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Research Article

Interactive Effect of Residue Quality and Agroecologies Modulate Soil C- and N-Cycling Enzyme Activities, Microbial Gene Abundance, and Metabolic Quotient

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Understanding interactive effect of agroecology explained by rainfall, temperature, elevation, and biochemical composition of residues on soil microbial abundance and functions is crucial for unraveling soil ecological processes. This study aimed to investigate how agroecology and residue quality influence enzymatic activities, gene abundance, and metabolic quotient (qCO_2). A field experiment was conducted using *Leucaena leucocephala* (LL) (high-quality residue) and *Acacia decurrens* (AD) (low-quality residue) in soils of highland and midland agroecologies. These residues differed in decomposability, characterized by a ratio of (lignin + polyphenol)/N of 5.0 for high-quality residue versus 21.0 for low-quality residue. Two experimental setups were employed: soil with litter mixture in polyvinyl chloride (PVC) tubes and residues buried in the surface soil using litterbags. Soil samples were collected after 30, 120, and 270 days of incubation and analyzed for biochemical properties, enzyme activities, and the abundance of nitrifying and total archaea and bacteria. Soil respiration was also measured at different intervals in the field. qCO_2 was calculated using microbial biomass (MBC) and daily respiration (DCO_2). Linear mixed model ($P < 0.05$) revealed that combined factors of agroecologies and residue qualities affected enzymatic activities, microbial abundance, soil properties, and qCO_2. Agroecological differences exerted a greater influence than residue qualities. Positive and negative significant correlations ($P < 0.05$, $r = 0.27$ to 0.67) were found between different C and N pools as well as enzymatic activities. Positive correlations ($P < 0.05$) were observed between the abundance of total bacteria, total archaea, and ammonia-oxidizing bacteria versus leucine-aminopeptidases. qCO_2 was influenced more by β-xylosidase, leucine-aminopeptidases, and thermolysin-like neutral metalloproteases (TLP) than by β-D-glucosidase and β-D-cellobiohydrolase. Leucine-aminopeptidases and TLP were identified as rate-limiting factors for protein and peptide decomposition, while β-xylosidase controlled hemicellulose degradation. In summary, this study provides insights into the intricate relationships between agroecology, residue quality, enzymatic activities, and microbial communities, shedding light on key processes governing soil ecological functions.

1. Introduction

Organic residues are a major source of nutrients and energy for plants and soil microbes in tropical agroecosystems. Understanding the mechanisms of organic matter transformation and nitrogen (N) mineralization in soil ecosystems could help to synchronize nutrient release from organic residues with crop nutrient demand throughout the growing season [1]. Plant residue quality determined the extent of decomposition and accumulation of organic carbon. Furthermore, residue quality affected soil nitrogen (N) cycling, enzymatic activities, and prokaryotic microbial abundance in the terrestrial ecosystem [2, 3]. In this regard, prokaryotic microbial abundance also plays a vital role in the processes of C and N mineralization, including proteolysis, which is a key soil ecological process that can be modulated by both environmental factors such as soil pH and residue biochemical quality [2]. In addition, the efficiency of soil microorganisms

in decomposing organic matter is influenced by the quality of plant residue [4].

In situ agroecological conditions explained by rainfall, temperature, and elevation modulate not only the efficiency of soil microbial decomposition but also the nitrification process [5, 6]. The effects of environmental factors such as temperature [7, 8] and soil pH [9, 10] on soil ecological processes have been extensively studied under laboratory conditions. However, understanding these processes in natural field conditions is challenging due to the complex interplay of multiple environmental factors [11]. On the other hand, due to climate extremes such as droughts [12, 13], heat waves, and floods [14] cause major fluctuations in the structure and functioning of ecosystems and, in some cases, pave the way for abrupt changes from one ecosystem state to another [15]. Even though the effect of extreme events such as drought [12], a combination of extended dry periods and high-intensity rainfall [16] on belowground microbial communities, has been studied, there is scanty information on the effect of high rainfall and low temperature agroecological conditions on soil microbial abundance, enzymatic activities, and metabolic quotient in the tropics. Furthermore, a detailed understanding of the interactive effect of agroecological conditions with residue quality on soil microbial abundance, enzymatic activities, and metabolic quotient under field conditions is lacking. In Ethiopia, predicted climate scenarios showed an increase in extreme rainfall and warming in the extreme temperature [17]. Furthermore, Esayas et al. [18] reported an increasing trend of very wet days and coldest nights in highland agroecologies.

To address these challenges, we designed a field experiment to study the interplay between agroecology and residue quality on microbial abundance, enzymatic activities, and metabolic quotient under field conditions. The experiment was undertaken in two different agroecological conditions which represent (1) high rainfall and low temperature (highland agroecology considered as predicted climatic scenarios) while (2) medium rainfall and high temperature (midland agroecology considered as existing/normal agroecology) in Ethiopia.

Therefore, the objectives of this study were (1) to examine how soil microbial processes and functions in soil C- and N-cycling are influenced by the interplay of agroecological conditions and biochemical quality of organic residues, (2) to explore the impact of low temperature and high rainfall agroecological conditions on microbial abundance and functions in Ethiopian highlands. This is because there are reports that showed the future climate change prediction in Ethiopia resulted from an increase in rainfall and a decrease in temperature [17, 18]. Our first hypothesis was that more microbial abundance and activity response would be observed in the high-quality-amended soils in midland agroecology as compared to low-quality residue-amended soils in the highland agroecology. This may be because of the low energy investment to decompose high-quality residues as compared to low-quality residues and the favorable climatic conditions in midland than highland agroecologies.

The second hypothesis was that prokaryotic gene abundance would be more related to proteolysis and metabolic quotient than C mineralization. This might be because proteolysis of protein and peptides is a rate-limiting process and may affect the metabolic quotient for organic residue-amended soils [4].

2. Materials and Methods

2.1. Study Site Description. This study was carried out in two different agroecologies in Ethiopia: a highland agroecology characterized by high rainfall and low temperatures and a midland agroecology with medium rainfall and high temperatures. The classification of these agroecologies was based on traditional categories in Ethiopia considering factors such as elevation and climate (rainfall and temperature) [19]. The highland agroecology lies between 2300 and 3200 meters above sea level, with a wet and cold climate having temperatures ranging from 12 to 18°C and annual rainfall exceeding 1400 mm. On the other hand, the midland agroecology ranges from 1500–2300 meters above sea level, with a wet-subhumid climate, temperatures from 18 to 25°C, and annual rainfall between 1431 and 1578 mm. These areas are considered favorable for agricultural activities [19, 20]. Specifically, the highland site was situated in Injibara (11°10′N latitude and 36°15′E longitude) in the Banja Shikudaded district of the Awi administrative zone, Amhara region. This district is located in the northwestern highlands, around 455 km from Addis Ababa, the capital city of Ethiopia. The midland site, on the other hand, was based in the Koga Irrigation Research Station at the Adet Agricultural Research Center (11°25′N latitude and 37°10′E longitude) in the North Mecha district of the West Gojam administrative zone in the Amhara region [21] (Figure 1).

In Injibara, the rainy season typically spans from April to November (refer to Figure 2(a)). The Koga site, located at an altitude of 1960 meters above sea level, experiences a dry season from October to May (see Figure 2(b)). The highland and midland agroecologies represent the primary traditional agroclimatic classification system in Ethiopia, according to Hurni et al., [22]. Detailed climatic data covering nine months for both study sites can be found in Figures 2(a) and 2(b).

