Integrated Impacts of Soil Salinity and Drought Stresses on the Decomposition of Plant Residues

Abdul Qadeer 1, Abdul Wakeel 1,*, Sardar Alam Cheema 2, Tanvir Shahzad 3 and Muhammad Sanaullah 1,*

1 Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad 38040, Pakistan; qadeermalik25@gmail.com
2 Department of Agronomy, University of Agriculture, Faisalabad 38040, Pakistan
3 Department of Environmental Sciences, Government College University Faisalabad, Faisalabad 38000, Pakistan; tanvirshahzad@gcuf.edu.pk
* Correspondence: abdulwakeel77@gmail.com or abdul.wakeel@uaf.edu.pk (A.W.); sanasial@gmail.com (M.S.)

Abstract: Soil salinity and drought are major environmental challenges that significantly affect soil functioning and soil organic matter (SOM) decomposition. Despite their importance, the combined effects of drought and salinity on residue decomposition are not well understood. This study addresses this gap by evaluating the decomposition of maize residue under salinity and drought stresses over a 75-day incubation period at 20 °C under controlled conditions. The experiment included two moisture levels: optimum moisture at 80% water-holding capacity (WHC) and drought conditions at 30% WHC, in both normal (ECe = 1.48 dS m$^{-1}$) and saline (ECe = 8 dS m$^{-1}$) soils, with 5 g DM kg$^{-1}$ soil maize residues mixed in. A control treatment without maize residue addition was also included. The results indicated that salinity stress reduced maize residue decomposition, as evidenced by lower soil respiration, decay constant, metabolic quotient (qCO$_2$), and soil extracellular enzyme activities. While drought did not affect total soil respiration in the presence of maize residue, it significantly decreased soil extracellular enzyme activities and decay constant rates. Combined drought and salinity stress further diminished maize residue decomposition, marked by reduced soil respiration, decay constant, microbial biomass carbon, and soil extracellular enzyme activities, while dissolved organic carbon (DOC) and qCO$_2$ increased significantly. Similarly, extracellular enzyme activities were significantly reduced under abiotic stresses and further diminished under combined stress conditions. In conclusion, the simultaneous occurrence of drought and salinity can have compounded detrimental effects on microbial functioning, particularly in the presence of fresh plant residues.

Keywords: soil salinity; drought; plant residues; soil respiration; microbial biomass carbon; extracellular enzyme activities

1. Introduction

Climate change is driving shifts in patterns of rainfall, increasing the frequency of drought periods, and exacerbating the problem of salinity [1]. Soil salinity is a major stress factor influenced by climate change, leading to the deterioration of soil ecosystems [2]. Worldwide salt-affected soils cover 831 Mha of agricultural land [3]. Soil salinity causes harmful impacts on soil functionality, microbial activities, and nutrient cycling, posing significant challenges for plant growth [4]. Elevated salt concentrations can alter the carbon (C) mineralization process by affecting microbial respiration and reducing soil organic matter (SOM) decomposition [5]. However, the combined effects of drought and salinity stresses on soil microbial processes, particularly residue mineralization, remain uncertain.

Environmental stresses, such as drought, negatively impact soil microbes by reducing soil enzyme activity and limiting the cycling of nutrients in soil ecosystems (e.g., C, N,
and P), which in turn diminishes soil fertility and crop productivity, leading to economic losses [6]. Salinity stress induces oxidative and osmotic stress, causing nutrient imbalance and disrupting metabolic functions in soil ecosystems [7]. Soil salinity can cause various physical changes in the soil, such as the flocculation or dispersion of soil particles, and affect the solubility of SOM, thus limiting soil microbial activity and resulting in the slowing of SOM decomposition. Soil drying increases evaporation and soil salinization, affecting the solubility and mobility of dissolved organic carbon (DOC). Therefore, CO₂ emissions are influenced by soil drying and salinity stress. While soil microbes can adapt to individual stresses of drought and salinity, their activities are likely limited under combined stress conditions [8]. Microbial metabolism drives CO₂ emissions, a major greenhouse gas (GHG), contributing to global warming (GW) [9,10]. Severe drought stress and high salt concentrations limit microbial activities due to osmotic stress [11], as microbial communities face desiccation and physiological stress under dry conditions. Understanding the effects of salt stress and soil moisture regimes on SOM mineralization is crucial, as reduced soil moisture hampers water availability for metabolic activity, thereby decreasing substrate diffusion to microbes [12]. Investigating the complex relations among various environmental factors affecting soil respiration and soil microbial activities is essential for soil and environment management.

