Front. Agr. Sci. Eng.
https://doi.org/10.15302/J-FASE-2024546

RESEARCH ARTICLE

Mineralization and humification of chicken manure and composted kitchen waste in soils based on an *in situ* litter-bag experiment: impacts of organic inputs and microbial community

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KEYWORDS

Decomposition, humic substances, humic substance precursors, microbial communities, organic amendments, soil carbon sequestration

HIGHLIGHTS

- Chicken manure and composted kitchen waste had similar mineralization but different humification.
- The carbon:nitrogen ratio of organic inputs and microbial community composition determined the mineralization and humification of organic inputs.
- Enhanced humification led to greater carbon loss and nitrogen release.

Received September 24, 2023;
Accepted January 9, 2024.

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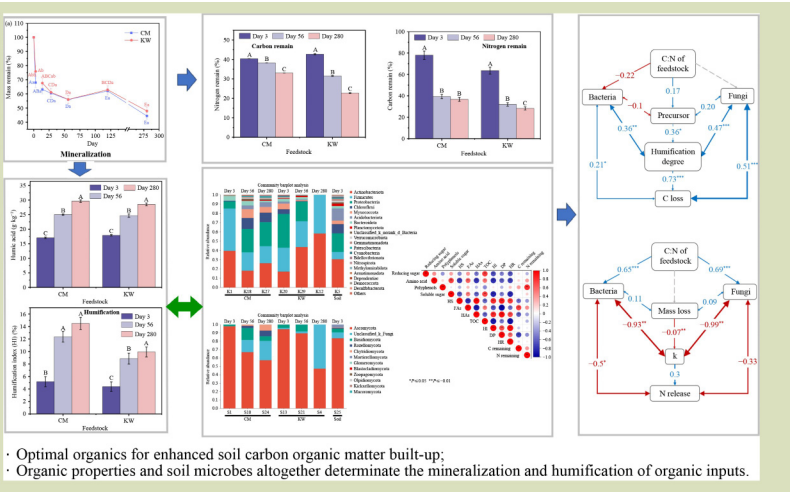
GRAPHICAL ABSTRACT



ABSTRACT

Organic inputs are key to increasing soil organic carbon in agricultural soils. This study aimed to unravel the process of mineralization and humification of chicken manure (CM) and composted kitchen waste (KW) using an *in situ* litter-bag incubation experiment. The results indicated that over 50%, 64% to 72%, and 62% to 85% of the initial mass, carbon and nitrogen, respectively, were lost through incubation with a marked loss occurring during the first 28 days. Increased humic acids (HAs), humus (HS) and degree of humification, along with a decrease in the level of fulvic acids and precursors for humic substances were observed through incubation. By comparison, CM demonstrated higher carbon and nitrogen conservation efficiencies and greater humification compared to KW. Additionally, a higher degree of humifaction and larger quantities of HAs and HS were not favorable for carbon and nitrogen conservation. Further structural equation modeling indicated that microbial

community had a strong effect on carbon loss and nitrogen release, while stoichiometric properties of organic inputs were the main determinant of the mineralization and humification processes. These findings will enhance understanding of litter decomposition in soils and provide valuable references for soil carbon sequestration with organic inputs.

1 Introduction

Increasing soil organic carbon (SOC) is currently receiving more attention as a way to mitigate climate change, improve soil health and ensure agricultural sustainability[1,2]. Increasing SOC by 4% annually could offset the annual amount of anthropogenic carbon emissions from fossil fuels (2.4×10^{18} g) [2]. Additionally, SOC is the key to soil function by impacting the biological, chemical and physical properties of agricultural soils, hence impacting the crop yield[3].

Research has indicated that sufficient and continuous organic inputs are key to increasing SOC in agricultural soils[4]. According to Zhao et al.[5], crop and straw return, as well as manure application, contributed to approximately 40% and 30% of the total SOC increase in China (4.34 Mg·ha^{-1} C) from 1980 to 2010. Also, biogas digestate, compost, manure or slurry has also been shown to increase SOC storage, with more organic inputs leading to greater SOC accumulation[6]. However, the impacts of different exogenous organic compounds on SOC can vary markedly[7]. For example, the remaining carbon in straw after one thermal year decreased in the following order: rice (40.3%) > soybean, rapeseed, wheat (34.7% to 37.8%) > maize (30.9%)[8]. Also, a global meta-analysis showed that sheep manure had the greatest effect in increasing SOC (mean effect size of 32.7%), followed by manure from pig (29.1%), cattle (27.2%), poultry (23.1%) and horse (17.5%) [6]. Basically, the carbon sequestration potential of organic inputs is determined by the two contrasting processes: carbon loss through mineralization and carbon stabilization through humification[9]. First, biomass mineralization leads to significant carbon loss through CO_2 emissions with the breakdown of organic macromolecules, which also leads to the release of available plant nutrients (e.g., NH_4^+-N, PO_4^{3-}-P, NO_3^--N, and K^+)[10]. Then, humification, which involves the poly-condensation of humic substance precursors (e.g., ammonia acid, reducing sugar, polyphenols, and carboxyl), greatly enhances the stability of organic compounds[11]. Therefore, it is necessary to explore the impact of the organic constitution and properties on the mineralization and humification of organic inputs as well as their carbon sequestration potential (i.e., the division of carbon inputs through organic application and SOC increase in a period).

Studies have indicated that stoichiometric characteristics, especially C:N ratio, greatly impact the decomposition of organic in soils. For example, Zhou et al.[12] found that the stoichiometric traits of foliar had a greater impact on the humification process than other environmental factors. In addition, it has been shown that degradation products of organic materials, such as glucose-C, are more stable than recalcitrant carbons, such as lignin, due to their rapid fixation into microbial biomass, thus effectively increasing the stable soil C pool[13]. Microbial communities also affect the mineralization and humification process of organic compounds, as they are the driving force of soil carbon dynamics[14]. For example, Wang et al.[15] found that the application of exogenous cellulose-degrading bacteria facilitated humus synthesis during co-composting of cattle manure and maize straw by directly participating in cellulose degradation and increasing the abundance of bacteria for cellulose degradation. Also, the integrated plant–soil microbial community system greatly influences litter decomposition in soils[7]. For these reasons, an exploration of the mineralization and humification process of an organic input and an in-depth analysis of the correlation among such processes, properties of organic inputs, and the dynamic of microbial communities are critical for evaluating the carbon sequestration potential of organics.

