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Effect of Coal Mining Subsidence on Soil Enzyme Activity in Mining Areas with High Underground Water Levels

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Abstract: In order to investigate the changes in soil enzyme activity and their influencing factors in coal mining subsidence areas with high underground water levels, in this study, we collected soil samples at different depths (SL: 0–20 cm; ML: 20–40 cm; DL: 40–60 cm) in a deep coal seam subsidence area (T1), a shallow coal seam subsidence area (T2), and control non-subsidence areas (W1 and W2) in eastern China. Soil physicochemical properties and enzyme activities were determined, and the mechanism of the latter's response to coal mining subsidence was investigated based on correlation analysis, redundancy analysis, and structural equation modeling. The results show the following: (1) In the coal mining subsidence areas, the soil pH value (pH), soil available nitrogen (AN), available phosphorus (AP), available potassium (AK), and soil organic matter (SOM) contents were lower than those in the non-subsidence areas, while the soil water content (SWC) and bulk density (BD) were higher than those in the non-subsidence areas and increased with depth. (2) The activities of soil urease (URE), sucrase (SUC), alkaline phosphatase (ALP), and catalase (CAT) gradually decreased with depth and were all lower than those in the non-subsidence areas; the largest decreases with respect to the latter were 24.33%, 18.73%, 38.89%, and 5.88%, respectively. (3) The soil nutrient environment had a highly significant and direct positive effect on enzyme activity, with AN, AP, and SOM contents having the greatest impact. (4) Soil BD had a highly significant and direct negative effect and an indirect negative effect (by affecting nutrients) on enzyme activity. The results of this study on the effects of soil physicochemical properties on enzyme activity provide a basis for the ecological restoration of mines.

Keywords: coal mining area with high underground water level; surface subsidence; physicochemical property; enzymatic activity; structural equation model



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1. Introduction

In coal mining areas with high underground water levels, coal mining leads to land cracking, subsidence, and water accumulation, all of which not only destroy surface vegetation but also have a significant impact on the soil ecosystem, altering its physicochemical properties, reducing the availability of soil nutrients, inhibiting the growth and reproduction of microorganisms, and thus affecting the activity of various soil enzymes [1–3].

Soil enzymes are special protein-based macromolecular active compounds produced through plant and microbial activities, possessing biocatalytic capabilities and serving as the main regulatory substances in soil biochemical processes. Soil enzyme activity can characterize soil ecological environment, fertility, and quality, reflecting the intensity and direction of various biochemical processes occurring in the soil [4]. The expression of soil enzyme activity is regulated by the supply of environmental nutrients, primarily including available nutrients such as soil organic matter (SOM), available nitrogen (AN), available phosphorus (AP), and available potassium (AK). At the same time, it is also

susceptible to physical and biological factors in the environment [5]. It can be said that soil physicochemical properties directly or indirectly affect soil enzyme activity.

Studies have shown that SOM is a natural carrier of soil enzymes and that its composition and content affect the stability of soil enzymes [6]. The reason for this is that a high content of SOM promotes the metabolism of soil microorganisms to produce enzymes, thus increasing enzyme activity [7]. Changes in the contents of nutrients such as nitrogen, phosphorus, and potassium in soil are also related to changes in soil enzyme activity [8,9]. For example, Liu found that soil sucrase (SUC) activity had a significant positive correlation with SOM, AP, and total phosphorus (TP) contents and that urease (URE) activity was significantly positively correlated with AK, AP, AN, total potassium (TK), and SOM contents [10]. Jiao analyzed the correlations between soil enzyme activity and nutrients in the reclamation area of the Hedaigou mine and found that soil total nitrogen (TN) showed a significant positive correlation with URE and alkaline phosphatase (ALP), indicating that the increase in soil nutrients had a significant promoting effect on the increase in soil enzyme activity [11]. In addition to soil nutrients, other physicochemical factors also affect soil enzyme activity. For example, the pH value (pH) is significantly negatively correlated with soil enzyme activity [12], because high pH inhibits soil microbial activity and reduces the rate of enzyme production [13]. Moreover, soil bulk density (BD) also has a strong direct effect on soil URE activity [14]. Obviously, the factors affecting enzyme activity and its intrinsic mechanisms are very complex, and the use of ordinary correlation analysis is not sufficient to determine the complex relationships among them. Structural equation modeling is extremely suited to dealing with multiple independent and dependent variables and can be employed to intuitively describe hidden variables [15], meaning that it can be used to more effectively explore the causal relationships between enzyme activity and environmental factors.

At present, reports regarding soil enzymes in coal mining subsidence areas mainly focus on arid and semi-arid mining areas in western China. For instance, Ma pointed out that, in arid and semi-arid regions, the SUC, URE, and ALP activities in subsidence areas decreased compared with those in control areas [1]. Ruan found that the activities of β -1,4-*N*-acetylglucosaminidase (NAG), SUC, β -glucosidase (EC3.2.1.21), and ALP in an Inner Mongolia open-pit mining area were decreased by soil water content (SWC) and nutrient contents [16]. Through the study of soil properties in the coal mining subsidence area of the Lingwu coal mine in Ningxia Hui Autonomous Region, Guo concluded that the activities of β -1,4-glucosidase (BG), NAG, ALP, and catalase (CAT) were reduced [17]. Additionally, Song, in their research study, also found that mining ground fractures in northern Shaanxi mining area had negative effects on the activities of ALP, SUC, CAT, and URE [18]. On the whole, there are few studies on eastern China coal mining areas with high underground water levels, and the understanding of changes in soil physicochemical properties caused by coal mining subsidence, as well as how soil enzyme activity responds to these changes, remains sparse. Therefore, in this study, we focused on coal mining areas in eastern China with high underground water levels. The specific research objectives were to analyze the changes in both soil physicochemical properties and enzyme activity after coal mining subsidence and determine the coupling relationship between physicochemical properties and enzyme activity by using structural equation modeling. We sought to determine and quantify the causal relationships between soil physicochemical factors and enzyme activity so as to provide theoretical references for soil ecological restoration and quality improvement in subsidence areas.

2. Materials and Methods

2.1. Study Area

The study area was within a coal mining area with high underground water levels in eastern China (35°13'~35°19' N, 116°34'~116°43' E), featuring a typical alluvial plain terrain. The main soil types in the study area are fluvo-aquic soils and lime concretion black soils; the area has a warm temperate continental monsoon climate, with an average

annual temperature of 13.8 °C and an average annual precipitation of 712.6 mm. The study area is mainly used for cultivated land with a winter wheat–summer maize rotation system. Following coal mining, the area has seen surface subsidence and the upwelling of groundwater form a large body of accumulated water, affecting the soil, ecological, and human environments of the cultivated land (Figure 1). On the premise of consistency in parent material, climate, biology, topography, sample soil type, and cultivation management measures and according to the depth of the coal seam, the sampling areas selected in this study were the deep coal seam subsidence stabilization area (T1), the shallow coal seam subsidence stabilization area (T2), and the corresponding neighboring areas of normal, non-subsidence cultivated land (W1 and W2).