2.2. Plant Analysis. To assess the impact of different qualities of residues on the ecological functions of soil microbes, aboveground residues such as leaves and twigs from the tropical shrubs *Leucaena leucocephala* (considered as high-quality residue) and *Acacia decurrens* (considered as low-quality residue) were collected in Ethiopia. These legume residues are among the most cultivated perennial plants in Ethiopia, utilized for purposes such as animal feed, charcoal production, and soil erosion control. Before their application, the biochemical quality of the plant residues was evaluated by measuring parameters such as neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) according to VDLUFA (2012) standards (refer to Table 1). In addition,

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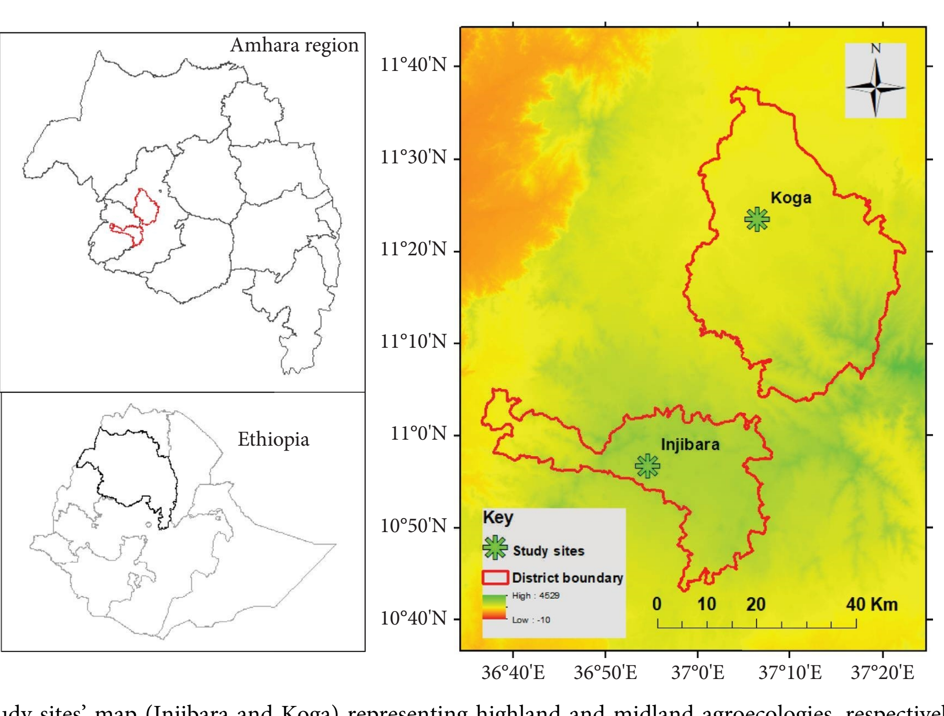
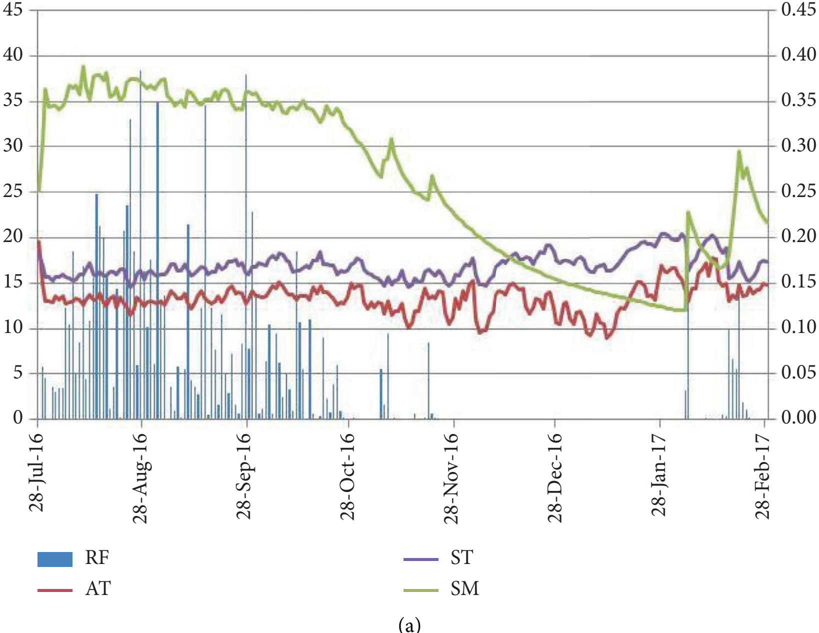


Figure 1: Study sites' map (Injibara and Koga) representing highland and midland agroecologies, respectively, Ethiopia.



(a)

Figure 2: Continued.

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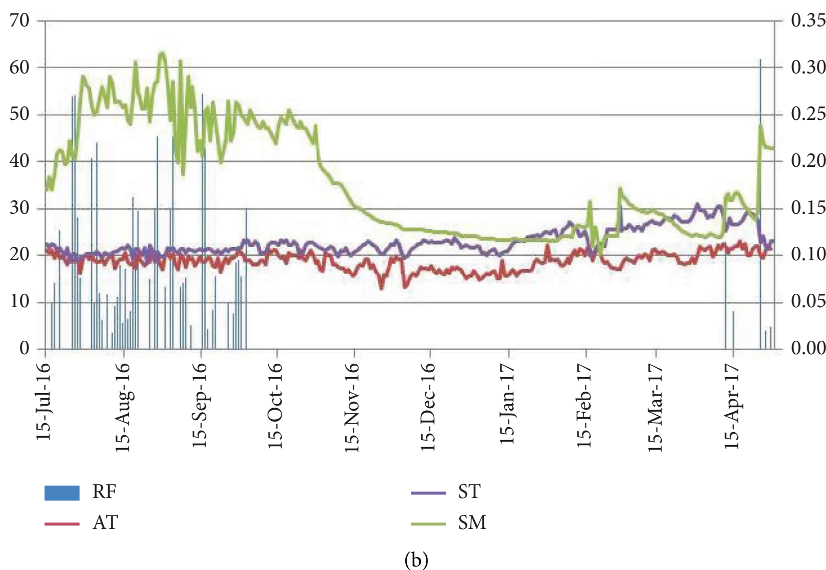

(b)

Figure 2: (a) Daily rainfall, air and soil temperature, and soil moisture data for highland agroecology (Injibara) during the experimental period. (b) Daily rainfall, air and soil temperature, and soil moisture data for midland agroecology (Koga) during the experimental period.

Table 1: Analysis of the biochemical composition of residues from *Acacia decurrens* (Willd.) (LQR residues) and *Leucaena leucocephala* (Lam.) (HQR) collected in Ethiopia.

RT	C ($g{\cdot}kg^{-1}$)	N ($g{\cdot}kg^{-1}$)	C/N ratio	PP ($g{\cdot}kg^{-1}$)	Hem ($g{\cdot}kg^{-1}$)	Cell ($g{\cdot}kg^{-1}$)	L ($g{\cdot}kg^{-1}$)	PP/N	L/N	(L + PP)/N
LQR	468.9	20.4	23.0	150.9	62.7	167.3	276.6	7.4	13.6	21.0
HQR	424.4	36.6	11.6	120.8	176.2	125.4	62.4	3.3	1.7	5.0

RT = residue type, C = carbon, N = nitrogen, C/N = carbon to nitrogen ratio, PP = polyphenol, Hem = hemicelluloses, Cell = cellulose, L = lignin, PP/N = ratio of polyphenol to nitrogen, L/N = ratio of lignin to nitrogen, (L + PP)/N = ratio of the sum of polyphenol and lignin to nitrogen.

the dry matter contents of total carbon (TC), total nitrogen (TN), and total extractable polyphenol (PP) of the residues were analyzed. Hemicellulose and cellulose concentrations were determined by subtracting ADF from NDF and ADL from ADF, respectively. Instruments such as the Euro EA 3000 elemental analyzer (HEKAtech, Wegberg, Germany) were used for measuring TC and TN, while dry matter content was assessed following the AOAC (1990) guidelines. Total PP was determined in line with the method outlined by Makkar et al. [23]. The decomposability of the plant residues, referring to their biochemical quality [1], was primarily characterized by their (L + PP)/N ratios, following [24]. Specifically, the ratio was 5.0 for *Leucaena leucocephala* (LL) and 21 for *Acacia decurrens* (AD). *Acacia decurrens* (AD) was a low-quality residue with a high C/N ratio of 23 and significantly higher lignin to *N* ratio than to LL (1.7 for LL and 13.6 for AD). Conversely, the nitrogen-rich LL displayed an 11% higher hemicellulose content in comparison to AD, contributing to the structural stability of LL (as shown in Table 1). These variations result in differing decomposition rates of residues and nutrient availability, particularly noticeable in the low-quality residue due to its elevated levels of recalcitrant compounds. Detailed biochemical data of the plant residues are presented in Table 1.