Soil organic matter is a critical indicator of soil quality and productivity, influencing biological, chemical, and physical soil processes. Plant litter serves as a key source of soil organic carbon (SOC) fraction [13]. Previous studies have shown that the chemical composition of the plant residue directly and indirectly affects organic C fractions [14,15]. Water-soluble residue compounds can directly add organic C to soil through leaching [16]. The chemical structure of residues also impacts soil microbial activities responsible for decomposition, ultimately affecting organic C production. However, there is a significant knowledge gap regarding the interactive effect of drought and salinity stresses on residue decomposition, particularly in agroecosystems. Addressing this gap is crucial for understanding residue decomposition responses under various climate stress factors.

This study was conducted to determine the decomposition of maize residue under the combined effects of drought and salinity stresses. We hypothesized that (i) maize residue decomposition would be restricted under drought or salinity stress, and (ii) the combination of these stresses would exacerbate the negative effects on decomposition. To test this, an incubation experiment was conducted to assess maize residue decomposition under salinity and drought stress. Specifically, we predicted that climate stress factors would lead to variations in soil respiration, soil MBC, and soil extracellular enzyme activities, thereby influencing maize residue decomposition.

2. Materials and Methods

2.1. Soil Sampling and Preparation

Soil samples were collected from the top layer (0–20 cm) of agricultural fields in Faisalabad, Pakistan. After sampling, the soil was air-dried, and visible crop debris and minor stones were removed from the soil before sieving with a 2 mm sieve. The obtained soil was characterized as silt loam in texture (silt: 62%, clay: 22%, and sand: 16%) [17]. The water-holding capacity (WHC) of 20.5% was measured using the oven drying method [18], and electrical conductivity (ECe) = 1.48 dS m⁻¹ [19], sodium adsorption ratio (SAR) = 7.45 (mmol L⁻¹)¹/², a pH of 7.8, and SOM of 0.87% were measured using the Walkley–Black method [20]. Soil salinity (ECe 8 dSm⁻¹, SAR 14 (mmol L⁻¹)¹/²) was developed by following the quadratic equation method. To develop the desired salinity level in soil samples, the calculated quantities of the following four salts—calcium chloride (CaCl₂, 281 mg kg⁻¹ of soil), sodium sulfate (Na₂SO₄, 704 mg kg⁻¹ of soil), magnesium sulfate (MgSO₄, 101 mg kg⁻¹ of soil), and sodium chloride (NaCl, 391.4 mg kg⁻¹ of soil)—were added in soil in solution form [21]. Briefly, the soil was spiked with these salts and kept for 30 days, undergoing alternating drying and wetting periods at 70% saturation percentage to achieve the desired EC and SAR levels. After the 30-day period, the soil ECe and SAR were
measured again to confirm the development of desired levels. The saline soils in this region mostly contain sodium, magnesium, and calcium salts; therefore, the above-mentioned salts were used for soil salinity development.

2.2. Soil Incubation

An incubation study was conducted under controlled conditions at a temperature of 20 °C in an incubator (SANYO MIR 253). For this study, 50 g of both normal and saline soils were placed into 200 mL incubation glass jars. Two soil moisture levels were maintained: 80% WHC (optimum soil moisture) and 30% WHC (drought stress), using distilled water over a period of 75 days. Moisture losses were replenished by adding distilled water at each trap change interval (as specified in Section 2.3).