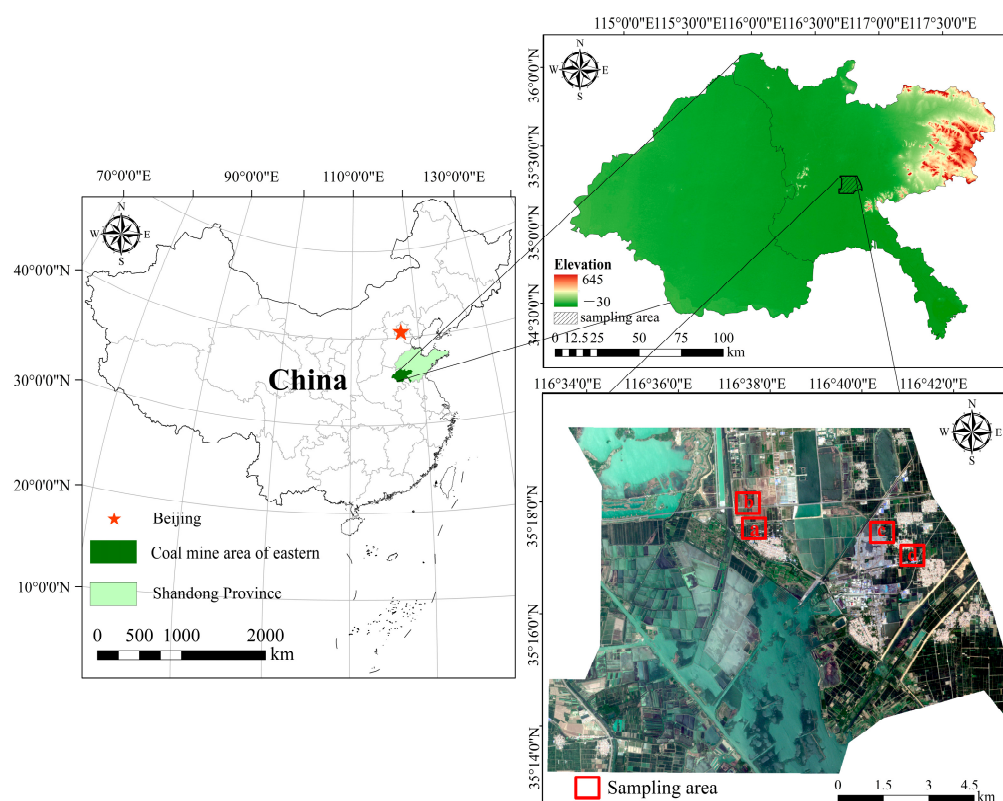


Figure 1. Geographical location of study area. Note: (a) Deep coal seam mining subsidence area (T1), (b) non-subsidence area (W1), (c) shallow coal seam mining subsidence area (T2), and (d) non-subsidence area (W2).

2.2. Soil Sampling

In October 2021, four sampling areas were established in the study area, and nine sampling points were randomly distributed in each sampling area, for a total of 36 sampling points. Each sampling point was divided into three layers of 0–20 cm (SL), 20–40 cm (ML), and 40–60 cm (DL). Each layer was sampled three times and the specimens mixed well, with each soil sample weighing approximately 1 kg, a total of 108 soil samples were collected in this study. At the same time, samples were taken by the ring-knife method and then brought back to the laboratory after having been sealed and numbered. After debris was removed from the bagged soil samples, the latter were divided into two parts, of which one was stored in a refrigerator at 4 °C for the determination of soil enzyme activity and the other was air-dried, ground, and screened for the determination of soil physicochemical properties.

2.3. Soil Environmental Factor Measurement

2.3.1. Soil Physicochemical Properties

Available nitrogen (AN) was determined using the alkali N-proliferation method, available phosphorus (AP) using the sodium hydrogen carbonate solution–Mo–Sb anti-spectrophotometric method, available potassium (AK) using the ammonium acetate extraction–flame photometric method, soil organic matter (SOM) using the potassium dichromate volume–external heating method, and soil pH using potentiometry. Finally, soil water content (SWC) and bulk density (BD) were measured by drying the cut soil samples at 105 °C for 24 h in an oven [19].

2.3.2. Soil Enzyme Activity

Soil urease (URE) was determined with the sodium phenol–sodium hypochlorite colorimetric method, and its activity ($\text{mg g}^{-1} 24 \text{ h}^{-1}$) was expressed as the amount of ammonia (NH_4^+-N) in milligrams that could be hydrolyzed to air-dried soil per gram after 24 h. Soil sucrase (SUC) was determined with the 3,5-dinitrosalicylic acid colorimetric method, and its activity ($\text{mg g}^{-1} 24 \text{ h}^{-1}$) was expressed as the milligrams of glucose produced per gram of soil after 24 h. Soil catalase (CAT) was determined with the potassium permanganate titration method, and its activity ($\text{mL g}^{-1} \text{ h}^{-1}$) was expressed as the volume of $0.1 \text{ mol L}^{-1} \text{ KMnO}_4$ solution consumed per gram of soil. Soil alkaline phosphatase (ALP) was determined with the disodium phenyl phosphate colorimetric method; its activity (mg g^{-1}) was expressed as milligrams of phenols per gram of soil [20,21].

2.4. Descriptive Statistics and Correlation Analysis

Microsoft Excel 2010 and IBM SPSS 26.0 software were used for the statistical analysis of the data. One-way ANOVA was used to test significance among treatments, and Duncan's test was used for multiple comparisons ($p < 0.05$). Pearson correlation analysis was used to calculate the correlation relationships between soil enzyme activity and physicochemical properties. Redundancy analysis (RDA) was performed using Canoco 5.0 to analyze the relationships between soil enzyme activity and environmental factors. SPSS and AMOS 26 were used for structural equation path analysis. Origin 2022 software was used for plotting.

3. Results

3.1. Soil Physicochemical Property Analysis

Coal mining leads to surface subsidence, which alters the geomorphic landscape and changes the soil structure, thus affecting the retention and movement of water, air, heat, and nutrients in the soil, which in turn causes significant changes in soil physicochemical properties (Figure 2 and Appendix A), resulting in a decline in soil quality.