Thus, this study investigated the mineralization and humification of two organic inputs using an *in situ* litter-bag incubation experiment. The objectives of this study were: to examine the mineralization and humification process of representative organic inputs, and to evaluate the influence of humic substances and microbial communities on the carbon and nitrogen release during organic decomposition. We hypothesized that: the C loss and N release from organic materials were the co-effects of both organic properties and soil microorganisms, and all the dynamic of C and N was correlated with the mineralization and humification of organic

inputs. The findings from this study will serve as a valuable reference for managing solid organic wastes and promoting soil carbon sequestration.

2 Materials and methods

2.1 Experimental sites

An *in situ* litter-bag incubation experiment, as commonly used to assess litter decomposition, was conducted to investigate the mineralization and humification of organic inputs in soils in the presented study. This experiment was conducted in citrus fields located at the National Purple Soil Fertility and Fertilizer Benefit Monitoring Base of Southwest University, Beibei District, Chongqing, China (29°48′36″ N, 106°24′33″ E). This region has a subtropical humid monsoon climate, with an average annual temperature of 18.3 °C and precipitation of 1087 mm[16]. The soil in the experimental site is purple, a form of Pup-Orthic Entisols (Chinese taxonomy) or Regosols (FAO taxonomy), which is typical gray-brown purple soil weathered from purplish rocks under tropical and subtropical climatic conditions. The properties of soils were listed as follows: pH 6.81, total SOC 7.02 g·kg^{-1}, total nitrogen (TN) 0.51 g·kg^{-1}, total phosphorus (TP) 0.88 g·kg^{-1}, total potassium 25.8 g·kg^{-1}, alkali-hydrolyzed nitrogen 52.4 mg·kg^{-1}, available phosphorus 74.5 mg·kg^{-1}, and available potassium 95.3 mg·kg^{-1}.

2.2 Experimental design and litter-bag preparation

For litter-bag sample preparation, two types of organic organic inputs, i.e., chicken manure (CM) and composted kitchen waste (KW) were used. The CM used in this study was collected from a commercial poultry farm located in Beibei, and the composted KM was obtained from Golden Way Biotechnology Co., Ltd. Each organic input weighed 50 g and was pre-dried at 60 °C. The dried organic inputs were then smashed to < 2 mm, and then filled into polyamide litter-bags (250 mm × 200 mm with a mess size of 74 μm). The litter bags were then closed with plastic clips and buried vertically in the soils at 200 mm deep. Twenty-eight bags from each organic input were buried in soils, and the basic properties of the organic inputs are presented in Table 1.

The litter bags were buried in soil from April 25, 2022 to January 30, 2023. After specific exposure periods of 3, 7, 14, 28, 56, 120, and 280 days, 4 bags from each organic-input treatment were collected for further analysis. Upon collecting, any soil adhering to the litter bags, visible roots, and fauna were meticulously removed using forceps and brushes, and the bags were then weighted. Samples from each litter bag were divided into three parts: one part of the sample was dried at 105 °C for 12 h to determine the water content for mass loss calculation; the remaining samples from each organic input were combined, with one part of the samples air-dried and grounded for chemical analysis, and the other part of the samples was stored at −80 °C for microbial analysis.

The percentage of remaining litter mass during the incubation experiment and a single exponential decay model[17] as:

$$R_t = \left(1 - \frac{\text{Mass}_{\text{residual litter\_}t}}{\text{Mass}_{\text{input}}}\right) \times 100\% \quad (1)$$

$$\frac{\text{Mass}_{\text{residual litter\_}t}}{\text{Mass}_{\text{input}}} = a \times e^{-k \times t} \quad (2)$$

$$t_{0.95} = \frac{-\ln 0.05}{k} \quad (3)$$

where, R_t (%) is the percentage of remaining organic-input mass (dry weight basis) in the litter bags at the sampling time t (yr), $\text{Mass}_{\text{residual litter\_}t}$ and $\text{Mass}_{\text{\_input}}$ is the remaining and initial mass of organic input of the experiment, respectively. The constant a and the litter decomposition rate k (yr^{-1}) are also included in the equation, with a larger value for faster decomposition. The lifetimes of residues were determined using the Eq. (3), where $t_{0.95}$ is the time required for residues to decompose by 95%.

Table 1 Properties of organic inputs used in the litter-bag incubation experiment

Type	TOC (g·kg^{-1})	TN (g·kg^{-1})	TP (g·kg^{-1})	C:N	N/P	Moisture (%)	Organic constitution (%)				Chemical composition of organic compounds (%)				
							Crude protein	Crude fiber	Crude fat	Carbohydrate	Alkyl C (0–50 ppm)	O-alkyl C (50–110 ppm)	A/A-O	Aromatic C (110–160 ppm)	Carbonyl C (160–210 ppm)
CM	288	58.8	48.2	4.89	1.22	29.5	7.95	19.0	1.59	61.2	18.2	45.5	0.40	22.7	13.6
KW	488	29.1	5.2	16.8	5.59	30.2	8.46	14.6	1.97	65.2	44.4	37.0	1.19	41.1	7.4

2.3 Chemical analysis

Total organic carbon (TOC) and TN content of the samples were determined following the $K_2Cr_2O_7$-H_2SO_4 external heating method and Kjeldahl methods, respectively[18]. The TP content was determined using the ascorbic acid/molybdate reagent blue color method after digesting by $HClO_4$-H_2SO_4. The percentage of residual C or N in the litter at the sampling time t was calculated as:

$$R\_C_t = \left(C_t \times Mass_{residual\ litter\_t}\right) / (C_{initial} \times Mass_{input}) \times 100\% \quad (4)$$

$$R\_N_t = \left(N_t \times Mass_{residual\ litter\_t}\right) / (N_{initial} \times Mass_{input}) \times 100\% \quad (5)$$

where, R_C_t (%) and R_N_t (%) are the percentages of residual carbon and nitrogen at sampling time t, respectively; C_t and N_t are the contents of C and N at the time t, respectively; $C_{initial}$ and $N_{initial}$ are the C and N contents of organic input at the beginning of the experiment.