The aerial parts of maize plants were collected after harvesting, air-dried, and ground into pieces smaller than 2 mm size. These maize residues were thoroughly mixed into the soil at the rate of 5 g DM kg$^{-1}$ of soil. The biochemical composition of the added residues was determined by the peroximate analysis method [22], resulting in the following composition: carbon 35.9%, nitrogen 1.11%, C: N ratio 32.4, cellulose 41.2%, hemicellulose 28.0%, and lignin 1.17%. Four replicates were maintained for each treatment.

2.3. Soil Respiration

Soil respiration was measured by trapping total CO$_2$ at 1, 3, 5, 10, 15, 20, 30, 45, 60, and 75 days of incubation. For this purpose, 1 M NaOH solution was used and measured by titrating against 0.1 M HCl in the presence of 2 M BaCl$_2$ solution, with phenolphthalein as an indicator [23].

2.4. Soil Microbial Biomass Carbon

For determining soil MBC, the chloroform fumigation–extraction method was used [24]. Briefly, 5 g of fresh soil was fumigated by using chloroform for 24 h. After fumigation, soil samples were extracted with 20 mL of 0.05 M K$_2$SO$_4$ solution. Another 5 g of fresh soil, not subjected to fumigation, was also extracted with 20 mL volume of 0.05 M K$_2$SO$_4$. Both sets of soil samples were shaken on a mechanical shaker at 280 rpm for 30 min. The total C concentration of the extract was analyzed by titrating with 0.033 M ferrous ammonium sulfate. Soil microbial biomass carbon was calculated by subtracting the non-fumigated values from the fumigated values. The non-fumigated values are considered as dissolved organic carbon [25]. In addition, the ratio of C-CO$_2$ emissions to soil microbial biomass, known as the metabolic quotient (qCO$_2$), was calculated for all treatments in both normal and saline soils [26].

2.5. Enzyme Assays

Soil extracellular enzyme activities were measured using fluorogenically labeled substrates: MUF-β-D-glucopyranoside (EC 3.2.1.21) for β-glucosidase, MUF-N-acetyl-β-D-glucosaminide dehydrate (EC 3.2.1.52) for chitinase, MUF-phosphate monoester (EC 3.1.3.2) for acid phosphatase, and L-Leucine-7-amino-4-methylcoumarin (EC 3.4.11.X) for L-Leucine aminopeptidase [27,28].

Briefly, a soil suspension was prepared by adding 0.5 g fresh soil in 50 mL of sterilized (autoclaved) water and shaking it on a mechanical shaker for 30 min. From this suspension, 50 µL was pipetted into the 96-well microplate. Buffer solutions (50 µL, MES or Trizma) were added in each well containing soil suspension to maintain a pH of 6.0–6.5. Finally, 100 µL of specific substrates (200 µM) was added to make a final volume of 200 µL. The fluorescence was measured after 2 h at an excitation wavelength of 360 nm and an emission wavelength of 460 nm by using a microplate reader (SYNERGY-HTX, BioTek, CA, USA). Enzyme activities were expressed as MUF or AMC release in nmol per g of dry soil per hour (nmol g$^{-1}$ h$^{-1}$).
2.6. Statistical Analysis

To compare the impacts of treatments (salinity and drought) on maize residue decomposition, a two-way analysis of variance (ANOVA), followed by least significant difference test, was performed at probability (\(p\)) level < 0.05, using Statistix (v 8.1) software.

For the quantification of the kinetics of cumulative soil respiration (mg g\(^{-1}\) dry soil), SIGMA PLOT 12.5 by Jandel Scientific was used for data fitting under single exponential rise to maximum curves (Equation (1)):

\[ f = a \times (1 - \exp(-b \times t)) \]  

where “f” is cumulative soil respiration, “a” is the size of active C pool, “b” is the 1st-order kinetic constant for the active C pool, and “t” is time in days. Residue decomposition under drought and salinity stresses was analyzed by using principal component analysis for all parameters by using Minitab 17 version [29].