In this study, seven sets of soil physicochemical properties were selected as representatives, and their changes were analyzed in different coal seam sampling areas and sampling depths. The soil chemical properties included AN, AP, AK, SOM, and soil pH, and the soil physical properties included SWC and BD. As can be seen from Figure 2, the soil nutrient contents in the coal mining subsidence areas were lower than in the non-subsidence areas and decreased with the sampling depth. Compared with the non-subsidence areas, soil AN and AP contents in the 20–40 cm soil layer of the subsidence areas showed the most significant changes, with decreases of 39.96% and 51.89%, respectively, in the deep coal seam area (T1) and decreases of 45.59% and 52.01%, respectively, in the shallow coal seam area (T2). The contents of soil AK and SOM changed significantly at 40–60 cm, with the values in T1 decreasing by 9.43% and 29.43%, respectively, and those in T2 decreasing by 9.29% and 25.09%, respectively. The soil pH in the subsidence areas increased slightly with soil depth, and the variation range was 7.49–7.84, which was slightly lower than that in the non-subsidence areas. SWC in the subsidence areas changed significantly with depth, with the middle and deep layers' moisture contents being significantly higher than those in the non-subsidence areas, increasing with respect to the latter by 26.44% (T1–W1 at ML),

25.91% (T1–W1 at DL), 18.62% (T2–W2 at ML), and 17.17% (T2–W2 at DL). Soil BD in the subsidence areas increased with the depth of the soil layer and was greater than that in the non-subsidence areas, while that in T1 was lower than that in T2.

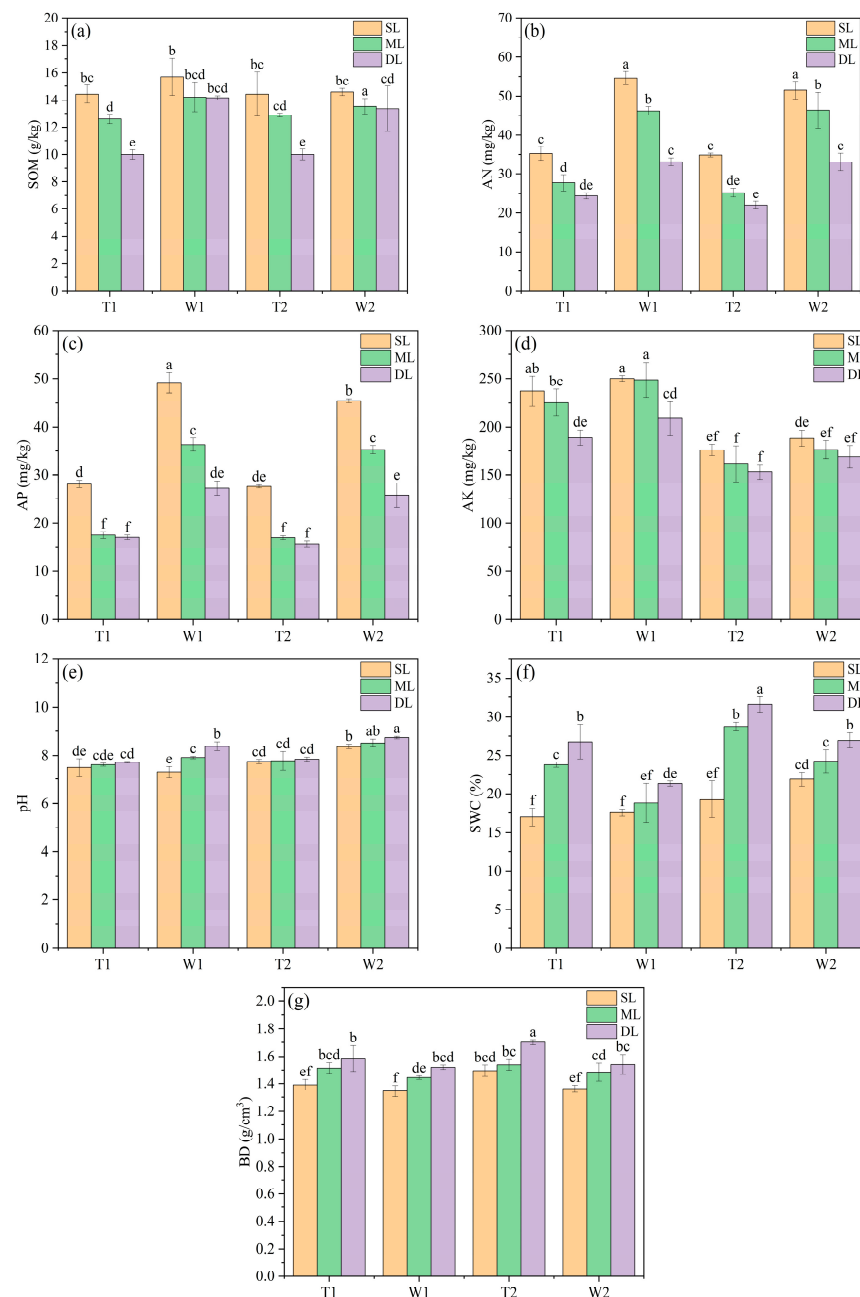


Figure 2. Soil physicochemical properties in different sampling areas. Note: (a) soil organic matter content (SOM); (b) available nitrogen content (AN); (c) available phosphorus content (AP); (d) available potassium content (AK); (e), soil pH value (pH); (f) soil water content (SWC); (g) soil bulk density (BD). The data in the figure are the averages of 3 repeats from 108 soil samples, and different letters indicate significant differences between different soil samples at the level of $p < 0.05$. Abbreviations: T1, deep coal seam mining subsidence area; W1, non-subsidence area 1; T2, shallow coal seam mining subsidence area; W2, non-subsidence area 2. SL, soil depth of 0–20 cm; ML, soil depth of 20–40 cm; DL, soil depth of 40–60 cm.

