_3^--N content (Zou et al., 2023), and the reason for these inconsistent results may be differences in soil moisture content, organic matter and biochar quality (Hale et al., 2023). The NH_4^+-N content was also increased with the DCD application ($P < 0.05$, Fig. 2c). The reasons for the accumulation of NH_4^+-N may be that the nitrification inhibition by DCD led to a reduction in NH_4^+-N transformation to NO_3^--N (O'Callaghan et al., 2010).

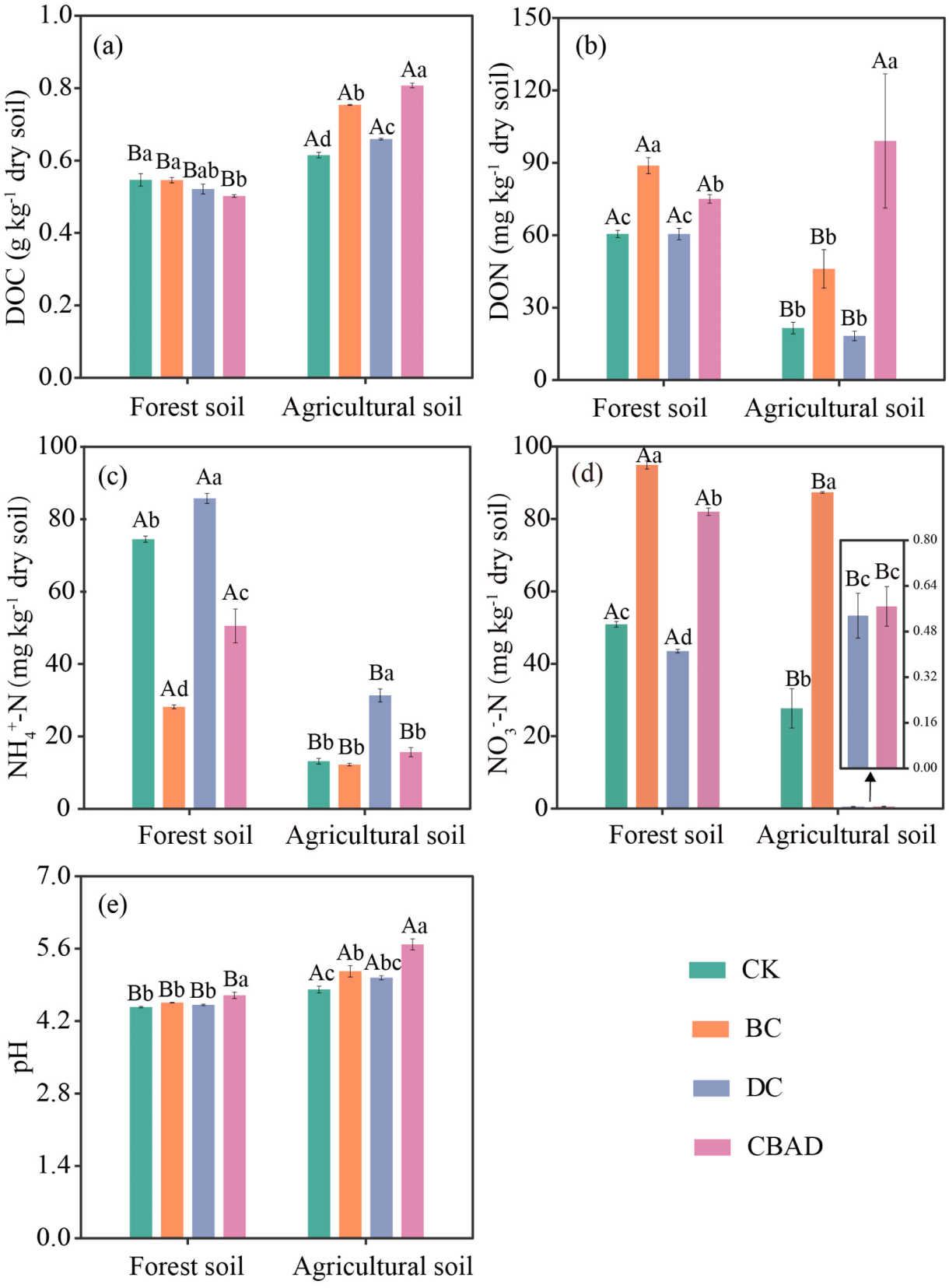


Fig. 2. Effects of biochar and DCD applications on the (a) DOC, (b) DON, (c) NH_4^+-N, (d) NO_3^--N and (e) pH of forest and agricultural soils. CK: blank control; BC: biochar application; DC: DCD application; CBAD: combined biochar and DCD application. Different capital letters represent significant differences between forest and agricultural soils, and different lower-case letters represent significant differences among the treatments, at $P < 0.05$.