3. Results

3.1. Soil Respiration

Without residue addition, individual abiotic stresses had opposing effects; salinity stress significantly increased soil respiration (1911 ± 66 mg C kg\(^{-1}\) soil), while drought stress resulted in decreased soil respiration 1384 ± 51 mg C kg\(^{-1}\) soil) compared with optimum conditions (Figures 1 and 2A). Interestingly, under combined stresses, the impacts balanced out, resulting in no significant change in soil respiration. In addition, while individual drought and salinity stresses did not affect the decay constant, the combined stresses of drought and salinity significantly decreased the decay rate constant (Figure 2B).

**Figure 1.** Cumulative C-CO\(_2\) emissions at optimum conditions (80\% WHC) (A, B) and drought stress (30\% WHC) (C, D) with residue incorporation for both normal and saline soils. Data represented as mean ± SE (\(n = 4\)).
When fresh crop residue was added, salinity stress decreased soil respiration, while drought stress had no impact. Combined salinity and drought stresses further reduced cumulative C-CO₂ emissions compared to individual stresses (Figures 1 and 2A). The decay constant significantly decreased both under individual and combined stresses of salinity and drought (Figure 2B).

3.2. Soil Microbial Biomass Carbon and Metabolic Quotient

In the absence of crop residues, individual salinity and drought stresses did not affect microbial biomass carbon (Figure 3A). However, combined stress significantly increased the MBC by 21% compared to control, while the metabolic quotient (qCO₂) significantly decreased under combined stress conditions (Figure 3B).
In contrast, with residue addition, the combined stresses of salinity and drought resulted in decreased MBC compared with individual stresses (Figure 3A), while qCO₂ was significantly increased (Figure 3B).

3.3. Dissolved Organic Carbon

In the absence of crop residue, DOC was decreased by 45%, 38%, and 50% under salinity, drought, and combined stresses, respectively (Figure 4), whereas in the presence of maize residue, DOC contents significantly increased when the combined stresses of drought and salinity were applied (Figure 4).

![Figure 4. Dissolved organic carbon at optimum conditions (80%WHC) and drought stress (30%WHC) with residue incorporation for both normal and saline soils. Data represented as mean ± SE (n = 4). Letters above the bars indicate significant differences between treatments according to LSD test (p < 0.05). Small letters on bars indicate significant differences between the treatments without residue and capital letters on bars indicate treatments with residue addition.](image-url)

3.4. Soil Extracellular Enzyme Activities

Under salinity stress, β-glucosidase activity increased by 23% as compared to control (no salinity and optimum moisture conditions), while salinity stress had no significant effects on alkaline phosphatase, acid phosphatase, leucine aminopeptidase, and chitinase activities as compared to the control. Under drought stress, β-glucosidase and leucine aminopeptidase activities increased by 25% and 20%, respectively, whereas alkaline phosphatase and acid phosphatase activities decreased by 54% and 19%. Drought stress did not significantly affect chitinase enzyme activities.

Combined salinity and drought stresses decreased the alkaline phosphatase activities by 78%, acid phosphatase by 15%, and chitinase activities by 49%, while glucosidase activities and leucine aminopeptidase activities increased by 41% and 17%, respectively. Maize residue addition under salinity stress decreased the glucosidase enzyme activities by 101%, alkaline phosphatase by 85%, leucine aminopeptidase by 79%, chitinase by 38%, and acid phosphatase enzyme activities by 49%. Maize residue incorporation under drought stress decreased the glucosidase activity by 67%, alkaline phosphatase by 158%, chitinase by 48%, and acid phosphatase by 25% while maize residue incorporation under drought stress showed a non-significant effect on leucine aminopeptidase enzyme activities (Figure 5). When maize residue was added under the combined stresses of salinity and drought, glucosidase activity decreased by 149%, alkaline phosphatase by 25%, leucine aminopeptidase by 126%, acid phosphatase by 76%, and chitinase by 117%.
Figure 5. Extracellular enzyme activities of (A) β-glucosidase, (B) alkaline phosphatase, (C) leucine aminopeptidase, (D) acid phosphatase, and (E) chitinase at optimum conditions (80%WHC) and drought stress (30%WHC) with residue incorporation for both normal and saline soils. Data represented as mean ± SE (n = 4). Letters above the bars indicate significant differences between treatments according to LSD test (p < 0.05). Small letters on bars indicate significant differences between the treatments without residue and capital letters on bars indicate treatments with residue addition.