3.2. Soil Enzyme Activity Analysis

The enzyme activity of different soils varied with the degree of coal mining subsidence; on the whole, that in the subsidence areas was lower than in the non-subsidence areas and decreased with the sampling depth. The soil URE activity in the deep coal seam area (T1) was higher than that in the shallow coal seam area (T2), and this difference was particularly significant in the middle and lower layers of the soil. The URE activity in T2 decreased significantly with the sampling depth, with a decrease of 13.22% from the surface layer to the middle one and a decrease of 22.27% from the middle layer to the deep one. Compared with the W2 area, the DL layer in the T2 area had the most significant decrease, 24.33%. There was no significant difference in soil URE activity in the vertical direction for non-subsidence area W1 (Figure 3a). Similarly, there was no significant difference in soil CAT activity among different sampling sites and different soil depths, but in the subsidence areas it was still slightly lower than that in the non-subsidence areas; this parameter showed the most significant decrease in the SL layer in the T1 area, 5.88%, and decreased slightly with an increase in sampling depth (Figure 3b). Compared with the non-subsidence areas, soil SUC activity changed more significantly among different soil depths in the subsidence areas, especially in T2, with the maximum decrease being 18.66% from the middle layer to the deep one. Compared with the W2 area, the DL layer in the T2 area decreased more significantly, by 24.33% (Figure 3c). Among the four types of soil enzymes, soil ALP activity changed the most significantly with the sampling depth. This enzyme's activity in the 0–20 cm and 20–40 cm soil layers in the subsidence areas was significantly lower than in the non-subsidence areas; compared with the latter, in T1, the activity level in the surface layer decreased by 19.05%, and that in the middle layer decreased by 36.84%, while in T2, that in the surface layer decreased by 30%, and that in the middle layer decreased by 38.89% (Figure 3d).

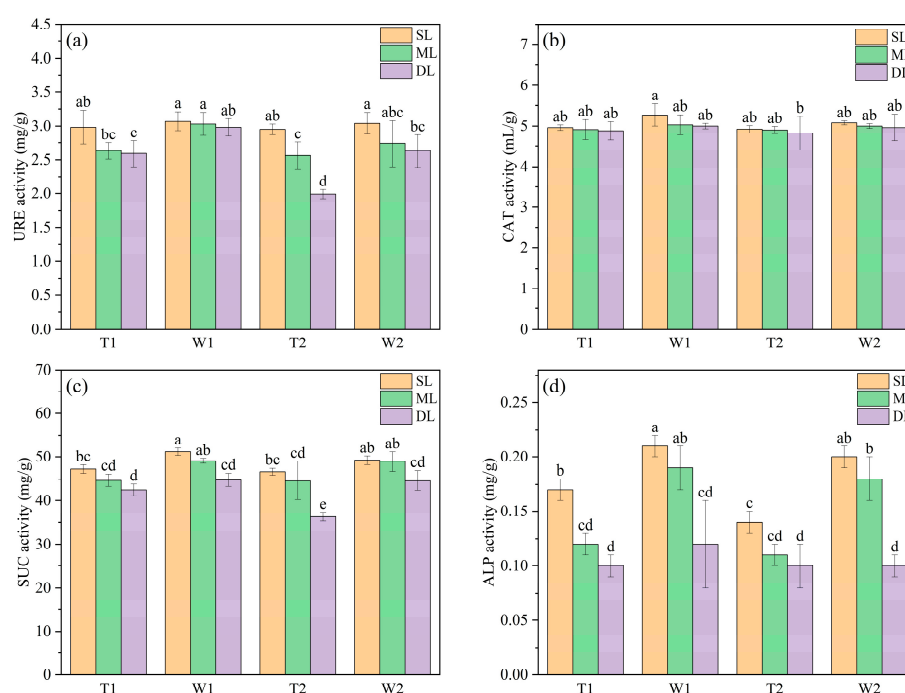


Figure 3. Soil enzyme activity analysis in different sampling areas. Note: (a) urease activity (URE); (b) catalase activity (CAT); (c) sucrose activity (SUC); (d) alkaline phosphatase activity (ALP). The data in the figure are the averages of 3 repeats from 108 soil samples, and different letters indicate significant differences between different soil samples at the level of $p < 0.05$. Abbreviations: T1, deep coal seam mining subsidence area; W1, non-subsidence area 1; T2, shallow coal seam mining subsidence area; W2, non-subsidence area 2. SL, soil depth of 0–20 cm; ML, soil depth of 20–40 cm; DL, soil depth of 40–60 cm.

3.3. Correlations between Soil Enzyme Activity and Physicochemical Properties

In this study, the interrelationships among soil enzyme activity levels and physicochemical factors were analyzed with RDA (Figure 4) combined with Pearson correlation heat maps (Figure 5). Axis 1 and Axis 2 explain 70.14% and 3.47% of the variation in soil enzyme activity, respectively; these findings are of biostatistical significance and effectively reflect the relationships between soil enzymes and physicochemical properties. URE showed highly significant positive correlations with AN, AP, AK, and SOM (correlation coefficients of 0.67, 0.66, 0.57, and 0.71, respectively) and highly significant negative correlations with SWC and BD (correlation coefficients of -0.78 and -0.78 , respectively). CAT was highly significantly positively correlated with AN and AP (correlation coefficients of 0.48 and 0.52, respectively), significantly positively correlated with SOM, and significantly negatively correlated with SWC. SUC had highly significant correlations with all six physicochemical factors, except for pH, showing positive correlations with AN, AP, AK, and SOM (correlation coefficients of 0.80, 0.76, 0.51, and 0.73, respectively) and negative correlations with SWC and BD (correlation coefficients of -0.71 and -0.83 , respectively). The degrees of correlation between ALP and each of the physicochemical factors were consistent with those seen for URE and SUC, showing highly significant positive correlations with AN, AP, AK, and SOM (correlation coefficients of 0.88, 0.87, 0.59, and 0.64, respectively) and highly significant negative correlations with SWC and BD (correlation coefficients of -0.69 and -0.77 , respectively). The effect of pH on enzyme activity was not significant and was positively correlated with URE and SUC, while it was negatively correlated with CAT and ALP.