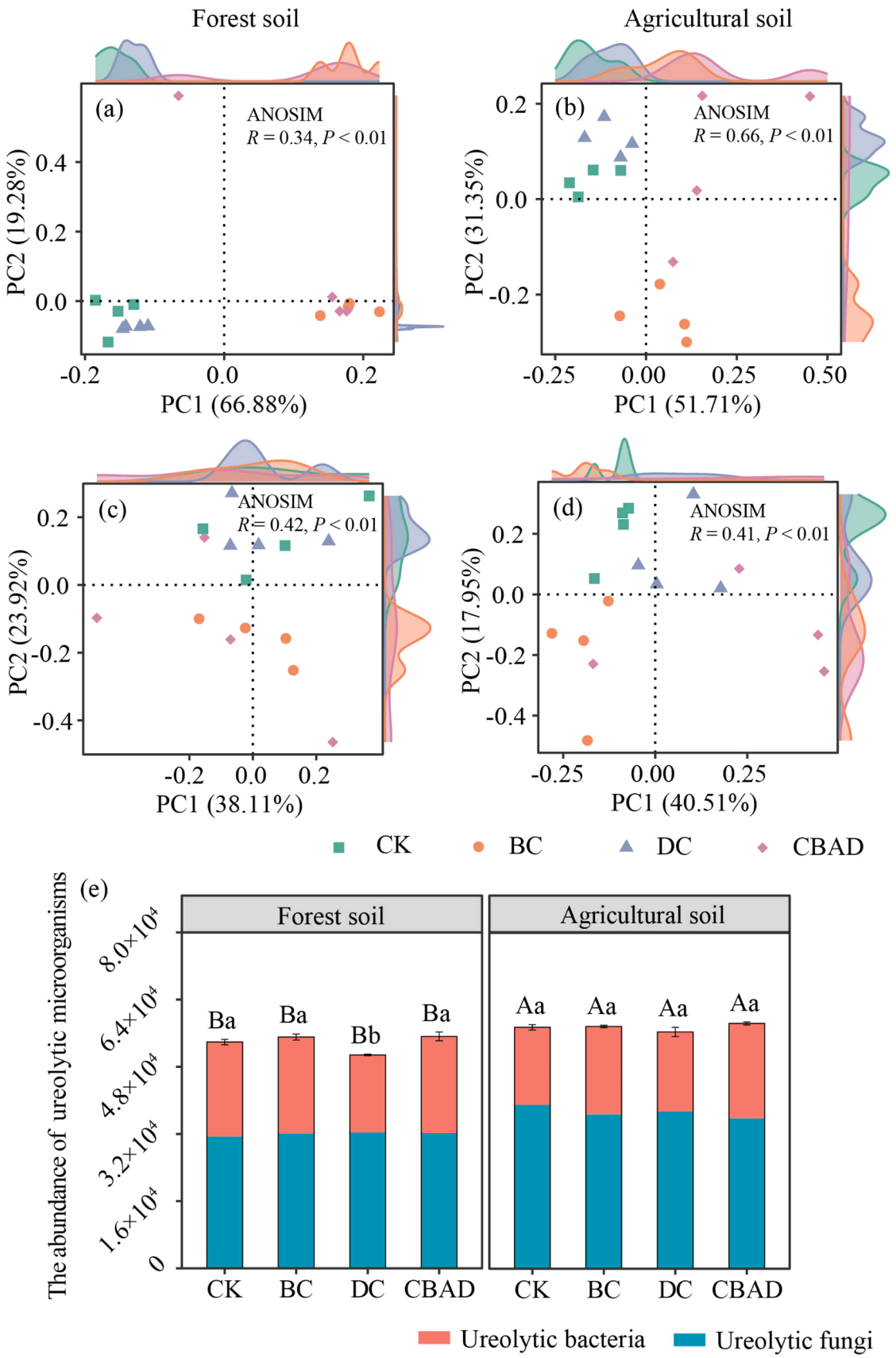


Fig. 3. Effects of biochar and DCD applications on bacterial community structure at genus level of the (a) forest and (b) agricultural soils, on fungal community structure at genus level of the (c) forest and (d) agricultural soils, and on the abundance of (e) soil ureolytic microorganisms. CK: blank control; BC: biochar application; DC: DCD application; CBAD: combined biochar and DCD application. Different capital letters represent significant differences between forest and agricultural soils, and different lower-case letters represent significant differences among the treatments, at $P < 0.05$.

Table 1
Effects of bamboo-derived biochar and DCD applications on bacterial community diversity of forest and agricultural soils.

Treatment	Forest soil				Agricultural soil			
	Shannon	Simpson	ACE	Chao1	Shannon	Simpson	ACE	Chao1
CK	3.73 ± 0.06 Ba	0.066 ± 0.006 Aa	391 ± 3 Bb	398 ± 2 Bb	4.79 ± 0.04 Aa	0.024 ± 0.001 Ba	722 ± 5 Aa	723 ± 4 Aa
BC	3.81 ± 0.04 Ba	0.063 ± 0.003 Aa	413 ± 7 Ba	424 ± 10 Ba	4.85 ± 0.02 Aa	0.018 ± 0.001 Bb	702 ± 3 Ab	701 ± 2 Ab
DC	3.69 ± 0.02 Ba	0.067 ± 0.001 Aa	402 ± 2 Bab	406 ± 1 Bab	4.78 ± 0.05 Aa	0.024 ± 0.003 Ba	704 ± 4 Ab	704 ± 4 Ab
CBAD	3.84 ± 0.03 Ba	0.059 ± 0.003 Aa	403 ± 1 Bab	420 ± 5 Ba	4.83 ± 0.05 Aa	0.018 ± 0.001 Bb	697 ± 7 Ab	701 ± 7 Ab

CK: blank control; BC: bamboo-derived biochar application; DC: DCD application; CBAD: combined bamboo-derived biochar and DCD application. Different capital letters represent significant differences between forest and agricultural soils, and different lower-case letters represent significant differences among the treatments, at $P < 0.05$.