The extraction and quantification of humic substance components, i.e., humus (HS), fulvic acids (FAs) and humic acids (HAs), from the incubated organic inputs followed the methods described by Zhang et al.[19]. Additionally, humification parameters, such as humification index (HI), humification ratio (HR) and degree of polymerization (DP) were calculated[20] as:

$$HI = \frac{C_{HA}}{TOC} \times 100\% \quad (6)$$

$$HR = \frac{C_{HS}}{TOC} \times 100\% \quad (7)$$

$$DP = C_{HA} / C_{FA} \quad (8)$$

C_{HA} and C_{HS} are the content of HAs and FAs, respectively, and TOC is the total organic carbon content at the time t.

The main humic substances precursors, including reducing sugars, amino acids, and polyphenols were analyzed using anthrone and dinitrosalicylate reagents, respectively[21].

2.4 Microbial analysis

Microbial diversity was assessed through high-throughput DNA sequencing. Genomic DNA was extracted from samples collected on days 3, 28 and 120 using the FastDNA SPIN Kit for Soil (Mo Bio Laboratories Inc., Carlsbad, CA, USA) following the manufacturer's protocol. DNA quality and quantity were measured using the NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). All extracted DNA was stored at −80 °C for further analysis. Two commonly used primer sets were applied to metabarcoding approaches to study microbial communities targeting bacterial 16S rRNA genes and fungal ITS genes. The high-throughput sequencing of the fungal ITS gene and bacterial 16S rRNA was performed by Shanghai Majorbio Biopharm Technology Co., Ltd. (Shanghai, China).

2.5 Statistical analysis

The statistical analysis was conducted using SPSS software (Version 22.0), and the figures were created using R 4.2.3, Excel 2019, and OriginPro 2021. One-way analysis of variance was performed to test the difference among samples with a significance level set at 0.05. Pearson's correlations were established to examine the relationships among humic substances, humic substances precursors, the properties of residual litter and the total carbon loss and nitrogen, with significant levels set at $P < 0.05$ and 0.01. Also, structural equation modeling (SEM) was conducted to clarify the direct and indirect relationships between the properties of organic input and the variables of the process of mineralization and humification. The $\chi^2/df < 3$, chi-square test > 0.1, goodness-of-fit index > 0.90, and root mean square error of approximation < 0.08 were adopted to fit the SEM.

3 Results and discussion

3.1 Mass loss patterns of the two organic inputs

As shown in Fig. 1(a), organic inputs experienced a rapid degradation (days 0 to 28) followed by a plateaus phase (day 28 to 120) and finally a continuous but slow degradation (day 120 until the end of the experiment) over the experimental period. Both organic inputs exhibited a similar pattern of mass loss as indicated in Table 1. Notably, over 40% of the mass loss within the first 28 days (~80% of the total mass loss) was observed and attributed primarily to the degradation of labile organic compounds (e.g., carbohydrate, lipid and protein) in organic inputs[22]. This rapid loss of mass during the initial phases was also observed in a 210-d *in situ* incubation with leaf and root litter[23]. Noticeably, a rapid mass loss was observed in our study, which can be attributed to the high temperature as temperature greatly impacts organic decomposition in soils [24] (Fig. S1). Also, the estimated whole lifetime of CM and KW, based on the exponential decay model, was 6.21 and 6.34 years (Table S1), respectively, indicating both organic inputs have similar persistence in soils[17].