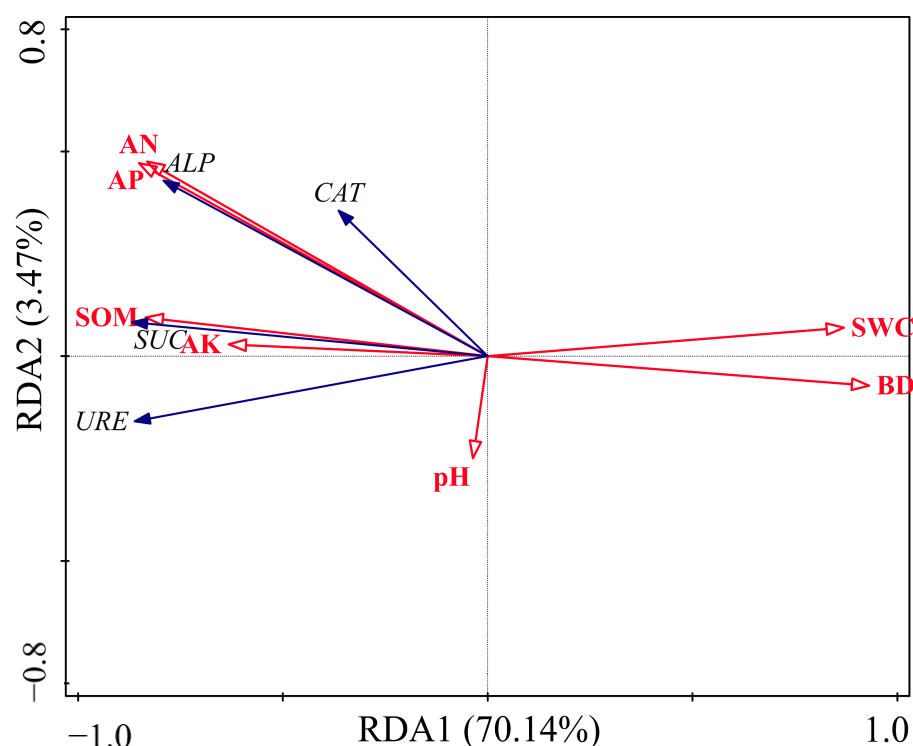


Figure 4. Redundancy analysis of soil enzyme activity and physicochemical properties. Note: Explanatory variables include SOM, soil organic matter; AN, available nitrogen; AP, available phosphorus; AK, available potassium; pH, pH value; SWC, soil water content; BD, bulk density; URE, urease; CAT, catalase; SUC, sucrase; ALP, alkaline phosphatase.

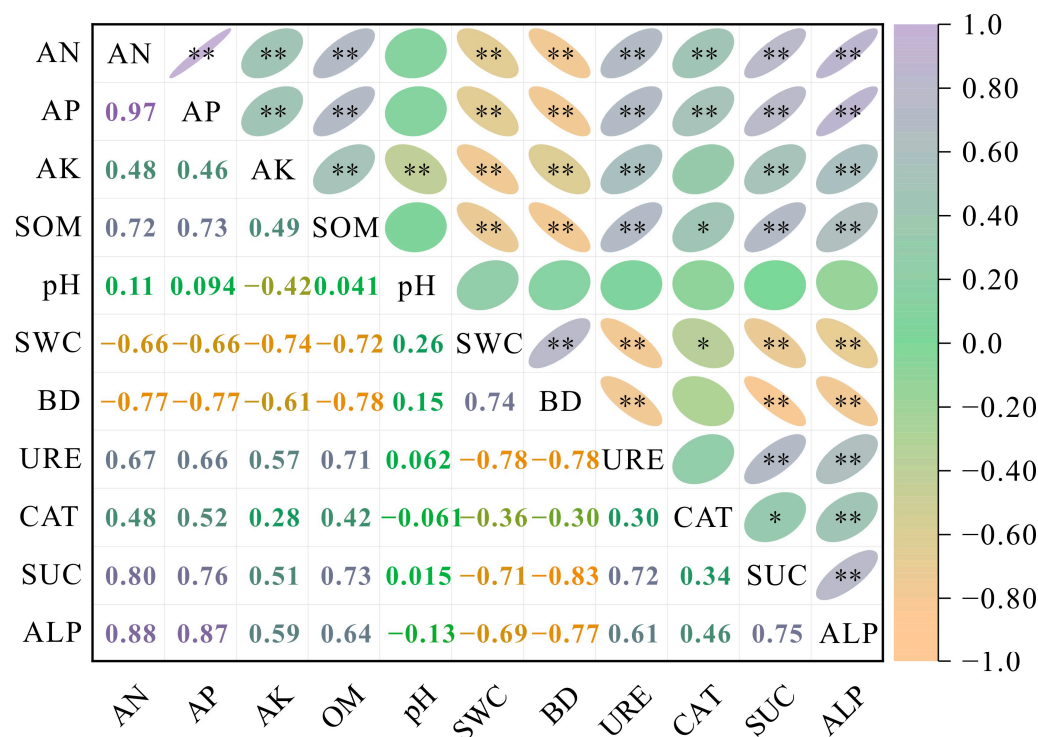


Figure 5. Heat map of correlations between soil enzyme activity and physicochemical properties. Note: Abbreviations include SOM, soil organic matter; AN, available nitrogen; AP, available phosphorus; AK, available potassium; pH, pH value; SWC, soil water content; BD, bulk density. URE, urease; CAT, catalase; SUC, sucrase; ALP, alkaline phosphatase. * $p \leq 0.05$, ** $p \leq 0.01$.

3.4. Structural Equation Modeling of Soil Enzyme Activity and Physicochemical Factors

Based on the results of the redundancy analysis, the physicochemical and enzyme factors with the most significant correlations were selected to establish a structural equation model (shown in Figure 6) to analyze the magnitude of influence of physicochemical factors on enzyme activity. The indicators selected for the nutrient environment included SOM, AN, AP, and AK. Considering the collinearity of certain indicators, such as the general linear relationship between soil BD and SWC, soil BD alone was selected as the indicator of the soil physical environment; this factor indicates soil density and has an effect on both the enzyme activity environment and the nutrient environment. The indicators of soil enzyme activity were URE activity, CAT activity, SUC activity, and ALP activity.

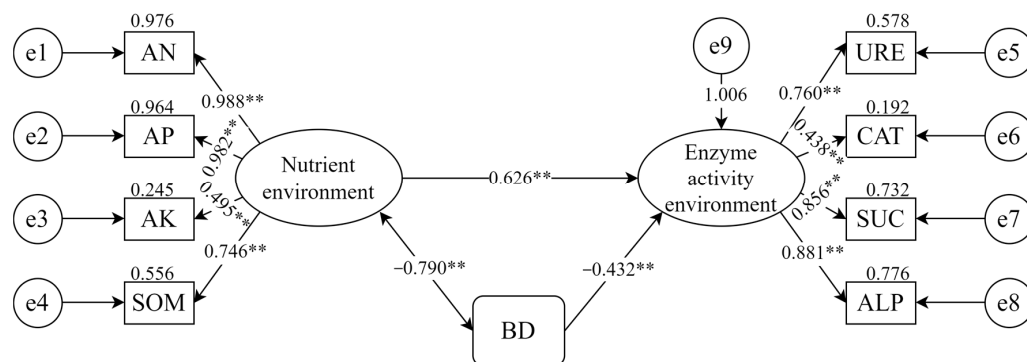


Figure 6. Structural equation model of soil enzyme activity and physicochemical factors. Note: The value on the arrow is the path coefficient (correlation coefficient) between different features. ** indicates a highly significant difference ($p < 0.01$). e1–e9 are error terms. Abbreviations: SOM, soil organic matter; AN, available nitrogen; AP, available phosphorus; AK, available potassium; BD, bulk density. URE, urease; CAT, catalase; SUC, sucrase; ALP, alkaline phosphatase.