Table 2
Effects of bamboo-derived biochar and DCD applications on fungal diversity of forest and agricultural soils.

Treatment	Forest soil				Agricultural soil			
	Shannon	Simpson	ACE	Chao1	Shannon	Simpson	ACE	Chao1
CK	2.97 ± 0.06 Ba	0.11 ± 0.01 Ab	178 ± 11 Ba	179 ± 12 Ba	3.32 ± 0.02 Aa	0.07 ± 0 Ba	249 ± 8 Aab	239 ± 7 Ab
BC	2.66 ± 0.11 Bb	0.17 ± 0.02 Aa	186 ± 6 Ba	185 ± 5 Ba	3.23 ± 0.04 Aa	0.08 ± 0 Ba	257 ± 4 Aa	264 ± 4 Aa
DC	2.91 ± 0.02 Bab	0.13 ± 0 Aab	191 ± 11 Ba	193 ± 12 Ba	3.26 ± 0.02 Aa	0.07 ± 0 Ba	236 ± 5 Abc	237 ± 6 Ab
CBAD	2.7 ± 0.09 Bb	0.17 ± 0.02 Aa	181 ± 17 Aa	179 ± 16 Ba	3.15 ± 0.14 Aa	0.09 ± 0.02 Ba	226 ± 7 Ac	224 ± 6 Ab

CK: blank control; BC: bamboo-derived biochar application; DC: DCD application; CBAD: combined bamboo-derived biochar and DCD application. Different capital letters represent significant differences between forest and agricultural soils, and different lower-case letters represent significant differences among the treatments, at $P < 0.05$.

Table 3
The significance and contribution percentage of soil abiotic properties, the community structure and diversity of microorganisms to the variabilities of K_m of urease.

	Variables	R^2	*P*	Contribution percentage	Comprehensive percentage
Soil abiotic properties	DOC	0.109	0.028*	13.45 %	58.6 %
	DON	0.062	0.078	7.7 %	
	NH_4^+-N	0.160	0.001*	19.85 %	
	NO_3^--N	0.056	0.083	6.93 %	
	pH	0.086	0.045*	10.67 %	
Microbial community structure and diversity	Bacterial community structure	0.007	0.289	0.9 %	41.37 %
	Shannon index of bacteria	0.158	0.010*	19.6 %	
	Fungal community structure	0.003	0.304	0.36 %	
	Shannon index of fungi	0.166	0.011*	20.51 %	

The significance was analyzed by a Monte Carlo test with 999 permutations. The "*" indicate that the significance at $P < 0.05$.

Biochar changed bacterial and fungal community structure in forest and agricultural soils ($P < 0.05$, Fig. 3). Biochar, characterised by its carbon-rich, porous nature, and high pH, might improve the soil organic carbon content, soil aeration, and pH levels (Dai et al., 2021; Ding et al., 2023; Zheng et al., 2023). These alterations in soil chemical properties could change the microbial community structures (Geisseler et al., 2017; Tao et al., 2023). Biochar improved the fungal community diversity ($P < 0.05$, Table 2). The density and porosity of biochar could expend soil ecological niche, which might improve fungal community diversity (Ren et al., 2022). DCD had a negligible effect on the fungal community diversity in forest and agricultural soils (Table 2), and its impacts was influenced by soil type (Morales et al., 2015).

4.3. Soil urease kinetics

Biochar and DCD application caused a significant decrease in the urease kinetic K_m in both soils (Fig. 1a). The soil enzyme kinetics are sensitive to the alteration of their physical and chemical properties (Baker and Allison, 2017). The reduction in the kinetic K_m of urease with the applications of biochar and DCD may be due to the increased content of mineral N, which increases the affinity of urease for substrates (Hu et al., 2021a; Wang et al., 2023b). In addition, biochar can improve soil aggregate stability, which may enhance substrate affinity for urease (Frankenberger and Johanson, 1982; Zhao et al., 2023). Neither biochar nor DCD affected the urease kinetic *V*max (Fig. 1b). The urease kinetic V_{max} represents potential urease activity and relates to the overall isoenzyme concentration of urease (Tischer et al., 2015). Biochar and DCD may not change the isoenzyme concentration of urease because the abundance of soil ureolytic microorganisms remained unchanged with the BC, DC and CBAD treatments (Fig. 3e). In addition, we determined that changes in urease kinetic K_m did not correlate with urease kinetic V_{max} (Fig. 1). The urease kinetic K_m is regulated by its chemical structure of urease, while the urease kinetic V_{max} is regulated by its isoenzyme concentration of urease (Min and Suseela, 2020; Tischer et al., 2015). The uncorrelated relationship between urease kinetics, K_m and V_{max} may be related to differences in regulatory factors. The increased affinity of soil urease suggested that it could achieve its maximum reaction rate more rapidly (Marangoni, 2002), which might increase the pressure on soil nitrogen fixation. Therefore, N fixation measures should be promptly implemented in soils treated with biochar and nitrification inhibitors, such as inoculating N-fixing strains and protecting core microbiota associated with N fixation (Chang et al., 2025; Chen et al., 2024).