Along with mass loss, the content of C and N in the residual

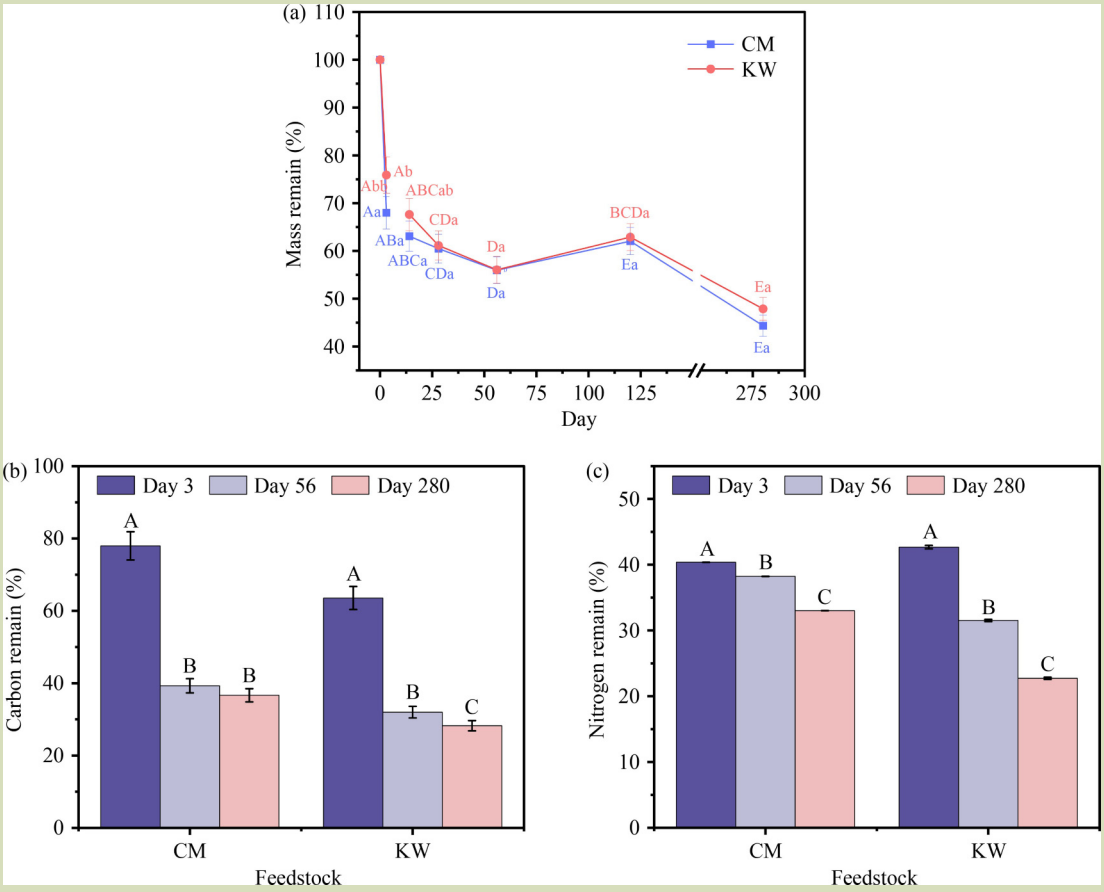


Fig. 1 Changes in the percentage of litter mass remained (a), carbon remained (b), and nitrogen remained (c) for chicken manure (CM) and composted kitchen waste (KW) through an *in situ* litter-bag incubation experiment. Error bars represent standard deviation. Uppercase letters indicate differences for a typical organic input with different incubation periods based on one-way ANOVA at $P < 0.05$ and lowercase letters indicate differences for the two organic inputs at the same incubation period at $P < 0.05$.

biomass decreased, and a significant proportion of carbon (64% to 72%) and nitrogen (62% to 85%) was lost during incubation (Fig. 1(b,c)). These findings were consistent with previous studies[25]. However, it should be noted that in some studies, no net loss of N or an increase in N content in the residual biomass was recorded[26]. The variations in carbon loss and nitrogen release between studies can be attributed to differences in the C:N ratio of organic inputs. For example, low C:N ratio organic inputs (C:N < 20) result in net N mineralization whereas high C:N inputs (C:N > 20) induce net N immobilization[27]. In our study, both CM and KM had a C:N ratio lower than 20, which results in more nitrogen mineralization and nitrogen release.

3.2 Variability in humic substances and humification process

Changes in the level of humic substances, such as HS, FAs and HAs, are shown in Fig. 2(a–c). As expected, both HS and HAs exhibited an increasing trend, while FAs in the residual CM and KW gradually decreased during the incubation period. This is because FAs can serve as intermediate products in the formation of HAs and the subsequently insoluble and non-phytotoxic humin. Additionally, no significant difference was observed between the two organic inputs ($P > 0.05$).

Figure 2(d–f) shows a comparison of various humification indexes to indicate the dynamics of humification for the two organic inputs. For both organic inputs, a gradual increase in HI and HR indicated a more intense humification process for CM during the experiment. The increase DP values further reinforced the notion of the increasing complexity of humic substances and enhanced maturity in both residual organic inputs throughout the incubation period[23]. Also, the significant difference in HI and HR indicated the existence of variation in the humification process between CM and KW,

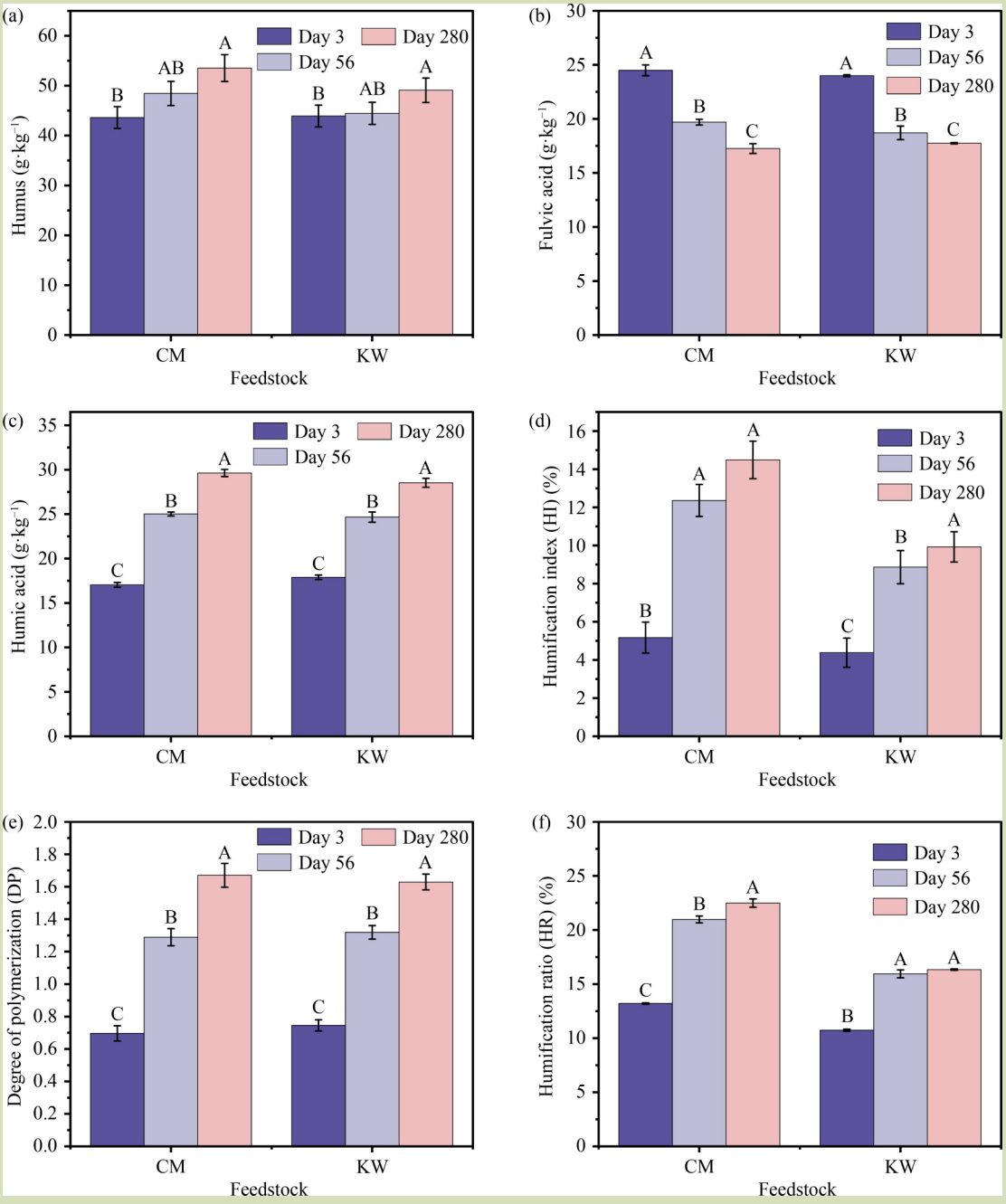


Fig. 2 Changes in the content of humus (a), fulvic acids (b), humic acids (c), humification index (d), degree of polymerization (e), and humification ratio (f) for chicken manure (CM) and kitchen waste (KW) treatments during an *in situ* litter-bag incubation experiment. Error bars represent standard deviation. Uppercase letters indicate differences for a typical organic input with different incubation periods based on one-way ANOVA at $P < 0.05$.

which can be attributed to the differing carbon and nitrogen releases during incubation (Fig. 1(b,c)). Additionally, the differences in the organic constitution of the organic input (Table 1) resulted in variation in humic substances and humification process by influencing the content of humic substance precursors[20].