According to the structural equation modeling test indicator, the model fitted well; the χ^2/df significance was greater than 0.05, and the null hypothesis was accepted. Table 1 shows the specific fitting results, according to which we can estimate the effect of the nutrient environment on the activities of URE, CAT, SUC, and ALP by using SOM, AN, AP, and AK. All physicochemical factors were extremely significant when used for estimating the nutrient environment ($p < 0.01$), with factor loadings of 0.746 for SOM, 0.988 for AN, 0.982 for AP, and 0.495 for AK, indicating that the four factors were greatly affected by the nutrient environment. The coefficients of AP and AN were larger, indicating that these nutrients had a greater effect on soil enzyme activity, while the coefficient of AK was smaller, indicating that AK had a lower correlation with other factors in the nutrient environment and its effect on enzyme activity was relatively low. The estimations of the effects on the enzyme activity environment of the four enzymes were also extremely significant ($p < 0.01$), with factor loadings of 0.760 for URE, 0.438 for CAT, 0.856 for SUC, and 0.881 for ALP. The influence coefficient of CAT was the smallest, which indicates that, in the enzyme activity environment, CAT was less correlated and less affected than the other three enzymes. The soil nutrients SOM, AN, AP, and AK had highly significant direct positive effects on the soil enzyme activity environment, with an influence coefficient of 0.626 ($p < 0.01$). Soil BD not only had a highly significant direct negative effect on soil enzyme activity ($p < 0.01$) but also indirectly affected soil enzyme activity through the soil nutrient environment, with a path coefficient of -0.790 ($p < 0.01$). Therefore, the impact of soil physicochemical factors on enzyme activity can be divided into two categories. One is the direct impact, which refers to the direct promotive effects of SOM, AN, AP, and AK and the direct inhibitory effect of soil BD. The other is the indirect impact, which refers to the indirect inhibitory effect of soil BD via soil nutrients. Together, these effects indicate a highly significant level of correlation between soil physicochemical properties and enzyme activity.

Table 1. Test of structural equation model fitting results.

Inspection Indicators	Acceptance Criteria	Model Results
RMR	the smaller the better	0.082
χ^2/df	<3	1.696
NFI	0~1, the bigger the better	0.925
CFI	>0.90	0.961
GFI	>0.90	0.938
AGFI	>0.90	0.808
NCP	the smaller the better	11.419

4. Discussion

4.1. Effects of Coal Mining Subsidence on Soil Physicochemical Properties

Coal mining leads to ground subsidence, which destroys the ecological environment, causing land destruction, soil erosion, vegetation degradation, and thus changes in soil physicochemical properties [22,23]. In this study, soil available nutrients were selected for analysis because they are considered more susceptible to changes in the soil environment. Both in the coal mining subsidence areas and the control areas, the contents of soil AN, AP, AK, and organic matter were higher in surface soil and lower in the deeper layers; further, their contents in the coal mining subsidence areas were lower than in the control areas (Figure 2).

SOM is the main source of nutrients for plants, promoting plant growth and development and improving soil texture. Within a certain range, the content of organic matter is positively correlated with the soil fertility level and can be used as an important indicator of this parameter [24]. The organic matter content in the surface soil was found to be relatively high, due to the release of a large amount of organic matter via the microbial decomposition of vegetation litter, and lower contents were found in the deeper layers. The content of SOM in the DL soil layer in the coal mining subsidence areas changed noticeably, while the spatial difference in organic matter content in the control areas decreased with soil

depth, indicating that coal mining has a significant impact on SOM (Figure 2). Phosphorus is an important aspect of soil fertility; a large number of elements essential to plant growth and development, including most of the phosphorus required by plants, come from the soil phosphorus pool [25]. Soil AP is an important indicator used to judge the surplus and deficit of soil phosphorus [26]. Here, soil AP content gradually decreased from the surface layer to the lower layers and tended to stabilize in the DL soil layer, indicating that the upper soil was the most affected by coal mining subsidence, with the effect gradually decreasing with soil depth (Figure 2). Soil nitrogen is an essential nutrient for crop growth and an important basic material for soil fertility [27]. The nitrogen in soil is mainly derived from the microbial fixation and decomposition of plant and animal residues, and its content directly affects soil fertility [28,29]. Soil AN is susceptible to soil hydrothermal conditions and biological activities, and its content in soil is extremely unstable. However, it can be used to reflect the most recent nitrogen supply capacity of the soil and is therefore an effective indicator of soil nitrogen. Soil AN content depends on the content of SOM and its degree of maturation [30]. In this study, soil AN showed a highly significant positive correlation with SOM (Figure 5). Soil AK, which is easily absorbed and utilized by plants, is one of the important indicators used to characterize soil potassium supply levels. There is a large spatial variation in its content, which is related to soil pH, soil moisture condition, soil texture, and clay mineral type [31]. In this study, soil AK content had a highly significant negative correlation with soil pH (Figure 5). In topsoil with lower pH values, the AK content was higher. The reason for this is that topsoil, as a whole, is weakly alkaline; the selective binding sites of potassium are not easily occupied by Al^{3+} or $Al(OH)_x$ and its polymer, and soil has a strong ability to fix potassium, meaning that K^+ is not easily lost in soil solutions, resulting in a high content of AK.

Soil pH reflects the acidity and alkalinity of soil, which are among its most important properties, directly affecting its physicochemical characteristics and thus crop growth [32,33]. In this study, the soil pH of the SL soil layer was the lowest and showed an increasing trend from the surface to the lower layers, with a small overall range of change; further, the value in the subsidence areas was slightly lower than in the non-subsidence areas (Figure 2). Coal mining subsidence led to changes in SWC; the highest SWC was determined in the DL soil layer (Figure 2), and this parameter was positively correlated with soil pH (Figure 5). SWC reflects the dynamic change in soil moisture [34], which affects the transformation of soil substances and determines the effectiveness of soil nutrients. As a result of coal mining subsidence, the soil structure is loosened and water evaporation is accelerated [35]. Underground coal mining leads to the subsidence of the surface, causing surface water accumulation, which significantly affects SWC. The underground water level in our chosen mining area was shallow. In the DL soil layer, SWC reached its maximum value, which was higher than that of the control areas (Figure 2). Soil BD reflects the compactness of soil. Surface cracks caused by mining subsidence cause fine particles in soil to settle downwards, which enhances compactness [36]. Therefore, the soil BD of the subsidence areas was found to be greater than that of the non-subsidence areas (Figure 2). Due to the presence of surface cracks, the cohesion of surface soil was reduced, so surface soil BD was found to be smaller than that in deeper soil layers [37].