2.3. Field Experiment Setup. Field experiments were conducted in two different agroecologies using the same soil samples and amended with two distinct residue types. A control soil sample without any amendment was also included. The experiments took place at Koga and Injibara over nine months to study the interaction effect of agrogeology and residue quality under rainy and dry seasons on prokaryotic microbial gene abundance, enzymatic activities, and metabolic quotient. Soil samples were transferred from Koga to Injibara to avoid soil variations. The soil was collected at a depth of 0–15 cm, with Koga's dominant soil type classified as nitisols [25–27]. The soil characteristics included TC (1.36–2.58%), TN (0.18–0.24%), pH H_2O (5.1–5.3), available P (Pav) (3.54–8.69), and a clay texture class. Fresh residues from leaves and twigs were obtained from the Adet Agricultural Research Center (AARC) and mixed with the soil in PVC tubes for the experiments. The treatments were arranged in a randomized complete block design (RCBD) with three replications and sampled at 30, 120, and 270 days after incubation to investigate the interacted effect of agroecologies with residue quality on potential enzymatic activities, microbial abundance, and metabolic quotient in the wet (rainy) and dry seasons. In addition, a litterbag study [28] was conducted using polyvinyl mesh bags to assess the weight loss of the residue types.

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2.4. Soil Sample Collection, Transport, and Storage. Fresh soil samples were mixed thoroughly and transported in cool conditions to the soil laboratory at the Amhara Regional Agricultural Research Institute (ARARI) in Bahir Dar, Ethiopia. Upon arrival, the samples were divided into three equal parts. One-third of the samples were stored at −20°C in the laboratory and later freeze-dried at Bahir Dar University at −80°C. After freeze-drying, these samples were maintained at −20°C at the ARARI for the analysis of enzymatic activities and prokaryotic gene abundance. Another third of the fresh soil samples underwent immediate analysis for ammonia and nitrate at the Soil Laboratory of Adet Agricultural Research Center. The remaining third of the fresh soil samples, which were not kept under cool conditions during transportation, were air-dried, ground, and sieved to <2 mm for the analysis of biochemical soil properties.

2.5. Microbial Activities

2.5.1. Respiration. The soil CO_2 respiration in the PVC tube was monitored using a closed soil respiration system from PP Systems, Inc., North America. This system includes the environmental gas monitoring (EGM) and the SRC-1 soil respiration chamber. The SRC-1 chamber has a surface area of 78 cm^2 and a system volume of 1171 ml. A fan inside the chamber mixes the air when it is placed on the soil. The EGM records the increase in CO_2 concentration every 8 seconds for 120 seconds, with the rate of increase expected to be linear. The respiration rate of each soil PVC was calculated as an average of 4 continuous recordings once the readings stabilized. Soil temperature at a depth of 5 cm was measured using a thermistor sensor connected to the respiration system. Soil respiration measurements were taken every 4–7 days for the first 30 days, weekly from 30–120 days, and monthly from 120–270 days [29].

2.5.2. Potential Enzymatic Activities. The purpose of analyzing potential C- and N-cycling enzymatic activities was to gain a clear understanding of soil microbial functions under field conditions [30, 31]. C-cycling enzymes involved in degrading glucose, cellulose, and hemicellulose include β-D-glucosidase (BGL), β-D-cellobiohydrolase (BCL), and β-xylosidase (BXL) [32, 33]. Similarly, leucine-aminopeptidases (LAP), alanyl-alanyl-phenyl aminopeptidase (AAP), and thermolysin-like neutral metalloproteases (TLP) were identified as important [2] and rate-limiting [34] N-cycling enzymes during plant residue decomposition.

The potential activities of these enzymes were determined using hydrolyzable substrates containing fluorescent compounds. The soil samples were prepared and analyzed using specific procedures outlined in the research of the authors in [4]. Enzymatic activity kinetics was calculated based on the measurements taken during the incubation period and fluorescence recordings. This comprehensive analysis provides valuable insights into the intricate processes of soil microbial functions related to carbon and nitrogen cycling.

2.6. Microbial Community Abundance. DNA was extracted from 0.5 g of each frozen soil sample using the FastDNA™ Spin Kit for Soil from MP Biomedicals, Solon, Ohio, USA, following the manufacturer's instructions with slight modifications. The concentration of the extracted DNA was measured using a spectrophotometer (NanoDrop™ 2000/2000C, Thermo Fisher Scientific, Waltham, MA, USA). To prevent contamination with humic acids [35], two additional washes with 500 μL of guanidine thiocyanate (5 M) were performed before washing with SEWS-M buffer. The quality of the isolated DNA was assessed using a 1.5% agarose gel, and the DNA extracts were stored at −28°C for further analysis. Target genes for total bacteria and archaea (16S rRNA gene), ammonia-oxidizing bacteria (bacteria amoA gene, AOB), and ammonia-oxidizing archaea (archaea amoA gene, AOA) were quantified following the protocol outlined by the authors in [36]. Plasmid standards were prepared and purified for each gene using the InviSorb Fragment CleanUp kit from Stratec Molecular GmbH, Berlin, Germany. Quantitative PCR (qPCR) was conducted on a StepOnePlus™ Real-Time PCR System from Applied Biosystems. Melting curves of the amplicons were generated for quality checks, and the reaction efficiency was determined using StepOne™ software version 2.2.2 from Applied Biosystems.

2.7. Ammonium and Nitrate Content in Soil. To analyze the levels of ammonium (NH_4^+-N) and nitrate (NO_3^--N) in the soil, the following process was followed [37].

Firstly, 25 g of fresh soil was mixed with 50 ml of 1 M KCl solution and shaken for an hour. The mixture was then filtered using Whatman 42 filter paper into an Erlenmeyer flask. For the determination of ammonium (NH_4^+-N), 25 ml of the extracted solution was combined with 100 ml of distilled water in a micro-Kjeldahl flask. To this mixture, 0.5 g of MgO powder and a few drops of phenolphthalein indicator were added until the color turned pink. The distillation process was carried out for about 20 minutes, collecting the evolving NH_4^+-N with boric acid. The collected distillate was then titrated using sulfuric acid (H_2SO_4) until the original color was restored.

After cooling down the Kjeldahl system, 1 g of Devarda's alloy and 100 ml of water were added to the residue sample for the determination of nitrate (NO_3^--N). The distillation process was repeated, and titration was performed using sulfuric acid (H_2SO_4) after 20 minutes.

To calculate the levels of NH_4^+-N and NO_3^--N, the following equations were used:

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$$NH_4+-N: \frac{(V1 - V0)}{(1 - wf)} \quad (1)$$

$$NO_3--N: \frac{(V2 - V0)}{(1 - wf)} \quad (2)$$

where V0 = volume of 0.01 N H_2SO_4 added to blank, V1 = volume of 0.01 N H_2SO_4 added to the soil sample for $NH4^+$-N titration, and V2 = volume of 0.01 N H_2SO_4 added to the soil sample for NO_3^--N titration.

In addition, the weight of the fresh sample was compared to the weight of the oven-dried sample to calculate Wf, using the formula provided.

Wf = (weight of fresh sample-weight of the oven-dried sample)/weight of the oven-dried sample.

The fresh samples were oven-dried at 105°C for 24 hours.

Overall, this detailed process allows for the accurate determination of ammonium and nitrate levels in the soil sample.

2.8. Microbial Biomass and Dissolved Organic C and N. The air-dried soil samples underwent a 10-day pre-incubation period to rejuvenate soil microbes for the assessment of microbial biomass carbon (MBC) and nitrogen (MBN). The determination of MBC and MBN was conducted through chloroform-fumigation-extraction as outlined by Vance et al. [38], utilizing conversion factors of KEC 0.45 and KEN 0.54 from Joergensen [39] and Joergensen and Mueller [40], respectively. Subsequently, 10 grams of both nonfumigated and fumigated soil subsamples were combined with 40 ml of a 0.5 M K_2SO_4 solution (at a 1 : 2, w/v, soil/extractant ratio). The mixture was then shaken for 30 minutes at 250 revolutions per minute on a horizontal shaker and centrifuged for 30 minutes at 4400 g. The levels of dissolved organic C (DOC) and N (DON) were determined in the supernatants of both fumigated and nonfumigated samples using a DOC/TN-analyzer (multi N/C 2100S from Analytik Jena, Jena, Germany). In addition, DOC and total extracted nitrogen (TEN) were calculated based on the total C and N concentrations in the supernatants of nonfumigated samples, following the methodology described in [41].