Urease had higher K_m values in agricultural soils compared to forest soils. (Fig. 1a). This difference could be attributed to the lower availability of mineral N in agricultural soils, as observed in our study (Fig. 2c, d). According to our previous discussion, the concentration of mineral N is a primary determinant of urease kinetics, and the richer the mineral nitrogen, the lower the K_m value. Our results showed that the higher V_{max} values of urease were obtained in agricultural soils compared to forest soils (Fig. 1b). This result may have been related to the abundance of soil ureolytic microorganisms (Fig. 3e). The V_{max} of

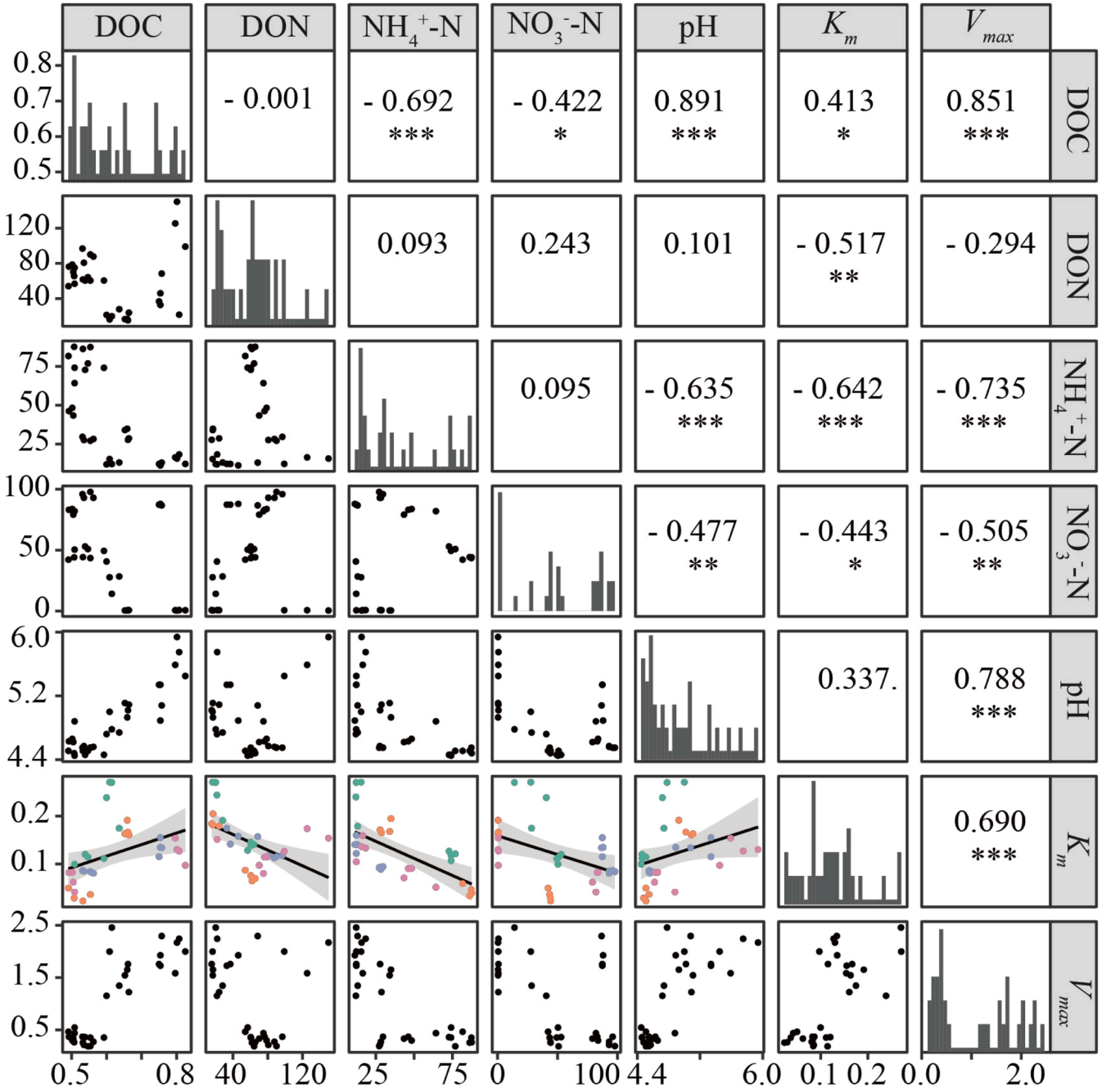


Fig. 4. The Pearson's correlations between the soil chemical properties and urease kinetic parameters. The "*, **, ***" indicate that the significances at $P < 0.05$, 0.01 and 0.001, respectively.

urease reflected the concentration of soil urease (Zhao et al., 2023). Soil urease mainly secreted from the soil ureolytic microorganisms (Hu et al., 2021b). Fisher et al. (2017) reported that ureolytic microbial community abundance was positively correlated with soil pH. Therefore, soil pH could also affect the V_{max} of urease, because ureolytic microbial community abundance was altered by soil pH (Wang et al., 2020a).