3.3 Changes in humic substance precursor concentrations

Previous research has indicated that humic substance precursors, including reducing sugar, soluble sugar, amino acids and polyphenols, are crucial in the humification of biomass[23,28]. Therefore, it is essential to investigate the

changes in the concentrations of these precursors during litter-bag incubation to gain a better understanding of the biomass humification process.

3.3.1 Changes in reducing sugar content

In the CM treatment, the level of reducing sugar remained high at ~10 g·kg^{-1}, with only a slight decrease observed ($P > 0.05$). In contrast, the level of reducing sugar in the residual KW initially decreased and then increased during the incubation period (Fig. 3(a)). The fluctuations in reducing sugar content can be attributed to the formation and consumption of humic substances through microbial metabolism, particularly during the initial phases (days 0 to 56)[24]. Also, the presence of nitrogen-rich organic compounds, indicated by the low C:N ratio, partly explains the consistent and increased level of reducing sugar in the residual CM for the decomposition of polysaccharides, as it facilitates the decomposition of polysaccharides[24].

3.3.2 Changes in the amino acid content

Amino acids are crucial precursors for the formation of humic substances, according to the lignin/phenol-protein and Maillard reaction theories[28]. As shown in Fig. 3(b), the level of amino acids decreased during the incubation experiment for both CM and KW, indicating their involvement in humic substance formation. This finding is consistent with a study that showed amino acid supplementation enhances humification during composting of lignocellulose-like biomass[20]. Also, the more marked decrease in CM treatments can be primarily attributed to their higher nitrogen content. In addition to contributing to humic substance formation, microbial metabolism and protein mineralization in the organic input also influence the dynamics of amino acids during the incubation process[28].

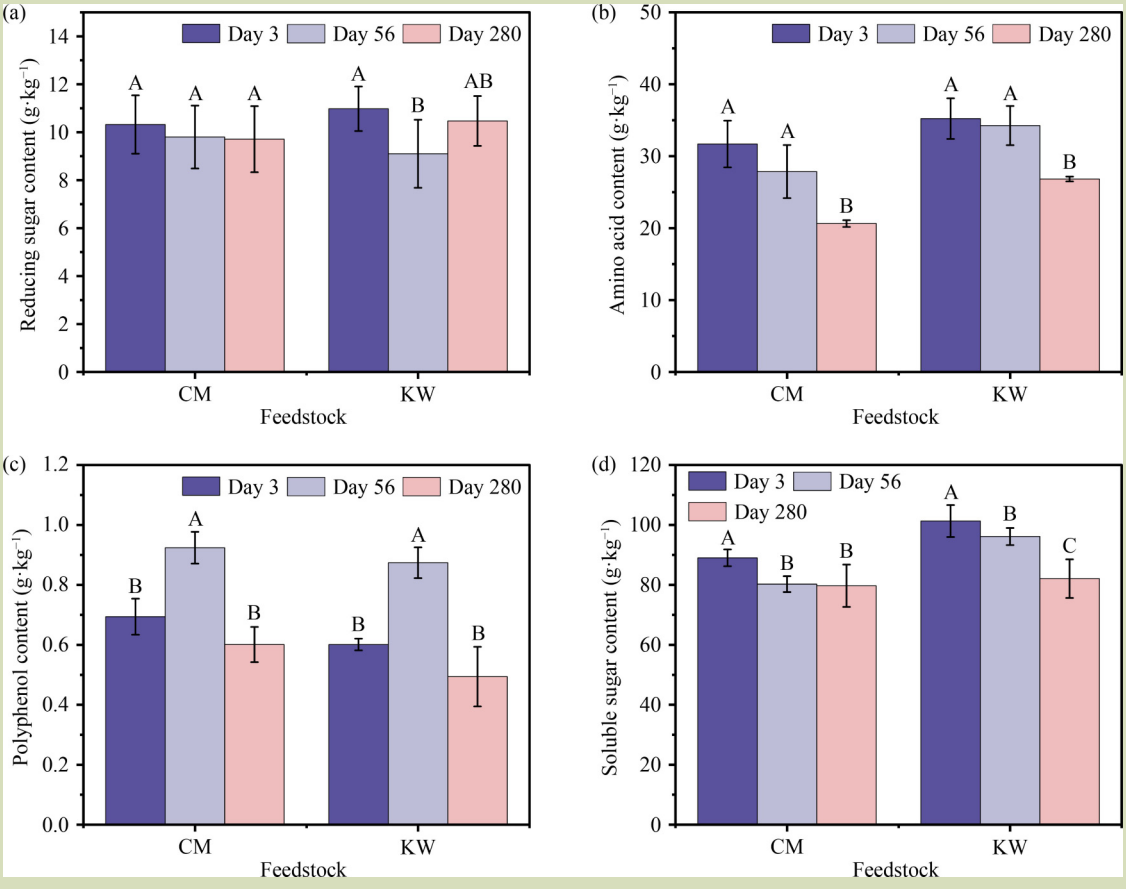


Fig. 3 Changes in the content of humic substance precursors, reducing sugar (a), amino acid (b), polyphenols (c), and soluble sugar (d) for chicken manure (CM) and kitchen waste (KW) treatment during an *in situ* litter-bag incubation experiment. Error bars represent standard deviation. Uppercase letters indicate differences for a typical organic input with different incubation periods based on one-way ANOVA at $P < 0.05$.