3.5. Principal Component Analysis

Principal component analysis of residue decomposition under drought and salinity stresses explained 71% variability with the first two factors: PC1 (47%) and PC2 (24%) (Figure 6). Normal and saline soils, both with and without drought stress, were separated into two distinct groups, indicating different behavior in maize residue decomposition. Carbon dioxide emissions and enzyme activities were positively correlated with principal components and served as controlling factors for both normal and saline soils (Figure 6). In contrast, soil MBC and soil DOC were correlated with the negative axis for saline soils.
Figure 6. Principle component analysis (PCA) with means of all parameters determined under salinity and drought stresses with maize residue incorporation.

4. Discussion

4.1. Maize Residue Decomposition under Drought Stress

Soil respiration was negatively affected by soil drying conditions, indicating that drought directly impairs the microbial activities through dehydration and decreased substrate transmission, due to the restricted resources [30]. Soil moisture is a critical variable in controlling soil CO$_2$ emissions [8]. Thus, drought can potentially reduce microbial activities, leading to decreased nutrient mineralization and soil extracellular enzyme activities. These negative effects of drought were observed for acid and alkaline phosphatase activities, reflecting negative effects of soil drying on microbial activities. Soil microbes consume the available labile C, which may be abundant during the early incubation periods. This heterotrophic utilization leads to rapid soil respiration exhausting labile C, which might decrease soil respiration [8].

When maize residue was incorporated into the soil under drought stress, the decomposition showed no change, as indicated by the lack of variation in soil respiration and MBC and DOC contents. This can be attributed to the prompt adaptation of soil microbes to the chemical composition of freshly incorporated maize residues, which provided a suitable substrate [31]. Soil microbes physiologically adjusted to the residues, maintaining their activities under low soil moisture by utilizing specific substrates. Our results established that soil respiration and MBC and DOC contents are significantly impacted by microbial access to SOM.

As the decomposition process begins and labile portions are added to the soil, the microbial response remains relatively consistent. Microbial growth in soil ecosystems largely depends on the C input [6]. Under stress, SOM decomposition may provide nutrients and energy for microbial metabolism, growth, and reproduction [32]. In this study, soil microbes adapted to drought stress and used energy from maize residue decomposition for respiration and metabolism. More energy was allocated to growth and reproduction, while less energy was used for soil respiration with CO$_2$.

The DOC represents the most active C fraction in soil, serving as a readily available substrate for microbial activities. DOC contents have been reported to decrease under soil drying [33]. High microbial activity can accelerate the decomposition of humus and residues, increasing DOC contents in soil [34]. The balance between desorption and adsorption of DOC in the soil matrix can be influenced by stress. Increased DOC under
optimum moisture conditions may result from desorption from soil particles with increased soil moisture [35]. Soil extracellular enzymes, synthesized by microbial communities, are closely associated with microbial biomass. In this study, enzyme activities were negatively affected by drought stress, linked to the positive correlation between soil moisture and enzyme activities [36]. Lower moisture reduced activities of soil enzymes due to decreased diffusion of soluble substrates and enzyme mobility, leading to poor substrate contact [37].

Microbial communities respond to cumulative soil moisture effects. Labile organic C fractions depend on the degradation of fresh residues and available SOM. Increased microbial functional diversity enhances enzyme activities, improving soil C decomposition efficiency [38,39] and microbial activity by transforming recalcitrant SOM and residues into soluble organic matter. Soil microbes and the extracellular enzymes primarily rely on easily decomposable organic matter, which serves as a substrate for microbial activities, metabolism, and growth [40].