2.9. Metabolic Quotient (qCO_2). Metabolic quotient (qCO_2) was used to quantify the microbial metabolic efficiency [42] for the field incubation period and was calculated based on (3) according to [30].

$$qCO_2 = \text{daily } CO_2 - \frac{C}{MBC}. \quad (3)$$

2.10. Statistical Analysis. Analysis of variance (ANOVA) was conducted according to the randomized complete block design (RCBD) with 54 observations, employing the proc mixed model within SAS software (version 9.0, SAS Institute, Cary, North Carolina, USA). The factors "plant residue quality" and "agroecology" were examined, along with their interaction, across different incubation periods. The main effects were compared using the MEANS statement with the least significant difference (LSD) test at a significance level of $p < 0.05$. Interaction means were compared utilizing the PDIFF STDERR option in the LSMEANS statement of the mixed procedure of SAS, with error terms for separating LSMEANS for interactions. Furthermore, Pearson linear correlations were performed using SAS to evaluate the relationships among microbial activities, gene abundance, C pools, and N nutrients.

3. Results

All measured biological and chemical soil properties, metabolic quotient, and microbial respiration responded significantly to the three factors "agroecologies," "residue quality," and "time," except dissolved organic carbon (DOC), microbial biomass nitrogen (MBN), nitrate (NO_3^--N), and Ala-Ala-Phe-AMC hydrochloride (AAP) for "agroecologies" and ammonium ($NH4^+$-N), NO_3^-–N, AAP, ammonium oxidized bacteria (AOB), and ammonium oxidized archaea (AOA) for residue quality (Table 2). Significant interactions of tested factors were found for microbial biomass carbon (MBC), DOC, NO_3^- –N, β-xylosidase (BXL), leucine-aminopeptidases (LAP), thermolysin-like neutral metalloproteases (TLP), and qCO_2 for "agroecologies × residue quality." Similarly, interaction effects were found for most biological and chemical soil properties except NO_3^-–N, TEN, LAP, and qCO_2 for "agroecologies × time," as shown in Table 2. The interaction effects were recorded for most biological and chemical soil properties except NH_4^+-N, NO_3^- –N, β-D-glucosidase (BGL), β-D-cellobiohydrolase (BCL), AAP, and AOA for "residue quality × time." In the contrary, nonsignificant interactions were found for most biological and chemical soil properties except MBC, DOC, BXL, LAP, and TLP.

3.1. Carbon and Nitrogen Mineralization in Contrasting Agroecologies. Distinct responses were recorded in microbial biomass carbon (MBC), daily respiration (DCO_2), and dissolved organic carbon (DOC) between highland and midland agroecologies (Figure 3, $P < 0.05$). Microbial biomass carbon decreased across the decomposition period in the highlands while an increasing trend was observed in the midlands. In contrast, when we considered DOC, an increasing result was noted in the highland while a decreasing value was obtained in the midland across the decomposition period. The trend for DCO_2 decreased up to 120 days and increased from this day to 270 days in both agroecologies. The trend of MBC and DOC was in contrary with highland and midland. Similarly, significant differences were recorded in MBC, DCO_2, and DOC between high (*Leucaena leucocephala*, HQR) and low (*Acacia decurrens*, LQR) biochemical quality residue-amended soils. The highest MBC and DCO_2 were noted in soils amended by low-quality residues (LQR) in the midland while the lowest was in the control (no residue) at the highland after 120 days.

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Table 2: Analysis of variance to reveal significant effects and interactions of soil biological and chemical properties, microbial respiration, and metabolic quotient (qCO_2).

Chemical properties	Factors: Agroecological zone (Ag)	Residue quality (Rq)	Incubation time (T)	Ag*Rq	Ag*T	Rq*T	Ag*Rq*T
MBC	***	***	***	***	***	***	***
DOCac	NS	***	***	***	***	***	***
MBN	NS	***	***	*	***	*	NS
NH_4^+-N		NS		NS		NS	NS
NO_3^--N	NS	NS	***	***	NS	NS	NS
TEN	***	**	NS	NS	NS	*	NS
Enzyme activities							
BGL	***	***	***	NS	*	NS	NS
BCL	***	***	*	NS	*	NS	NS
BXL	***	***	***	***	***	***	***
AAP	NS	NS	NS	NS	**	NS	NS
LAP	***	***	***	***	NS	***	***
TLP	***	***	***	***	***	***	***
Gene abundance							
TB	***	***	*	NS	*	NS	NS
AOB	***	NS	***	NS	***	NS	NS
TA	***	*	***	NS	***	NS	NS
AOA	**	NS	***	NS	NS	*	NS
Metabolic quotient (qCO_2) and daily respiration CO_2 (DCO_2)							
qCO_2	***	NS	***	*	NS	**	***
DCO_2	***	***	***	NS	***	***	***

*** < 0.001, ** < 0.01, and * < 0.05.

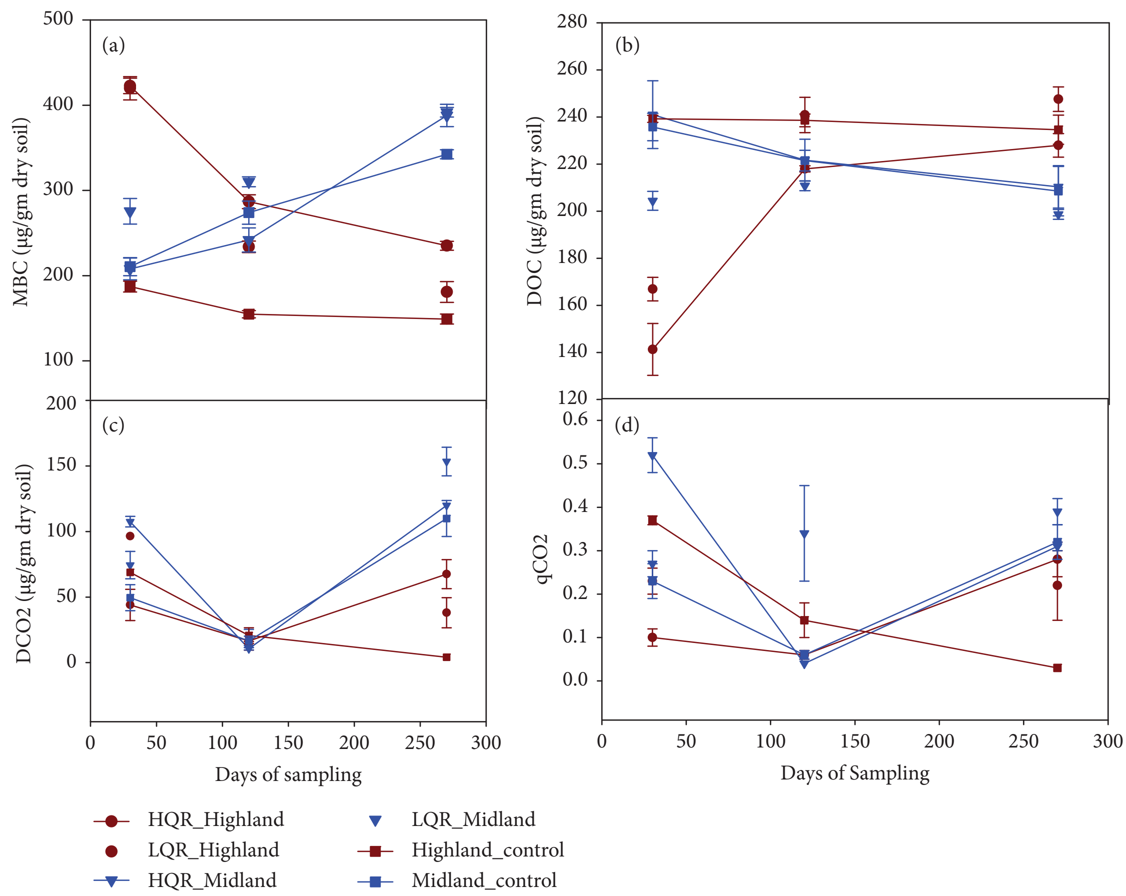

Figure 3: Estimated carbon pool values and metabolic quotient with standard error (SE) for *Leucaena leucocephala* (HQR) and *Acacia decurrens* (LQR) residue-amended soils in highland and midland agroecologies across the incubation period: (a) microbial biomass carbon, (b) dissolved organic carbon, (c) daily respiration, and (d) metabolic quotient.