5. Conclusion

The agricultural soils exhibited a higher catalytic potential and max catalytic rate of urease than forest soils. The applications of bamboo-derived biochar and DCD improved the catalytic potential of urease in the agricultural and forest soils, which will not be conducive to the retention of nitrogen in soils to which biochar or DCD has been added. Increased catalytic potential of soil urease under the application of biochar or DCD alone associated with increased mineral N content and fungal community diversity. Increased catalytic potential of soil urease under the combined biochar and DCD application related to the increased content of mineral N. In agricultural and forest soils treated with biochar and DCD, fungal community diversity was closely correlated with urease kinetics in agricultural and forest soils, but mineral N content may have played a more dominant role in influencing urease kinetics. Our results suggested that abiotic factors such as mineral N content or pH were more important drivers of soil urease kinetics than the structures and diversities of microbial communities. These findings highlighted the necessity of nitrogen-fixing measures in soils amended with bamboo-derived biochar or DCD and advanced our comprehension of how abiotic and biotic factors influence the urease kinetics in

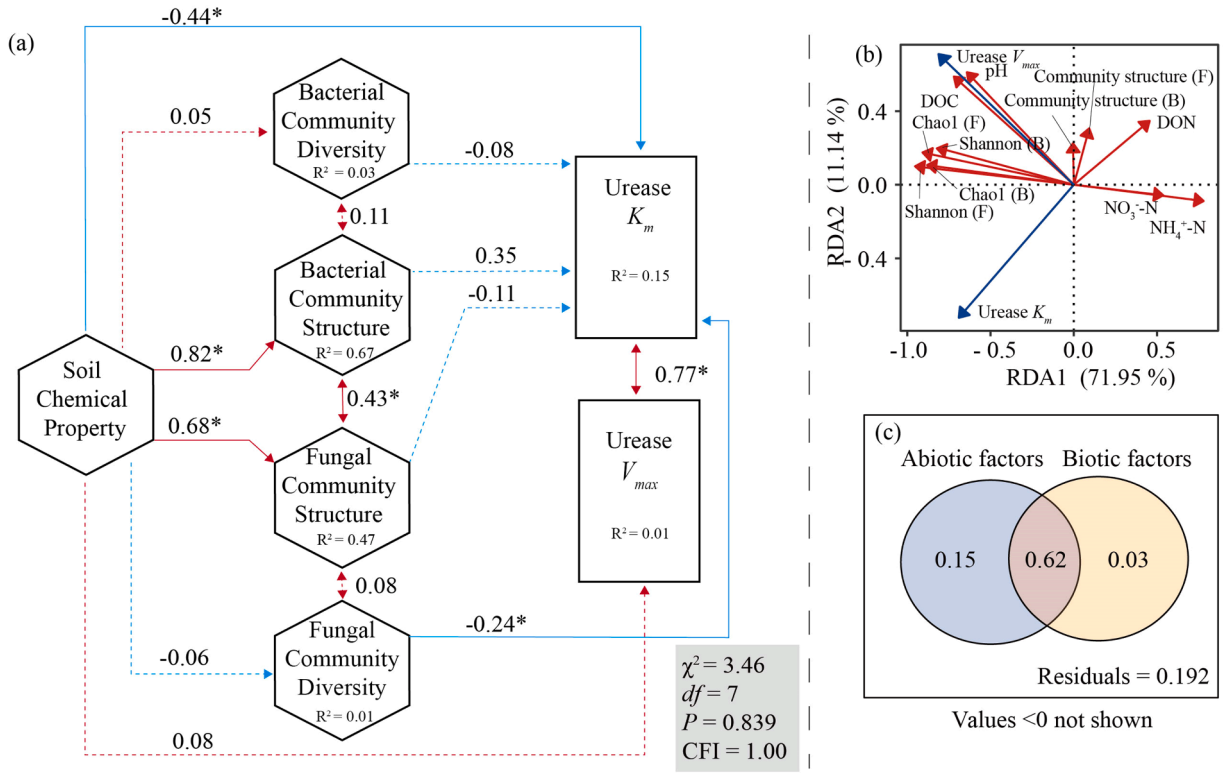


Fig. 5. Relationship between urease kinetic properties of forest and agricultural soils and soil abiotic and biotic factors. (a) Pathway analysis revealed the influencing modes of the soil chemical properties, diversity and communities of bacteria and fungi on urease kinetic properties. The variables in hexagon boxes represent the first principal components. The values near the path were standardized path coefficients. The red and blue lines indicate positive and negative path coefficients, respectively. The solid lines represent significant paths at $P < 0.05$ and dashed lines represent non-significant paths. *: $P < 0.05$, **: $P < 0.01$, and ***: $P < 0.001$. R^2 values represent the proportion of variance explained by each variable. (b) Comprehensive linkages among soil chemical properties, bacterial and fungal community structure and the urease kinetic parameters. Capital letters in brackets indicate the microbial taxa: B, bacteria; F, fungi. (c) Venn diagrams visualizing the variances in the K_m and V_{max} values of urease explained by soil abiotic (such as DOC, DON, NH_4^+-N, NO_3^--N, pH) and biotic factors (such as bacterial and fungal community characteristics).

agricultural and forest soils.

Funding

This work was supported by the National Natural Science Foundation of China (Grant No. 32360431), the Science and Technology Innovation Program of Jiangxi provincial department of forestry (No. 202310), and the Special Research Project on *Camellia oleifera* of Jiangxi provincial department of forestry (No. YCYJZX2023111).

CRediT authorship contribution statement

Qiyuan Zheng: Writing – original draft, Methodology. **Weijin Wang:** Writing – review & editing. **Pengcheng Zhang:** Writing – review & editing, Methodology. **Meng Zhang:** Writing – review & editing, Methodology. **Fen Yu:** Writing – review & editing, Methodology. **Lin Yu:** Writing – review & editing. **Weiyi Liu:** Writing – review & editing. **Wenyuan Zhang:** Writing – review & editing, Supervision, Investigation, Funding acquisition, Conceptualization. **Manyun Zhang:** Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.indcrop.2025.120761.

Appendix B. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.indcrop.2025.120761.