4.2. Maize Residue Decomposition under Salinity Stress

Salinity stress decreased maize residue decomposition, as indicated by reduced soil respiration, decay constant, metabolic quotient, and soil extracellular enzyme activities, although the soil MBC was higher. These effects could be linked to microbial colonization following substrate addition. Energy from maize residue decomposition might have been used for microbial growth and reproduction rather than metabolism, as indicated by soil respiration [41]. Soil salinity a significant stress factor and can adversely affect microbial processes through ion toxicity and osmotic stress [42], reducing microbial growth and activity and slowing decomposition processes. These adverse effects explain the reduced soil extracellular enzyme activities observed. However, higher C mineralization in response to an increased soil salinity has also been reported [8], with different salts affecting soil C mineralization differently. The metabolic quotient increased with an increase in SOM decomposition under salinity stress, as also reported earlier [43]. Higher soil respiration and soil MBC contents can be attributed to higher biodegradability of maize residues, owing to higher C/N and low lignin contents. Notably, lignin’s recalcitrance slows its degradation. Thus, salinity at 8 dS m$^{-1}$ negatively affected the decomposition of maize residue and higher salinity levels may have more severe effects [44].

4.3. Maize Residue Decomposition under the Combined Stresses of Drought and Salinity

Combined drought and salinity stresses negatively affected maize residue decomposition, as indicated by decreased soil respiration. These integrated stresses reduced microbial activities, likely due to lower osmotic potential [45]. Lower moisture and soil salinity could limit nutrient transport, leading to microbial starvation [46]. Under unfavorable conditions, soil microbes become dormant, reducing their activities and potentially altering the availability of added C. Freshly added residue particles might become inaccessible to soil microorganisms. This decrease in decomposition was also reflected by negative impacts on soil MBC and soil extracellular enzymatic activities involved in C, N, and P cycling. Reduced enzyme activities correlated with decreased soil respiration.

Principal component analysis showed that the soil respiration and soil extracellular enzyme activities were correlated, suggesting that decreased enzyme activities under stress conditions led to reduced soil respiration. The potential activity of C, N, and P cycling enzymes showed a strong correlation. Enzyme activities and soil MBC were most correlated under combined salinity and drought stress treatments. Thus, maize residue decomposition was significantly decreased under these combined stresses.

5. Conclusions

This study provides integrated and comprehensive results on soil respiration, soil microbial activities, and soil extracellular enzyme activities as indicators of maize residue decomposition under salinity and drought stress. We concluded that climate stress factors such as drought and salinity significantly impact maize residue decomposition. In our
study, under drought stress, maize residue decomposition was unaffected, as soil respiration, soil MBC, and soil DOC showed no variation, although soil extracellular enzyme activities decreased. Conversely, under salinity stress, maize residue decomposition was negatively affected, evidenced by decreased soil respiration, decay constant, qCO₂, and soil extracellular enzyme activities, while soil MBC was significantly increased. Combined climatic stress factors further decreased maize residue decomposition, as indicated by reduced soil respiration, soil MBC, and soil extracellular enzyme activities, despite a significant increase in soil DOC. We concluded that while drought and salinity separately affect maize residue decomposition, combined stresses further exacerbate the negative impact on this process.

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Conflicts of Interest: The authors declare no conflicts of interest.

References

13. Wei, Y.; Zhang, Y.; Wilson, G.W.; Guo, Y.; Bi, Y.; Xiong, X.; Liu, N. Transformation of litter carbon to stable soil organic matter is facilitated by ungulate trampling. Geoderma 2021, 385, 114828. [CrossRef]


42. Farouk, S.; Al-Huqail, A.A. Sustainable biochar and/or melatonin improve salinity tolerance in borage plants by modulating osmotic adjustment, antioxidants, and ion homeostasis. *Plants* **2022**, *11*, 765. [CrossRef] [PubMed]


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