4.2. Effects of Coal Mining Subsidence on Soil Enzyme Activity

Soil enzyme activity is highly sensitive to changes in soil environment and is one of the more important indicators reflecting the soil health status [38]. Soil enzymes participate in various physicochemical reactions within soil substances [39] and play a pivotal role in the soil ecosystem.

In this study, different soil enzyme activities were found to vary greatly in different regions and at different soil depths. With the increase in soil depth, the activities of soil URE, SUC, and ALP showed a downward trend, which is consistent with the findings of Zhang's study [40], while the changes in soil CAT activity were not significant (Figure 3). The soil enzyme activities in the coal mining subsidence area were significantly lower than

those in the non-subsidence area at some soil layers, which is consistent with the findings of Ma's study on the northwestern arid and semi-arid mining area [41]. The main reason for this was that the changes in soil structure in the subsidence area exacerbated soil nutrient loss, and the activities of soil enzymes were reduced accordingly. Enzyme reactivity is dependent on soil nutrients. In topsoil, the soil nutrient content is higher, meaning the enzyme activity is naturally higher as well, and with greater soil depth, nutrient reduction leads to a decrease in enzyme activity. In this study, soil URE and ALP both followed this pattern, whereby the clustering of soil ALP activity on the soil surface was more pronounced (Figure 3). Soil URE and ALP are the main factors regulating the nitrogen and phosphorus cycles [42], and the losses of N and P caused by surface subsidence lead to a decrease in enzyme activity [43]. Hydrogen peroxide is produced by biological respiration and biological oxidation reactions of SOM and is prevalent in soil and organisms. CAT enzymatically promotes the decomposition of hydrogen peroxide and the oxidation of compounds in the soil, thus eliminating the toxic effects of hydrogen peroxide on crops [44]. The CAT activity in the sampling area was relatively stable, and there were no significant differences in enzyme activity between the subsidence area and the non-subsidence area (Figure 3). One possible explanation for this is that soil microorganisms synthesize a large amount of CAT in adapting to unfavorable environments [45]. Soil SUC activity is closely related to soil nutrients and soil respiration intensity. Under conditions of coal mining subsidence, the nutrient content of deep soil decreases, the soil is compacted, and the soil respiration intensity is weak. Therefore, the soil SUC activity decreases with depth.

4.3. Response of Soil Enzyme Activity to Soil Physicochemical Properties

Active soil enzymes are sensitive to environmental changes in soil ecosystems, and their activities are affected by a variety of physicochemical properties. Based on the results of the correlation analysis between soil physicochemical properties and enzyme activity, in this study, we adopted structural equation modeling to reflect the soil environment and enzyme activity and then analyzed the effects of different soil physicochemical factors on the latter. The results show that soil enzyme activity was significantly correlated with soil fertility factors (Figure 5). Soil URE activity can be used to reflect soil nitrogen supply capacity [46]. Soil SUC can increase the soluble nutrients in soil, and its activity is closely related to the transformation, decomposition, and accumulation of SOM [47]. In this study, soil URE and SUC activities showed highly significant positive correlations with SOM and soil AN (Figure 5); this was attributed to the loss of SOM in coal mining subsidence areas with high underground water levels, which is conducive to soil denitrification [48]. The content of soil AN was reduced, resulting in a reduction in substrates for enzymatic reactions, and the activities of SUC and URE were reduced accordingly. Soil ALP catalyzes the hydrolysis of phospholipids or phosphoric anhydride, and its activity directly affects the decomposition and transformation of organic phosphorus. In this study, soil ALP activity was significantly positively correlated with soil AP content (Figure 5), which is consistent with the findings of Gao's research study [49]. A high content of organophosphorus in soil can induce the production of phosphatase and improve the content of AP in the soil to a certain extent. Soil CAT activity can reflect the accumulation of SOM, play an important role in the formation of humus, and be used as one of the indicators when monitoring soil quality and remediation [50]. In the coal mining subsidence areas with high underground water levels, CAT activity was found to be highly significantly positively correlated with soil AN and AP, significantly positively correlated with SOM and soil AK, significantly negatively correlated with SWC, and negatively correlated with soil BD and pH (Figure 5). The reason for this may be that soil CAT activity is related to soil respiration intensity and microbial activity; specifically, the lower the soil BD, the stronger the soil respiration intensity and microbial activity and thus the greater the CAT activity. Decomposition products or concomitant products of SOM, AN, AP, AK, and other nutrients affect the activity of CAT by promoting its production along with CAT enzymatic reactions [51]. This is consistent with the finding of many other studies indicating that SOM and nitrogen,

phosphorus, and potassium contents are the main factors affecting URE, CAT, ALP, and SUC [52–54].

Soil enzymes play an important role in soil nutrient turnover and functional stabilization. Among the factors affecting the activity of soil enzymes, the effects of soil moisture and BD are heterogeneous. Soil moisture regulates soil microbial activity by affecting the osmotic potential, nutrients, energy transfer, and microbial cell metabolism, thereby affecting overall enzyme activity [55]. In this study, the activities of URE, CAT, SUC, and ALP in the subsidence areas were lower than those in the non-subsidence areas (Figure 3) and showed significant or extremely significant negative correlations with SWC and BD (Figure 5). The structural equation model also indicated that soil BD had not only a highly significant direct inhibitory effect on soil enzyme activity but also an indirect inhibitory effect via soil nutrients (Figure 6). The reason for this is that mining subsidence and compaction in mining areas with high underground water levels leads to the destruction of the soil structure, an increase in BD, the reduction in soil pores, and the compaction of soil, resulting in a decrease in soil infiltration capacity, hindered downward dissolution and infiltration of soil nutrients, reductions in nutrient concentration, and ultimately, inhibition of enzyme activity [56]. In addition, SWC in coal mining subsidence areas with high underground water levels is higher than in non-subsidence areas; the soil texture here is sticky and heavy, and water permeability and aeration are reduced, which limits microbial activity and thus limits enzyme activity [57]. Soil pH shows different correlations with different soil enzymes. In this study, it was positively correlated with URE and SUC and negatively correlated with CAT and ALP (Figure 5). An increase in soil pH can change the spatial conformation, reaction base point, and microenvironment of the amino acid residues of soil enzymes; affect the stability of enzymes adsorbed by the soil; or change the activity of soil enzymes by changing the binding state between enzymes and soil particles [58,59]. For example, Shi found a significant positive correlation between soil pH and soil URE and invertase activities in an abandoned mine land soil improvement experiment [60], while soil pH and phosphatase activities in *Ligularia virgaurea* land were significantly negatively correlated [61]. Chen concluded that the levels of URE, phosphatase, and invertase essentially reflect changes in soil pH and permeability, as well as the intensity of the conversion of nitrogen, carbon, phosphorus, and potassium elements in soil [62].