3.3.3 Changes in the polyphenols content

During the incubation period, the concentration of polyphenols in the residual CM and KW increased, reaching its peak on day 56, and then gradually decreased until the end of the experiment (Fig. 3(c)). The initial increase in polyphenols content is attributed to the degradation of lignin-cellulose compounds[28], which also resulted in significant mass loss. Subsequently, the formation of humus and microbial consumption contributed to the decrease of polyphenols in the residual biomass[20].

3.3.4 Changes in the soluble sugar content

Due to the formation of humic substances and microbial consumption as other precursors, the soluble sugar content in both CM and KW also decreased during incubation (Fig. 3(d)), which is consistent with previous studies[23]. In addition, the higher soluble sugar content in the residual KW was primarily attributed to its higher carbohydrate content in the initial organic input (Table 1).

3.3.5 Relationships between the precursors, humification, carbon and nitrogen loss

The potential effects of the precursors on biomass humification and the loss of carbon and nitrogen were further analyzed. As shown in Fig. S2, negative correlations were observed between the levels of HS/HAs and the precursors (such as amino acid, polyphenols and soluble sugar), indicating that these precursors promoted the formation of stable humic substances through incubation[28]. Similarly, studies have also indicated that the addition of exogenous humic substance precursors (e.g., ammonia acids, catechol and maleic anhydride) promotes humus formation during aerobic composting[29]. Also, the negative correlation between HS, HAs, humification indexes and remaining carbon indicates that more carbon is lost through humification. According to Bui et al.[30], significant amounts of CO_2 would be lost through both biotic and abiotic-catalyzed humification. Additionally, enhanced humification also facilitates nitrogen release, although the impacts were not found to be statistically significant ($P > 0.05$).

3.4 Changes in the microbial community composition and structure

3.4.1 α-Diversity and β-diversity of microbial communities during litter-bag incubation experiment

Changes in bacterial and fungal α-diversity indices and amplicon sequence variants (ASVs) during the litter-bag incubation experiment are illustrated in Fig. 4(a,b), respectively. The bacterial Chao1, Shannon indices, and the number of bacterial ASVs for both CM and KW samples experienced significant increases on day 56 compared to day 3, indicating that incubation could bring a positive effect on the microbial community with the increase in its abundance and diversity[23]. However, these indices gradually decreased afterward due to the consumption of labile organic compounds[23]. In contrast, the fungal Chao1, Shannon indices, and the number of ASVs for CM samples continued to increase, indicating an increased abundance and diversity of fungi during incubation (Fig. 4(b)). Similarly, the fungal Chao1

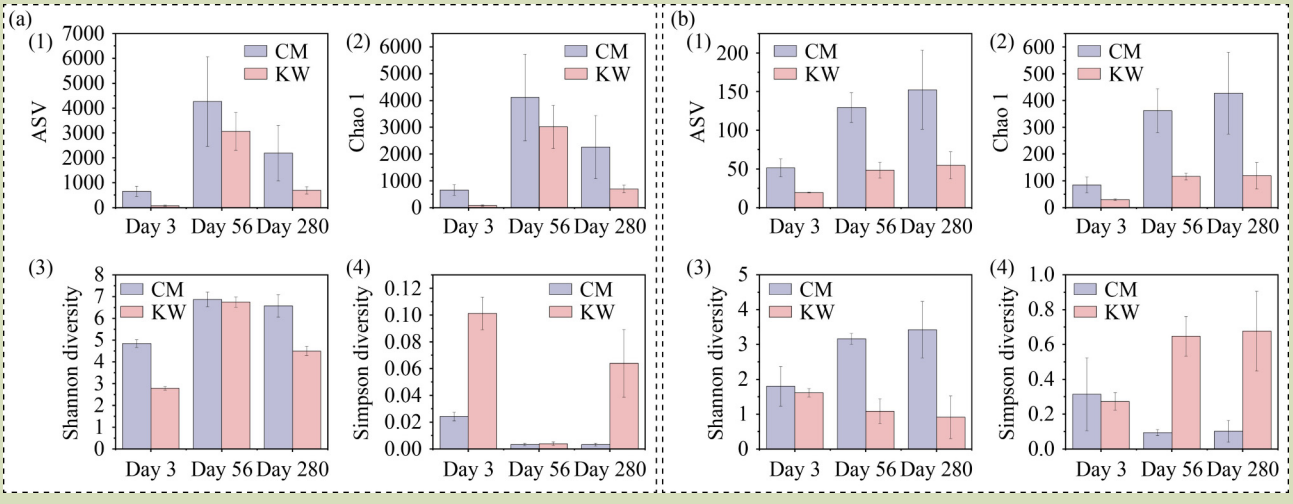


Fig. 4 α-Diversity and similarity of microbial communities during the litter-bag experiment: α-diversity of bacterial (a) and fungal (b) indicated by the number of amplicon sequence variants (ASV), Chao1, Shannon diversity and Simpson diversity.

and the number of ASVs also increased for KW samples with the prolonged incubation period. Our results differ from previous findings since changes in abiotic properties of biomass (e.g., TOC, TN and TP) strongly influence the dynamic compositional and functional succession of microbial communities[29].

The analysis of similarities indicated significant changes in the microbial communities during the litter-bag experiment (Fig. S3). As shown in Fig. S4(a), the bacterial communities were clustered into three distinguishable groups, corresponding to the three phases of decomposition (Fig. 1), indicating significant changes in the bacterial communities through the incubation period. In contrast, the fungal communities had high similarity between samples collected at the final phase (days 56 to 280) and the initial phases (days 3 to 56) for CM and KW, respectively (Fig. S4(a)). Additionally, the CM samples collected on days 3, 56 and 280 shared 174 bacterial and 40 fungal ASVs, while the KW samples shared 15 bacterial and 11 fungal ASVs (Fig. S4(b)).