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The highest metabolic quotient (qCO_2) was recorded in midland than in highland agroecology. Regarding the response of qCO_2 to residue quality, significantly ($P < 0.05$) higher qCO_2 values were recorded for LQR than the HQR in midland while the reverse was true for highland (Figure 3).

Similarly, the trend of N mineralization was significantly ($P < 0.05$) different in the HQR and LQR-amended soils in highland and midland agroecologies (Figure 4). A higher and increasing trend of MBN was noted in the LL-amended soils in the midland agroecology. Similarly, LL-amended soil gave higher MBN as compared to AD-amended soils with a decreasing trend in the highland agroecology across incubation periods (Figure 4). The contrasting effect of the agroecology on ammonia-N (NH_4^+-N) was shown after 120 days of incubation, with an increasing trend for midland and a decreasing trend for highland. The highest NH_4^+-N was found in soils amended with AD in the midland agroecology (Figure 4). In midland, the highest nitrate (NO_3^--N) was found in soils amended with LL as compared to AD-amended soils. While in highland, the highest nitrate (NO_3^--N) was recorded in the control soil after 120 days of incubation. An increasing trend of total extractable nitrogen (TEN) was noted in both agroecologies, even though the highest and lowest TEN values were found in the LL-amended soils in midland and AD-amended soils in the highland, respectively (Figure 4).

3.2. Potential Enzymatic Activities across Agroecologies and Residue Types. Higher β-D-glucosidase (BGL), β-D-cellobiohydrolase (BCL), and β-xylosidase (BXL) enzymatic activities were noted in LL-amended soils in the midland agroecology, while the lowest values of BGL and BXL activities were recorded in the control soils in the highland agroecology (Figure 5). Similarly, the lowest values of BCL activity were noted in control soils in the midland agroecology. The activities of LAP, AAP, and TLP were decreased over incubation periods in midland agroecologies regardless of residue types except LAP in LL-amended soils (Figure 5). The LAP activity in LL-amended soils was increased across incubation periods. Similarly, increasing activity of AAP was recorded across incubation periods in LL-amended soils in highland agroecology. A decreasing trend of the activity of TLP was recorded in both agroecologies. However, a contrasting trend was shown in AAP activities between midland and highland agroecologies after 120 days of incubation (Figure 5).

3.3. Gene Abundance in Contrasting Agroecologies and Contrasting Residue Quality-Amended Soils. Gene abundance was determined in soils amended with different biochemical quality residue types in contrasting agroecologies. Higher gene abundance of total bacteria, total archaea, ammonia oxidized bacteria (AOB), and ammonia oxidized archaea (AOA) were observed in longer than shorter incubation periods (Figure 6). The highest total bacteria copy number (16S rRNA gene) was recorded in AD-amended soils followed by LL-amended soils after 120 days of incubation in the midland agroecology. Furthermore, the highest total archaea copy numbers (16S rRNA gene) were noted in AD-amended soils after 120 days of incubation followed by the control at the midland agroecology. Lower copy numbers of total archaea (16S rRNA gene) were found after 120 days of incubation in LL- and AD-amended soils in the highland agroecology. The highest gene copy numbers of AOB and AOA were found in amended as well as control soils in the midland agroecology after 120 days of incubation. Furthermore, higher AOA copy numbers were recorded in LL-amended soils in the highland after 120 days of incubation than in the midland.

3.4. Relationship between Different Carbon and Nitrogen Pools and Their Potential Enzymatic Activities. Both positive and negative significant Pearson correlations ($P < 0.05$) were found between the different C- and N-pools with potential C- and N-cycling enzymatic activities (Table 3). The correlations of MBC with BGL ($r = 0.40$ $P < 0.01$) and TLP ($r = 0.40$; $P < 0.01$) and DCO_2 with TLP ($r = 0.36$; $P < 0.1$) were strong and positive while the correlations of DOC with BGL and TLP ($r = -0.47$; $P < 0.01$, $r = -0.34$; $P < 0.01$), respectively, were negative. Significant correlations were not observed between BCL, BXL, LAP, and AAP versus MBC, DCO_2, and DOC. The relationship between qCO_2 with β-xylosidase (BXL) and TLP was positive, but TLP was negatively correlated with LAP. The relationship between NH_4^+-N and LAP ($r = 0.31$; $P < 0.05$) was positive while the correlation with AAP ($r = -0.32$; $P < 0.05$) was negative. The correlations between NO_3^--N and BGL ($r = -0.53$; $P < 0.001$) and BXL ($r = -0.27$; $P < 0.05$) and TLP ($r = -0.32$; $P < 0.05$) were negative but positive with the LAP ($r = 0.33$; $P < 0.05$). The same correlation trend was followed between mineral N (Min N) and BGL, BXL, TLP, and LAP. There was a significant positive relationship between total extractable nitrogen (TEN), BCL, and BXL. However, there were no significant correlations between TEN and three of the N-cycling enzymatic activities. Similarly, significant correlations were not noted between NH_4^+-N and three of the potential C-cycling enzyme activities.

3.5. Relationship between Gene Abundance, Enzymatic Activities, Ammonia Nitrogen, and Soil Respiration. Significant ($P < 0.05$) positive correlations were recorded between the abundance of total bacteria ($r = 0.67$), total archaea ($r = 0.47$), and ammonia-oxidized bacteria (AOB) ($r = 0.61$) versus LAP (Table 3). Similarly, positive correlations were noted between NH_4^+-N versus total bacteria ($r = 0.53$; $P < 0.001$) and AOB ($r = 0.41$; $P < 0.05$). Negative correlations were found between DCO_2 and qCO_2 with total bacteria ($r = -0.46$; $P < 0.01$, $r = -0.42$: $P < 0.05$) and AOB ($r = -0.34$; $P < 0.05$, $r = -0.38$; $P < 0.05$), respectively. There were no significant relationships between gene abundance and three C-cycling enzymes as well as AAP and TLP activities. Furthermore, AOA did not correlate with all enzymatic activities, DCO_2, and NH_4^+-N.