Data availability

Data will be made available on request.

References

Aguilar-Pontes, M.V., Brandl, J., McDonnell, E., Strasser, K., Nguyen, T.T.M., Riley, R., Mondo, S., Salamov, A., Nybo, J.L., Vesth, T.C., Grigoriev, I.V., Andersen, M.R., Tsang, A., de Vries, R.P., 2018. The gold-standard genome of *Aspergillus niger* NRRL 3 enables a detailed view of the diversity of sugar catabolism in fungi. Stud. Mycol. 91, 61–78. https://doi.org/10.1016/j.simyco.2018.10.001.

Alizadeh, H., Kandula, D.R.W., Hampton, J.G., Stewart, A., Leung, D.W.M., Edwards, Y., Smith, C., 2017. Urease producing microorganisms under dairy pasture management in soils across New Zealand. Geoderma Reg. 11, 78–85. https://doi.org/10.1016/j.geodrs.2017.10.003.

Ameloot, N., Sleutel, S., Case, S.D.C., Alberti, G., McNamara, N.P., Zavalloni, C., Vervisch, B., Vedove, Gd, De Neve, S., 2014. C mineralization and microbial activity in four biochar field experiments several years after incorporation. Soil Biol. Biochem. 78, 195–203. https://doi.org/10.1016/j.soilbio.2014.08.004.

Baker, N.R., Allison, S.D., 2017. Extracellular enzyme kinetics and thermodynamics along a climate gradient in southern California. Soil Biol. Biochem. 114, 82–92. https://doi.org/10.1016/j.soilbio.2017.07.005.

Bhaduri, D., Saha, A., Desai, D., Meena, H.N., 2016. Restoration of carbon and microbial activity in salt-induced soil by application of peanut shell biochar during short-term incubation study. Chemosphere 148, 86–98. https://doi.org/10.1016/j.chemosphere.2015.12.130.

Chang, Y., Lin, L., Shen, J., Lin, Z., Deng, X., Sun, W., Wu, X., Wang, Y., Li, Y., Xu, Z., 2025. Enhanced nitrogen fixation and cd passivation in rhizosphere soil by biochar-loaded nitrogen-fixing bacteria: chemisorption and microbial mechanism. J. Hazard. Mater. 481, 136588. https://doi.org/10.1016/j.jhazmat.2024.136588.

Chen, Z., Zhang, K., Su, F., Wang, X., Wang, Z., 2024. Long-term sloping cultivation reduced core microbiota related to nitrogen fixation and soil hydrocarbon degradation. J. Environ. Manag. 370, 122482. https://doi.org/10.1016/j.jenvman.2024.122482.

Dai, Z., Xiong, X., Zhu, H., Xu, H., Leng, P., Li, J., Tang, C., Xu, J., 2021. Association of biochar properties with changes in soil bacterial, fungal and fauna communities and nutrient cycling processes. Biochar 3, 239–254. https://doi.org/10.1007/s42773-021-00099-x.

Ding, X., Li, G., Zhao, X., Lin, Q., Wang, X., 2023. Biochar application significantly increases soil organic carbon under conservation tillage: an 11-year field experiment. Biochar 5, 28. https://doi.org/10.1007/s42773-023-00226-w.

Douglas, G.M., Maffei, V.J., Zaneveld, J.R., Yurgel, S.N., Brown, J.R., Taylor, C.M., Huttenhower, C., Langille, M.G.I., 2020. PICRUSt2 for prediction of metagenome functions. Nat. Biotechnol. 38, 685–688. https://doi.org/10.1038/s41587-020-0548-6.

Fisher, K.A., Yarwood, S.A., James, B.R., 2017. Soil urease activity and bacterial ureC gene copy numbers: effect of pH. Geoderma 285, 1–8. https://doi.org/10.1016/j.geoderma.2016.09.012.

Frankenberger, W.T., Johanson, J.B., 1982. Effect of pH on enzyme stability in soils. Soil Biol. Biochem. 14, 433–437. https://doi.org/10.1016/0038-0717(82)90101-8.

Geisseler, D., Linquist, B.A., Lazicki, P.A., 2017. Effect of fertilization on soil microorganisms in paddy rice system–a meta-analysis. Soil Biol. Biochem. 115, 452–460. https://doi.org/10.1016/j.soilbio.2017.09.018.

Hale, L., Hendratna, A., Scott, N., Gao, S., 2023. Biochar enhancement of nitrification processes varies with soil conditions. Sci. Total Environ. 887, 164146. https://doi.org/10.1016/j.scitotenv.2023.164146.

Hamer, U., Meyer, M.U.T., Meyer, U.N., Radermacher, A., Götze, P., Koch, H.J., Scherber, C., 2021. Soil microbial biomass and enzyme kinetics for the assessment of temporal diversification in agroecosystems. Basic Appl. Ecol. 53, 143–153. https://doi.org/10.1016/j.baae.2021.03.014.

Hu, X., Liu, X., Qiao, L., Zhang, S., Su, K., Qiu, Z., Li, X., Zhao, Q., Yu, C., 2021b. Study on the spatial distribution of ureolytic microorganisms in farmland soil around tailings with different heavy metal pollution. Sci. Total Environ. 775, 144946. https://doi.org/10.1016/j.scitotenv.2021.144946.