3.4.2 Microbial community composition during litter-bag incubation experiment

The succession of bacterial and fungal communities in the two organic inputs through litter-bag incubation is shown in Fig. 5. It can be observed that Actinobacteriota and Firmicutes were

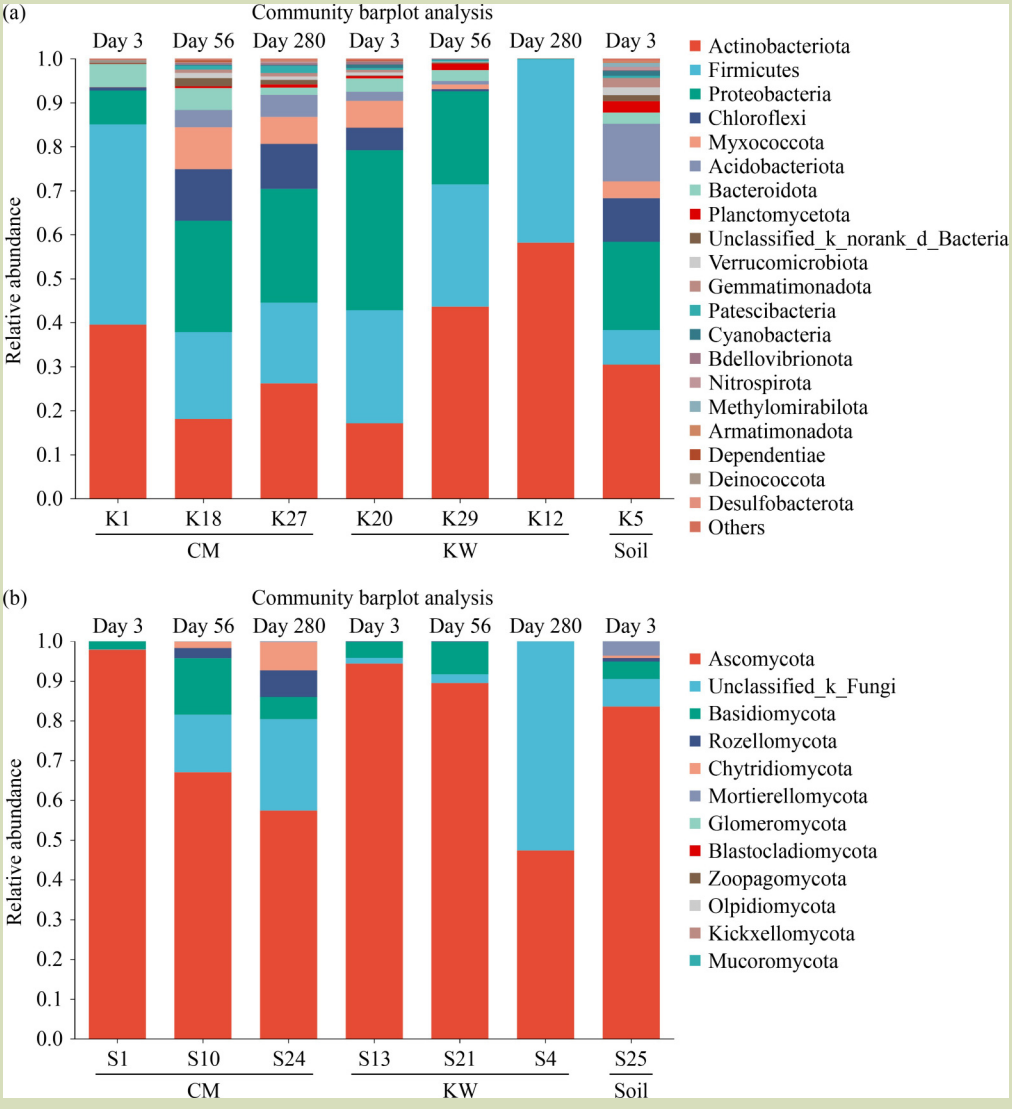


Fig. 5 Changes in the relative abundance of the top more than 20 bacterial (a) and 12 fungal (b) phyla during the *in situ* litter-bag incubation experiment.

the predominant bacterial phyla in the initial litter samples (day 3). However, there was a significant change in the microbial communities as the experiment proceeded. Also, CM samples exhibited more complex bacterial communities compared to KW samples, which is consistent with the alpha-diversity results shown in Fig. 4. In comparison to the initial litter samples, the relative abundance of Actinobacteriota and Firmicutes decreased, while the relative abundance of Proteobacteria, Chloroflexi and Myxococcota increased in the samples after fast degradation (Fig. 1). During the final phase of litter-bag incubation, the relative abundance of Actinobacteriota, Proteobacteria and Acidobacteriota increased, while Myxococcota, Chloroflexi and Bacteroidota decreased in the CM samples. It has been reported that bacteria in Firmicutes phylum are capable of decomposing sugar, protein, and hemicelluloses, which explains the decreased proportion of Firmicutes due to the consumption of labile organic compounds during incubation. Also, the increase in the proportion of Actinobacteriota can be attributed to its high efficiency in lignocellulose degradation. In contrast, the KW samples had an increase in the relative abundance of Actinobacteriota and Firmicutes, accompanied by a decrease in the proportion of Proteobacteria, Chloroflexi and Gemmatimonadota. It has been reported that Gemmatimonadota is positively correlated with nutrients such as carbon and nitrogen[31], which explains the decreased proportion of Gemmatimonadota on day 280 due to the release of nutrients. Ultimately, the distribution of microbial communities in the residual litters exhibited a high similarity to that of soils. Similarly, several studies have reported a high similarity between soil and litter microbial communities[29].

The fungal communities exhibited significant variation between the two organic inputs and samples collected at various time intervals, similar to the bacterial communities (Fig. 5(b)). Ascomycota emerged as the dominant fungal phylum in all residual biomass and soil samples. This finding is consistent with previous studies that have also identified Ascomycota as the most abundant and diverse fungal group as a key player in soils and composting processes[32]. In the CM samples, the proportion of Ascomycota decreased while Rozellomycota and Chytridiomycota increased over time. The decrease of the Ascomycota population is due to its high requirement of organic C and available nutrients, which are limited in the final phase of litter-bag incubation. During the composting, the Basidiomycota proportion was highest in the CM sample collected on day 56 whereas Rozellomycota and Chytridiomycota proportions had increased. The KW samples had a consistently high proportion of Ascomycota (~90%) throughout the incubation period, with a gradual increase in the relative abundance of Basidiomycota observed after day 56. Basidiomycota is slow-growing and oligotrophic and can exploit a broad range of substrates, preferentially synthesizing enzymes to decompose more complex polymers, making it adaptable to environmental conditions in the final phase of incubation.