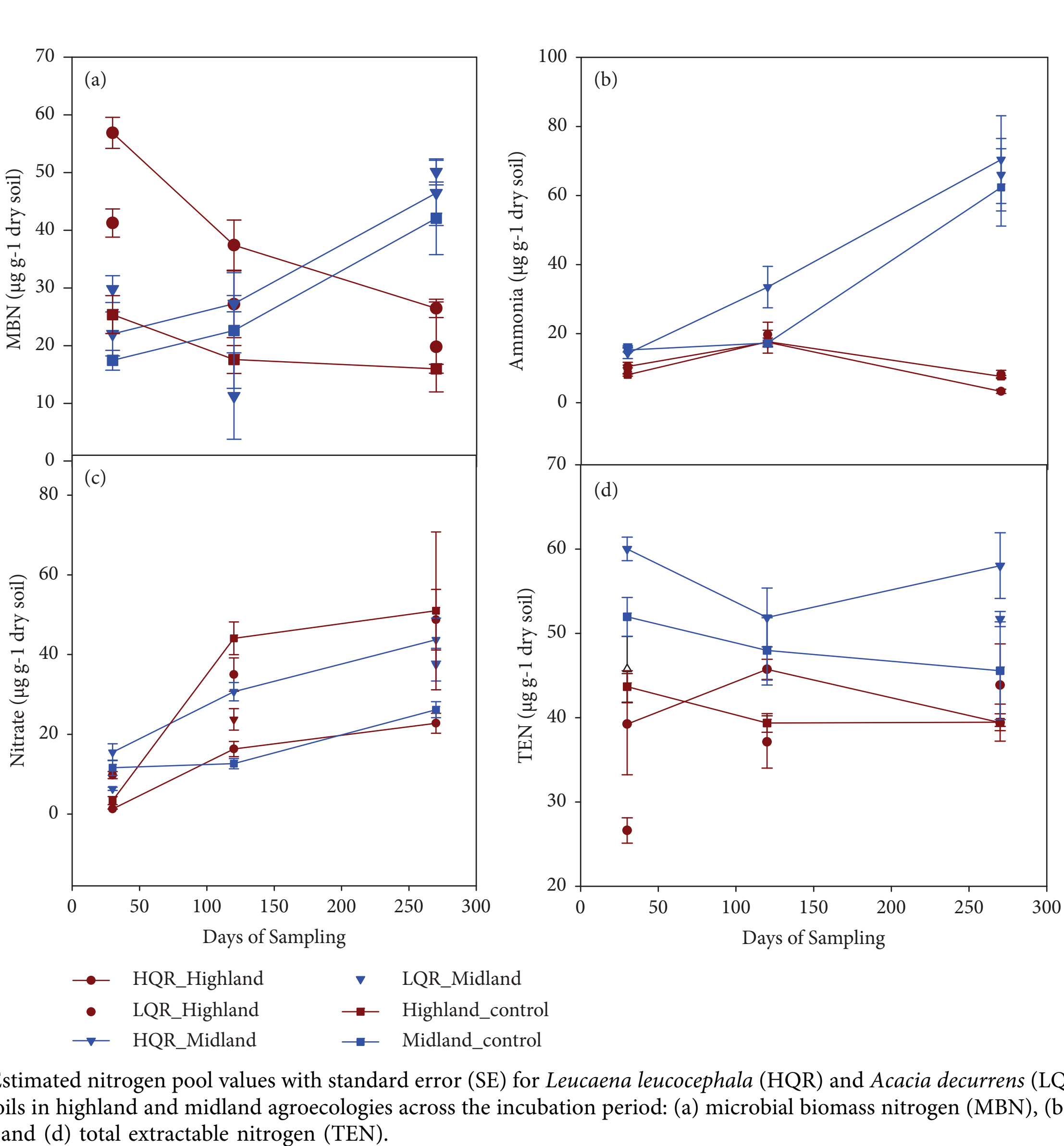

Figure 4: Estimated nitrogen pool values with standard error (SE) for *Leucaena leucocephala* (HQR) and *Acacia decurrens* (LQR) residue-amended soils in highland and midland agroecologies across the incubation period: (a) microbial biomass nitrogen (MBN), (b) ammonia, (c) nitrate, and (d) total extractable nitrogen (TEN).

4. Discussion

4.1. The Interaction Effect of Agroecology and Plant Residue Quality on Enzymatic Activities, Prokaryotic Gene Abundance, and Metabolic Quotient. We investigated how agroecology and residue quality interact to influence enzymatic activities, prokaryotic gene abundance, and metabolic quotient in a field study. In the midland agroecology, characterized by favorable temperature and rainfall, we found higher levels of C-degrading enzymatic activities, proteolytic activity, and nitrifier abundance compared to the highland agroecology, where temperatures are lower and rainfall is higher. This difference may be attributed to the more conducive conditions for soil microbial communities in the midland agroecology (Figures 2(a) and 2(b)). Regarding C-degrading enzymes, we observed greater activity in high-quality residues than in low-quality ones (Figure 5), likely due to higher concentrations of endogenous nutrients, particularly nitrogen (Table 1; [4]). This leads microbes to produce more C-degrading enzymes, accelerating litter decomposition and resulting in higher weight loss in higher quality residues (Sup. Figure 1) [43, 44]. This is further supported by the presence of higher microbial biomass carbon (MBC) in the midland agroecology. Furthermore, our findings are consistent with the concept that microbes at higher elevations invest more energy in scavenging for nutrients and energy from complex soil organic matter (SOM), while at lower elevations, nutrients may be more readily available [45]. This suggests that although decomposition is faster in the midland agroecology, resource utilization efficiency is lower compared to the highland agroecology when soil is amended with low-quality residues. In terms of proteolytic enzymatic activities, there was no clear difference observed except for TLP, which may be due to higher endogenous nitrogen suppressing the production of protein and peptide-degrading enzymes. Similarly, high-quality residues require less energy for decomposition compared to low-quality residues, as supported by recent

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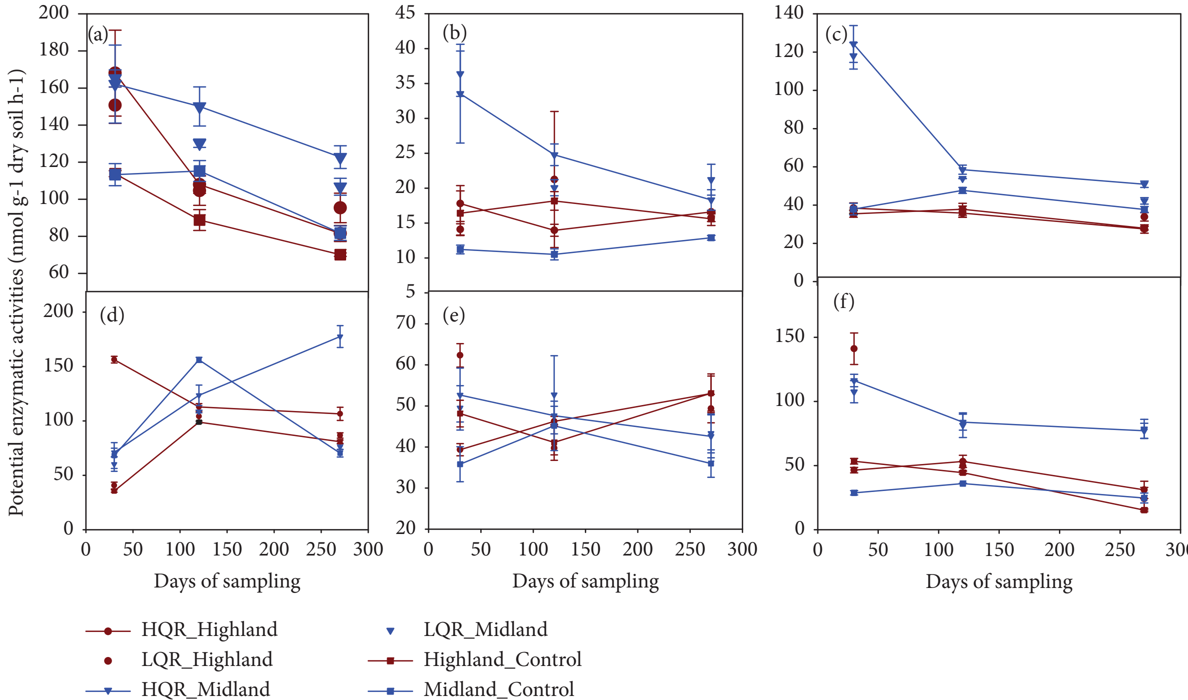

Figure 5: Estimated potential C (a–c) and N (d–f) cycling enzymatic activities with standard error (SE) during 30–270 days of incubation for *Leucaena leucocephala* (LL) and *Acacia decurrens* (AD) residue-amended soils in highland and midland agroecologies: (a) β-D-glycosidase (BGL), (b) β-D-cellobiohydrolase (BCL), (c) β-D xylanase (BXL), (d) leucine-aminopeptidases (LAP), (e) alanyl-alanyl-phenyl aminopeptidase (AAP), and (f) thermolysin-like neutral metalloproteases (TLP).

studies [2, 4]. In soils amended with high-quality residues in the midland agroecology, microorganisms were not nitrogen-limited for the first sixty days, leading to the utilization of available carbon for growth rather than increased respiration as a stress response seen in low-quality residue-amended soils in the highland agroecology. However, after 120 days, higher DCO_2 was produced in high-quality-amended soils in the midland agroecology, possibly due to nitrogen exhaustion in the high-quality plant residue. The interrelated effects of agroecology and organic residue quality suggest a treatment-specific modification of the microbial decomposer community, either by prompting all community members or selecting specific resource-use-efficient community members to enable functional accommodation to the environmental context [2]. This is in line with recent theories proposing soil microbial efficiency as a result of moderating or filtering microbial traits [46]. Under cooler agroecology with low-quality residues, the soil microbial decomposer community may undergo trait modification by selecting resource-use-efficient community members to sustain decomposition and growth. The negative synergistic effect of agroecology and residue quality parameters induces less efficient utilization of complex organic residues to acquire a unit of biomass carbon, as evidenced by a higher metabolic quotient (qCO_2) and stimulated activities of individual enzymes in the highland agroecology amended with low-quality residues. Similarly, Puttaso et al. [42] reported higher microbial qCO_2 in more lignified residues than less lignified ones in a long-term field experiment. The less lignified and nitrogen-rich plant residues in the midland agroecology stimulate soil microbial biomass, possibly due to enhanced microbial decomposition and the incorporation and preservation of dissolved organic carbon and nitrogen in the soil.