Hu, F., Xu, C., Ma, R., Tu, K., Yang, J., Zhao, S., Yang, M., Zhang, F., 2021a. Biochar application driven change in soil internal forces improves aggregate stability: based on a two-year field study. Geoderma 403, 115276. https://doi.org/10.1016/j.geoderma.2021.115276.

Krajewska, B., 2009. Ureases I. Functional, catalytic and kinetic properties: a review. J. Mol. Catal. B-Enzym. 59, 9–21. https://doi.org/10.1016/j.molcatb.2009.01.003.

Liu, Y., Tan, X., Wang, Y., Guo, Z., He, D., Fu, S., Wan, S., Ye, Q., Zhang, W., Liu, W., Shen, W., 2020. Responses of litter, organic and mineral soil enzyme kinetics to 6 years of canopy and understory nitrogen additions in a temperate forest. Sci. Total Environ. 712, 136383. https://doi.org/10.1016/j.scitotenv.2019.136383.

Luo, X., Chen, L., Zheng, H., Chang, J., Wang, H., Wang, Z., Xing, B., 2016. Biochar addition reduced net N mineralization of a coastal wetland soil in the Yellow River Delta, China. Geoderma 282, 120–128. https://doi.org/10.1016/j.geoderma.2016.07.015.

Marangoni, A.G., 2002. Characterization of enzyme activity. In: Marangoni, A.G. (Ed.), Enzyme Kinetics. John Wiley & Sons Inc, New York, pp. 44–60.

Min, K., Suseela, V., 2020. Plant invasion alters the Michaelis–Menten kinetics of microbial extracellular enzymes and soil organic matter chemistry along soil depth. Biogeochemistry 150, 181–196. https://doi.org/10.1007/s10533-020-00692-5.

Morales, S.E., Jha, N., Saggar, S., 2015. Impact of urine and the application of the nitrification inhibitor DCD on microbial communities in dairy-grazed pasture soils. Soil Biol. Biochem. 88, 344–353. https://doi.org/10.1016/j.soilbio.2015.06.009.

Nelissen, V., Rütting, T., Huygens, D., Staelens, J., Ruysschaert, G., Boeckx, P., 2012. Maize biochars accelerate short-term soil nitrogen dynamics in a loamy sand soil. Soil Biol. Biochem. 55, 20–27. https://doi.org/10.1016/j.soilbio.2012.05.019.

O'Callaghan, M., Gerard, E.M., Carter, P.E., Lardner, R., Sarathchandra, U., Burch, G., Ghani, A., Bell, N., 2010. Effect of the nitrification inhibitor dicyandiamide (DCD) on microbial communities in a pasture soil amended with bovine urine. Soil Biol. Biochem. 42, 1425–1436. https://doi.org/10.1016/j.soilbio.2010.05.003.

Proshlyakov, D.A., Farrugia, M.A., Proshlyakov, Y.D., Hausinger, R.P., 2021. Iron-containing ureases. Coord. Chem. Rev. 448, 214190. https://doi.org/10.1016/j.ccr.2021.214190.

Purich, D.L., 2010. An introduction to enzyme science. In: Purich, D.L. (Ed.), Enzyme Kinetics: Catalysis and Control. Elsevier, Boston, pp. 1–51.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41, D590–D596. https://doi.org/10.1093/nar/gks1219.

Ren, T., Feng, H., Xu, C., Xu, Q., Fu, B., Azwar, E., Wei, Y., Lam, S.S., Liu, G., 2022. Exogenous application and interaction of biochar with environmental factors for improving functional diversity of rhizosphere's microbial community and health. Chemosphere 294, 133710. https://doi.org/10.1016/j.chemosphere.2022.133710.

Sinsabaugh, R.L., Belnap, J., Findlay, S.G., Shah, J.J.F., Hill, B.H., Kuehn, K.A., Kuske, C. R., Litvak, M.E., Martinez, N.G., Moorhead, D.L., Warnock, D.D., 2014. Extracellular enzyme kinetics scale with resource availability. Biogeochemistry 121, 287–304. https://doi.org/10.1007/s10533-014-0030-y.

Song, L., Liu, P., Jiang, W., Guo, Q., Zhang, C., Basit, A., Li, Y., Li, J., 2019. α-Lys^{424} participates in insertion of FeMoco to MoFe protein and maintains nitrogenase activity in *Klebsiella oxytoca* M5al. Front. Microbiol. 10. https://doi.org/10.3389/fmicb.2019.00802.

Srinivasan, B., 2022. A guide to the Michaelis–Menten equation: steady state and beyond. FEBS J. 289, 6086–6098. https://doi.org/10.1111/febs.16124.

Stone, M.M., Weiss, M.S., Goodale, C.L., Adams, M.B., Fernandez, I.J., German, D.P., Allison, S.D., 2012. Temperature sensitivity of soil enzyme kinetics under N-fertilization in two temperate forests. Glob. Change Biol. 18, 1173–1184. https://doi.org/10.1111/j.1365-2486.2011.02545.x.

Sun, Q., Zhou, H., Xu, C., Ba, Y., Geng, Z., She, D., 2023. Effective adsorption of ammonium nitrogen by sulfonic-humic acid char and assessment of its recovery for application as nitrogen fertilizer. Sci. Total Environ. 867, 161591. https://doi.org/10.1016/j.scitotenv.2023.161591.

Tan, X., Machmuller, M.B., Cotrufo, M.F., Shen, W., 2020. Shifts in fungal biomass and activities of hydrolase and oxidative enzymes explain different responses of litter decomposition to nitrogen addition. Biol. Fertil. Soils 56, 423–438. https://doi.org/10.1007/s00374-020-01434-3.