In this study, a structural equation model was used to verify that the soil nutrient environment, especially as regards AN, AP, and SOM, was the most important factor affecting enzyme activity (Figure 6). We directly analyzed not only the effect of single physicochemical factors on enzyme activity but also the complex coupling phenomenon of multiple factors by establishing relationships among latent variables. Soil enzymes play an important role in soil nutrient cycling as catalysts for various biochemical reactions and as bioactive indicators in the measurement of soil quality and fertility. In this study, we explored the relationship between soil enzymes and physicochemical properties in order to fully utilize the biochemical characteristics of the former and leverage the advantages of their biological activity. The study of soil enzyme activity enables us to evaluate soil quality and health status in coal mining subsidence areas more comprehensively. This will contribute to more effective ecological restoration of the soil environment in coal mining subsidence areas with high underground water levels.

5. Conclusions

Following coal mining in areas with high underground water levels, the ensuing surface subsidence has a significant effect on soil physicochemical properties and enzyme activity. Our research findings show that the contents of soil nutrients including AN, AP, AK, and SOM in the coal mining subsidence areas analyzed were lower than those in non-subsidence areas and decreased with the increase in sampling depth. In the subsidence areas, soil pH was lower, and SWC and BD were higher, with all three factors increasing with soil depth. On the whole, the enzyme activity in the coal mining subsidence areas was lower than that in the non-subsidence areas, and it decreased with an increase in sampling

depth. The influence of coal mining subsidence on soil URE was more noticeable in the DL layer of the T2 area, with a decrease of 24.33%. The soil SUC also decreased significantly in the DL layer of the T2 area, with a decrease of 18.73%. The soil ALP decreased by 38.89% in the ML layer of the T2 area, while the related changes in soil CAT activity were not significant. In addition, the strong direct positive effects of the nutrient environment, especially in relation to soil AN, AP, and SOM, were found to be the dominant factors affecting soil enzyme activity in the coal mining subsidence areas with high underground water levels, with an effect size of 0.626. The extremely significant negative effect of soil BD on soil enzymes is a consolidation of direct and indirect effects, with effect sizes of -0.432 and -0.164 , respectively.

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Appendix A

Table A1. Soil physicochemical properties in different sampling areas.

Sampling Area	AN mg/kg	AP mg/kg	AK mg/kg	SOM g/kg	pH	SWC %	BD g/cm ³
T1-SL	35.24 ± 1.73 c	28.15 ± 0.75 d	237.00 ± 15.52 ab	14.43 ± 0.65 bc	7.49 ± 0.37 de	16.98 ± 1.13 f	1.3899 ± 0.0406 ef
T1-ML	27.71 ± 2.05 d	17.48 ± 0.73 f	225.33 ± 13.65 bc	12.59 ± 0.34 d	7.61 ± 0.07 cde	23.77 ± 0.34 c	1.5145 ± 0.0395 bcd
T1-DL	24.42 ± 0.83 de	17.01 ± 0.48 f	189.00 ± 8.00 de	10.00 ± 0.34 e	7.71 ± 0.03 cd	26.78 ± 2.24 b	1.5829 ± 0.0940 b
W1-SL	54.60 ± 1.74 a	49.15 ± 2.13 a	250.00 ± 3.00 a	15.69 ± 1.35 b	7.30 ± 0.23 e	17.53 ± 0.46 f	1.3441 ± 0.0403 f
W1-ML	46.15 ± 1.15 b	36.33 ± 1.40 c	248.67 ± 18.01 a	14.21 ± 1.09 bcd	7.91 ± 0.05 c	18.80 ± 2.50 ef	1.4446 ± 0.0137 de
W1-DL	33.15 ± 1.00 c	27.26 ± 1.50 de	208.67 ± 17.67 cd	14.17 ± 0.14 bcd	8.39 ± 0.18 b	21.27 ± 0.33 de	1.5204 ± 0.0164 bcd
T2-SL	34.86 ± 0.47 c	27.66 ± 0.35 de	176.00 ± 6.08 ef	14.43 ± 1.63 bc	7.74 ± 0.09 cd	19.26 ± 2.36 ef	1.4947 ± 0.0420 bcd
T2-ML	25.23 ± 1.11 de	16.92 ± 0.42 f	161.67 ± 18.72 f	12.87 ± 0.14 cd	7.77 ± 0.40 cd	28.73 ± 0.57 b	1.5380 ± 0.0408 bc
T2-DL	21.98 ± 1.05 e	15.63 ± 0.63 f	153.00 ± 7.55 f	10.00 ± 0.38 e	7.84 ± 0.09 cd	31.59 ± 0.98 a	1.7010 ± 0.0183 a
W2-SL	51.49 ± 2.21 a	45.46 ± 0.41 b	188.33 ± 8.50 de	14.58 ± 0.26 bc	8.38 ± 0.09 b	21.84 ± 0.91 cd	1.3610 ± 0.0257 ef
W2-ML	46.37 ± 4.58 b	35.26 ± 0.88 c	176.33 ± 9.71 ef	13.53 ± 0.58 a	8.52 ± 0.16 ab	24.22 ± 1.52 c	1.4843 ± 0.0671 cd
W2-DL	33.07 ± 2.24 c	25.79 ± 2.53 e	168.67 ± 11.93 ef	13.35 ± 1.65 cd	8.74 ± 0.05 a	26.96 ± 0.96 b	1.5406 ± 0.0730 bc

Note: The data in the table are the averages ± standard deviation of 3 repeats from 108 soil samples, and different letters indicate significant differences between different soil samples at the level of $p < 0.05$. Abbreviations: T1, deep coal seam mining subsidence area; W1, non-subsidence area 1; T2, shallow coal seam mining subsidence area; W2, non-subsidence area 2. SL, soil depth of 0–20 cm; ML, soil depth of 20–40 cm; DL, soil depth of 40–60 cm; SOM, soil organic matter; AN, available nitrogen; AP, available phosphorus; AK, available potassium; pH, pH value; SWC, soil water content; BD, bulk density.

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