4.2. Potential Enzymatic Activities Modulate Biochemical Soil Properties and Metabolic Quotient in Residue-Amended Soils. The breakdown of organic residues in soil ecosystems relies on microbial enzymatic activities, which in turn influence soil biochemical properties and the metabolic quotient [47]. The strong ($P < 0.01$) correlation between microbial biomass carbon (MBC) and the potential activities of β-glucosidase (BGL) ($r = 0.40$; Table 3) underscores the direct role of BGL enzymes in converting carbon from glucose into MBC by partitioning carbon from plant residues [48, 49]. Similarly, the significant ($P < 0.01$) positive correlation between MBC and TLP ($r = 0.40$; Table 3) indicates that carbon from the proteolysis of proteins or peptides is also transformed into MBC. This aligns with the findings of Enggrob et al. [50], suggesting that small plant-derived compounds, such as amino acids and sugars, rapidly turnover to microbial biomass during litter decomposition, eventually stabilizing soil organic matter (SOM). Furthermore, the negative but robust correlation between dissolved organic carbon (DOC) versus BGL and TLP indicates that glucose decomposition and protein or peptide proteolysis contribute more to microbial growth, such as MBC, than DOC [2]. The degradation of hemicellulose by β-xylosidase (BXL) and protein or peptide proteolysis using TLP enzymatic activities requires

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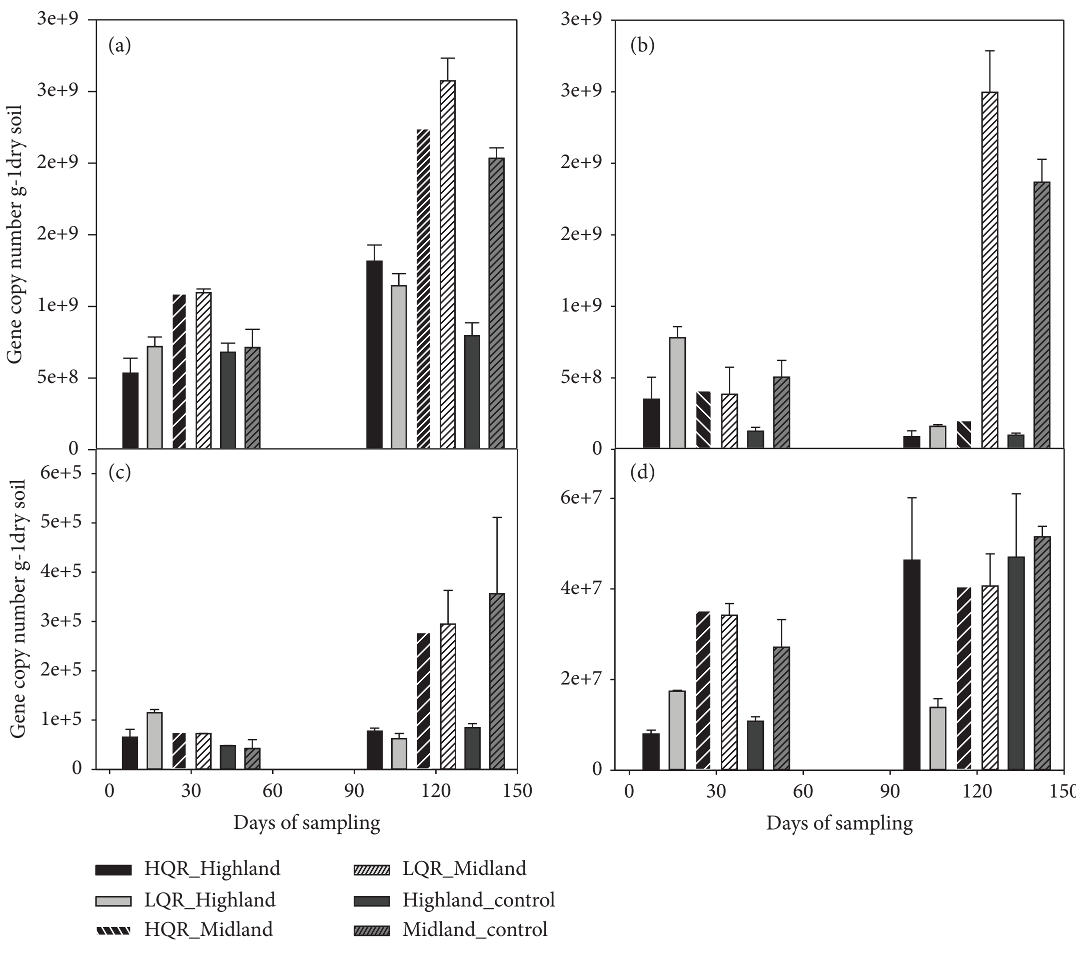

Figure 6: Estimated value of gen copy number with standard error (SE) for *Leucaena leucocephala* (HQR) and *Acacia decurrens* (LQR) residue-amended soils in highland and midland agroecologies across the incubation period: (a) total bacteria, (b) total archaea, (c) ammonia oxidized bacteria (AOB), (d) ammonia oxidized archaea (AOA).

more energy and contributes less to growth, as evidenced by their positive and significant correlations with the metabolic quotient (qCO_2) and DCO_2. Conversely, a negative and strong relationship was observed between qCO_2 and leucine aminopeptidase (LAP), suggesting that carbon produced from protein or peptide proteolysis by LAP enzymatic activities contributes more to growth than energy investment [51]. As the potential activities of cellulose- and hemicellulose-degrading enzymes increase, less nitrate (NO_3^-) is produced, indicating that the decomposition of cellulose and hemicellulose contributes less to NO_3^- production. However, as protein or peptide proteolysis using LAP enzymatic activities increases, NO_3^- and ammonium (NH_4+) levels increase, highlighting the relevance of LAP activity for nitrate and ammonia production [2]. This suggests that LAP activity may be a rate-limiting factor for proteolysis. However, this finding contrasts with Zhang et al.'s [52] report of a negative and absent relationship between LAP activity and NH_4^+-N and NO_3^--N, respectively, in the grassland soils of Mongolia. The negative but significant correlation between NH_4^+ and aminopeptidase (AAP), and NO_3^- and TLP, remains unexplained in this experiment.

4.3. Gene Abundance Moderates Proteolysis and C Mineralization. To facilitate plant residue decomposition and mineralization, specific genes and transcripts related to proteins, amino peptides, cellulose, and hemicellulose are essential [53]. With this premise in mind, our study aimed to explore the connection between the gene abundance of prokaryotic microorganisms and the processes of proteolysis and C mineralization. Our findings revealed significant ($P < 0.05$) positive correlations between the abundance of various bacterial groups, such as total bacteria ($r = 0.67$), total archaea ($r = 0.47$), and ammonia-oxidizing bacteria (AOB) ($r = 0.61$), and LAP (Table 3). This correlation can be attributed to the crucial role of ammonia oxidation, which acts as the rate-limiting step in nitrification. This process is catalyzed by a diverse array of microorganisms, including ammonia-oxidizing archaea (AOA) and AOB [54], thus confirming our second hypothesis. Furthermore, Balume et al. [2] noted a significant correlation between the abundance of AOB and AOA and the potential activities of proteolytic enzymes, albeit influenced by soil pH levels. In less acidic soils with less lignified residue amendments, Balume et al. [2] observed a positive relationship between AOA and AOB and AAP. However, during our field

Table 3: Pearson correlation of different estimated carbon and nitrogen pool values, microbial abundance with potential C and N enzymatic activities ($N = 54$) following the addition of high (L + PP)/N; 5.0) and low quality (L + PP)/N; 21) residues on soil across the incubation period at highland and midland agroecologies.