3.4.3 Correlations between microbial dynamics on the decomposition and humification of organic inputs

Spearman's correlation analysis was conducted to examine the relationship between the dominant bacterial and fungal communities and the formation of humic substances, as well as the loss of carbon and nitrogen, during the *in situ* litter-bag incubation experiment, and the results are presented in heat maps shown in Fig. S5(a,b). During composting, Bacteroidota facilitated HS formation due to the ability to decompose complex organic matter like proteins and polysaccharides during the mineralization stage of composting. Also, a positive correlation was found between HAs and the majority of bacterial phyla, except for Actinobacteria and Firmicutes. In contrast, these two phyla were positively correlated with the level of FAs in the remaining biomass. This could be because FA production is associated with Actinobacteria and is produced more during prophase, which supports the microbial polyphenol theory[33]. The phyla Basidiomycota, Rozellomycota, Chytridiomycota and Mortierellomycota were positively correlated with HS and HAs (Fig. S5(b)), which is indicative of their involvement in the conversion of HA during decomposition. However, these phyla have a negative correlation with humic substance precursors, indicating that the consumption of such precursors contributes to the formation of HS. The microorganisms obtain energy and assimilate organic carbon through metabolism, producing various compounds. These degraded small molecules serve as precursor substances for the formation of HS in the later stages of composting. Similarly, a positive correlation was observed between FAs and Ascomycota, reflecting the findings for bacterial phyla.

3.5 SEM analysis of humification, C loss and N release of the two organic inputs

SEM was used to identify the main variables driving C loss and N release both via direct effects as well as indirect effects. For the process of humification, the stoichiometric properties of organic input, especially C:N ratio, were found to be the main drivers (Fig. 6(a)). Organic input C:N ratio was inversely related to bacteria diversity, which was positively related to humification degree as well as the C loss. Fungi diversity was positively linked to the precursor, humification degree and the C loss. Also, the C:N ratio of the organic input affects the

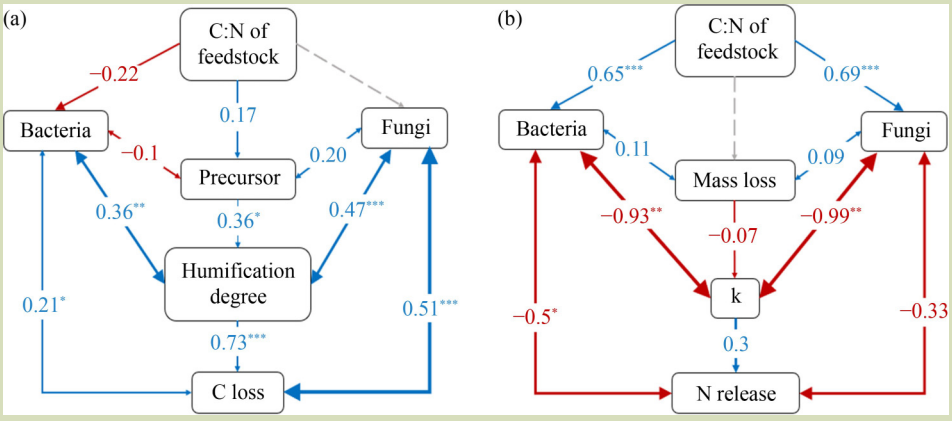


Fig. 6 Structural equation modeling revealing the direct and indirect relationships among C:N ratio of organic input, bacteria, fungi, precursor, humification index, and C loss (a) as well as C:N ratio of organic input, bacteria, fungi, mass loss, decomposition constant, N release (b). Bacteria and fungi are latent variables measured by Chao1 and Shannon diversity. Precursors are latent variables measured by reducing sugar, soluble sugar, amino acids, and polyphenols. Humification degree is a latent variable measured by humification index, humification ratio and degree of polymerization. The arrow width is proportional to the strength of the path coefficients. The red and blue arrows indicate significant negative and positive correlations, respectively, while gray dashed arrows indicate non-significant relationships. *, ** and *** indicate significance at $P < 0.05$, 0.01 and 0.001, respectively.

degree of humification and thus the amount of carbon lost by affecting the precursor. For nutrient release (Fig. 6(b)), the C:N ratio of organic input was still the important driver, specifically by positively affecting microbial diversity, which in turn was negatively linked to the N concentration. In addition, the microorganisms affect the amount of N released by affecting the decomposition constant (k). In summary, C:N ratio of CM and KM regulated the community of both bacteria and fungi, further influencing the amount of C and N (Fig. 6), indicating the properties of organic input have significant impacts on the mineralization and humification processes.

4 Conclusions

The study aimed to explore the processes of mineralization and humification in two organic inputs during an *in situ* litter-bag incubation experiment. The results showed that the rate of litter decomposition decreases over time, with most of the mass loss occurring during the initial stages of decomposition (days 1 to 56). Both CM and KW exhibited a similar pattern of mass loss, and their whole lifetime was 6.21 and 6.34 years, respectively. The study also found an increase in humification for both organic inputs, although they differed in the levels of humic substance precursors and subsequent humic substance formation, which had a significant impact on the preservation of carbon and nitrogen during incubation. Additionally, the study showed that the stoichiometric properties of organic inputs and the dynamics of microbial communities, as well as the distribution of specific bacterial and fungal phyla, greatly influenced the mineralization and humification of CM and KW.

Supplementary materials

The online version of this article at https://doi.org/10.15302/J-FASE-2024546 contains supplementary materials (Figs. S1–S5; Table S1).

Acknowledgements

This work is funded by the Key Research and Development Project for the Xizang Autonomous Region (XZ202101ZD003N, XZ202201ZY0003N), the National Key Research and Development Program of China (2022YFD1901402), and the Key Basic Research Foundation of Chongqing Academy of Agricultural Sciences (cqaas2023siczzd003).

Compliance with ethics guidelines

Yujia Shi, Haixia Zeng, Linfa Fang, Yue Deng, and Ran Xiao declare that they have no conflicts of interest or financial conflicts to disclose. This article does not contain any studies with human or animal subjects performed by any of the authors.

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