Tan, X., Nie, Y., Ma, X., Guo, Z., Liu, Y., Tian, H., Megharaj, M., Shen, W., He, W., 2021. Soil chemical properties rather than the abundance of active and potentially active microorganisms control soil enzyme kinetics. Sci. Total Environ. 770, 144500. https://doi.org/10.1016/j.scitotenv.2020.144500.

Tao, S., Veen, G.F., Zhang, N., Yu, T., Qu, L., 2023. Tree and shrub richness modifies subtropical tree productivity by regulating the diversity and community composition of soil bacteria and archaea. Microbiome 11, 261. https://doi.org/10.1186/s40168-023-01676-x.

Tischer, A., Blagodatskaya, E., Hamer, U., 2015. Microbial community structure and resource availability drive the catalytic efficiency of soil enzymes under land-use change conditions. Soil Biol. Biochem. 89, 226–237. https://doi.org/10.1016/j.soilbio.2015.07.011.

Wang, Y., Pang, J., Zhang, M., Tian, Z., Wei, T., Jia, Z., Ren, X., Zhang, P., 2023b. Is adding biochar be better than crop straw for improving soil aggregates stability and organic carbon contents in film mulched fields in semiarid regions?—Evidence of 5-year field experiment. J. Environ. Manag. 338, 117711. https://doi.org/10.1016/j.jenvman.2023.117711.

Wang, S., Thi Thu Hoang, D., The Luu, A., Mostafa, T., Razavi, B.S., 2023a. Environmental memory of microbes regulates the response of soil enzyme kinetics to extreme water events: drought-rewetting-flooding. Geoderma 437, 116593. https://doi.org/10.1016/j.geoderma.2023.116593.

Wang, Z., Tian, H., Lei, M., Megharaj, M., Tan, X., Wang, F., Jia, H., He, W., 2020b. Soil enzyme kinetics indicate ecotoxicity of long-term arsenic pollution in the soil at field scale. Ecotoxicol. Environ. Saf. 191, 110215. https://doi.org/10.1016/j.ecoenv.2020.110215.

Wang, L., Xiong, X., Luo, X., Chen, W., Wen, S., Wang, B., Chen, C., Huang, Q., 2020a. Aggregational differentiation of ureolytic microbes in an Ultisol under long-term organic and chemical fertilizations. Sci. Total Environ. 716, 137103. https://doi.org/10.1016/j.scitotenv.2020.137103.

Weiske, A., Benckiser, G., Herbert, T., Ottow, J., 2001. Influence of the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) in comparison to dicyandiamide (DCD) on nitrous oxide emissions, carbon dioxide fluxes and methane oxidation during 3 years of repeated application in field experiments. Biol. Fertil. Soils 34, 109–117. https://doi.org/10.1007/s003740100386.

Wu, Y., Zhou, H., Sun, W., Zhao, Q., Liang, M., Chen, W., Guo, Z., Jiang, Y., Jiang, Y., Liu, G., Xue, S., 2022. Temperature sensitivity of soil enzyme kinetics under N and P fertilization in an alpine grassland, China. Sci. Total Environ. 838, 156042. https://doi.org/10.1016/j.scitotenv.2022.156042.

Xia, Y., Wang, S., Zhang, X., Fu, F., Deng, H., Zhao, Y., Yu, H., Ge, C., 2024. Deciphering how endogenous mangrove litterfall influences organic matters transformation driven by microbes in sediment with exogenous microplastics inputs. J. Hazard. Mater. 480, 135763. https://doi.org/10.1016/j.jhazmat.2024.135763.

Yang, Y., Yin, Z., Li, L., Li, Y., Liu, Y., Luo, Y., Li, G., Yuan, J., 2022. Effects of dicyandiamide, phosphogypsum and superphosphate on greenhouse gas emissions during pig manure composting. Sci. Total Environ. 846, 157487. https://doi.org/10.1016/j.scitotenv.2022.157487.

Yang, L., Zhang, L., Geisseler, D., Wu, Z., Gong, P., Xue, Y., Yu, C., Juan, Y., Horwath, W. R., 2016. Available C and N affect the utilization of glycine by soil microorganisms. Geoderma 283, 32–38. https://doi.org/10.1016/j.geoderma.2016.07.022.

Zhao, Z., Wu, Y., Chen, W., Sun, W., Wang, Z., Liu, G., Xue, S., 2023. Soil enzyme kinetics and thermodynamics in response to long-term vegetation succession. Sci. Total Environ. 882, 163542. https://doi.org/10.1016/j.scitotenv.2023.163542.

Zheng, Q., Wang, W., Wen, J., Wu, R., Wu, J., Zhang, W., Zhang, M., 2023. Non-additive effects of bamboo-derived biochar and dicyandiamide on soil greenhouse gas emissions, enzyme activity and bacterial community. Ind. Crop. Prod. 194, 116385. https://doi.org/10.1016/j.indcrop.2023.116385.

Zou, Y., An, Z., Chen, X., Zheng, X., Ben, Z., Zhang, S., Chang, S.X., Jia, J., 2023. Effects of co-applied biochar and plant growth-promoting bacteria on soil carbon mineralization and nutrient availability under two nitrogen addition rates. Ecotoxicol. Environ. Saf. 266, 115579. https://doi.org/10.1016/j.ecoenv.2023.115579.