	TA	TB	AOA	AOB	DCO_2	NH_4^+	NO_3^-	MinN	MBC	DOC	MBN	TEN	qCO_2	BGL	BCL	BXL	LAP	AAP	TLP
TA	1.00	0.64***	0.59***	0.60***	−0.14^{NS}	−0.05^{NS}	−0.07^{NS}	−0.06^{NS}	0.28^{NS}	−0.13^{NS}	−0.3^{NS}	0.12^{NS}	−0.23^{NS}	0.04^{NS}	0.05^{NS}	−0.01^{NS}	0.47**	0.19^{NS}	0.04^{NS}
TB		1.00	0.53***	0.72***	−0.46**	0.53***	0.28^{NS}	0.40*	−0.00^{NS}	0.18^{NS}	−0.3^{NS}	0.35*	−0.42*	0.04^{NS}	0.21^{NS}	0.11^{NS}	0.76***	0.21^{NS}	0.05^{NS}
AOA			1.00	0.49**	−0.14^{NS}	0.16^{NS}	0.14^{NS}	0.16^{NS}	−0.03^{NS}	0.11^{NS}	−0.2^{NS}	0.33^{NS}	−0.11^{NS}	−0.00^{NS}	0.14^{NS}	0.11^{NS}	0.32^{NS}	−0.1^{NS}	−0.0^{NS}
AOB				1.00	−0.34*	0.41*	0.14^{NS}	0.26^{NS}	0.12^{NS}	−0.02^{NS}	0.00^{NS}	0.12^{NS}	−0.38*	0.00^{NS}	0.03^{NS}	−0.03^{NS}	0.61***	0.07^{NS}	0.00^{NS}
DCO_2					1.00	0.54***	−0.08^{NS}	0.33*	0.51***	−0.26^{NS}	0.42**	0.22^{NS}	0.87***	0.14^{NS}	0.22^{NS}	0.24^{NS}	−0.24^{NS}	0.01^{NS}	0.36**
NH_4^+						1.00	0.30*	0.85***	0.47***	−0.14^{NS}	0.43**	0.37**	0.25^{NS}	−0.10^{NS}	0.10^{NS}	−0.00^{NS}	0.31*	−0.32*	0.04^{NS}
NO_3^-							1.00	0.76***	−0.27*	0.37**	−0.04^{NS}	0.00^{NS}	−0.11^{NS}	−0.53***	−0.16^{NS}	−0.27*	0.33*	−0.16^{NS}	−0.32*
Min N								1.00	0.18^{NS}	0.1^{NS}	0.27*	0.25^{NS}	0.11^{NS}	−0.36**	−0.02^{NS}	−0.16^{NS}	0.40**	−0.31*	−0.15^{NS}
MBC									1.00	−0.83***	0.53***	−0.05^{NS}	0.1^{NS}	0.40**	0.09^{NS}	−0.04^{NS}	0.00^{NS}	−0.02^{NS}	0.40**
DOC										1.00	−0.39**	0.25^{NS}	0.08^{NS}	−0.47***	−0.05^{NS}	0.04^{NS}	0.21^{NS}	−0.07^{NS}	−0.34*
MBN											1.00	−0.23^{NS}	0.21^{NS}	0.16^{NS}	0.01^{NS}	−0.05^{NS}	−0.09^{NS}	−0.21^{NS}	0.07^{NS}
TEN												1.00	0.27*	0.10^{NS}	0.31*	0.43***	0.34^{NS}	−0.17^{NS}	0.08^{NS}
qCO_2													1.00	0.09^{NS}	0.23^{NS}	0.39**	−0.32*	0.04^{NS}	0.27*
BGL														1.00	0.62***	0.60***	−0.15^{NS}	0.16^{NS}	0.69***
BCL															1.00	0.74***	−0.03^{NS}	0.06^{NS}	0.43**
BXL																1.00	−0.07^{NS}	0.18^{NS}	0.59***
LAP																	1.00	−0.03^{NS}	−0.12^{NS}
AAP																		1.00	0.42**
TLP																			1.00

*** < 0.001, ** < 0.01, * < 0.05, and $^{NS:}$no significance. TA: total archaea; TB: total bacteria, AOA: ammonia-oxidizing archaea and AOB; ammonia-oxidizing bacteria, DCO_2: daily respiration, NH_4^{+}: ammonia, NO_3^- –N; nitrate, Min-N mineral nitrogen, MBC; microbial biomass carbon, DOC: dissolved organic carbon, MBN: microbial biomass nitrogen, TEN: total extractable nitrogen, qCO_2: metabolic quotient, BGL: β-D-glucosidase, BCL: β-D-cellobiohydrolase, BXL: β-xylosidase, LAP: leucine-aminopeptidases, AAP: alanyl-alanyl-phenyl aminopeptidase, and TLP: thermolysin-like neutral metalloproteases.

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experiment, we did not find a significant relationship between AOA and AOB and AAP or TLP, possibly due to multifactorial influences warranting further investigation. Interestingly, our study did not uncover any relationship between prokaryotic gene abundance and three potential C-cycling enzymes responsible for glucose, cellulose, and hemicellulose degradation. This suggests that while cellulose and hemicellulose degradation are essential processes, they may not directly contribute to nitrification.

5. Conclusion

This study highlights how the biochemical composition of residues interacts with different agroecological settings to influence the functional relationship between proteolytic enzyme activities and the abundance of total and nitrifying bacteria compared to total and nitrifying archaeal communities. This relationship is particularly pronounced in midland agroecologies with high-quality-amended soils, contrasting with highland and low-quality amended soils, indicating a niche distinction influenced by agroecology and resource quality between bacteria and archaea. Moreover, this study demonstrates that specific enzymatic activities governing nitrogen (N) and carbon (C) cycling, which act as rate-limiting factors, regulate the microbial metabolic quotient. Specifically, our research indicates that hemicellulose-degrading enzymes (BXL) working in tandem with protein-degrading enzymes (particularly LAP and TLP) play a pivotal role in litter decomposition under field conditions. This suggests that predictive models for residue decomposition should integrate these enzymatic parameters. These conclusions are particularly pertinent within the studied environmental context, as they elucidate the interconnected impacts of agroecology and organic residue quality on soil microbial processes such as decomposition, proteolysis, mineralization, nitrification, and microbial quotient (qCO_2). However, it is worth noting that we were unable to analyze the carbon and nitrogen content of undecomposed residues at each sampling time, which is necessary for calculating carbon use efficiency (CUE). Thus, further research is required to determine CUE under field conditions for residue-amended soils [22, 55].

Data Availability

The data used to support the findings of this study are available from the corresponding author on request. However, the data used to produce this paper are from a PhD study supported by the International Institute of Tropical Agriculture (IITA) with its Legume CHOICE project.

Additional Points

Highlights. (i) Interacted effect of agroecologies and residue qualities modulates prokaryotic *microorganis*ms' abundance, functions, and soil biochemical properties. (ii) Agroecological differences and residue biochemical quality exerted more influence on prokaryotic microorganisms' abundance and functions.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Birhanu Agumas conceptualized the project, performed data curation and statistical (formula) analysis, investigated the study, proposed methodology, and wrote the original draft. Getachew Agegnehu reviewed and edited the manuscript. Tesfaye Feyisa was responsible for resources and reviewed and edited the manuscript.

Supplementary Materials

Supplementary Figure 1: weight loss of *Leucaena leucocephala* (HQR) and *Acacia decurrens (*LQR) residue in highland and midland agroecologies across the incubation period. (*Supplementary Materials*)

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