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Short-Term Effect of Nitrogen Fertilization on Carbon Mineralization during Corn Residue Decomposition in Soil

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Abstract: The effect of N fertilization on residue decomposition has been studied extensively; however, contrasting results reflect differences in residue quality, the form of N applied, and the type of soil studied. A 60 d laboratory incubation experiment was conducted to ascertain the effect of synthetic N addition on the decomposition of two corn (*Zea mays* L.) stover mixtures differing in C:N ratio by continuous monitoring of CO_2 emissions and periodic measurement of microbial biomass and enzyme activities involved in C and N cycling. Cumulative CO_2 production was greater for the high than low N residue treatment, and was significantly increased by the addition of exogenous N. The latter effect was prominent during the first month of incubation, whereas N-treated soils produced less CO_2 in the second month, as would be expected due to more rapid substrate depletion from microbial C utilization previously enhanced by greater N availability. The stimulatory effect of exogenous N was verified with respect to active biomass, microbial biomass C and N, and cellulase and protease activities, all of which were significantly correlated with cumulative CO_2 production. Intensive N fertilization in modern corn production increases the input of residues but is not conducive to soil C sequestration.

Keywords: aerobic incubation; CO_2 production; microbial metabolism; enzyme activities; active biomass; gross mineralization/immobilization

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1. Introduction

Modern cereal production relies on intensive N fertilization to increase grain yield and thereby enhances the input of crop residues. The latter effect is important for agricultural sustainability, as residues protect the surface soil against erosion loss, serve as a source of nutrients for plants and soil microbes, and sustain the soil microflora by supplying C as an energy source. Moreover, crop residues are essential to the formation of soil organic matter, although the efficiency of this conversion is necessarily reduced by liberation of CO_2 during microbial decomposition, which depends not only on environmental factors (temperature and moisture) but also N availability and residue quality (i.e., chemical composition and C:N ratio) [1].

The effect of exogenous N addition on residue decomposition has been the subject of numerous investigations, but contrasting results have been reported. A positive effect has been found in some studies [2,3], whereas in others, N addition has reduced C mineralization [4–6] or has had no net effect [7–9]. The disparities can be attributed in part to the type of residue, which affects the proportions of cellulose, lignin, and other constituents that differ in their ease of decomposition [10], and further interactions arise in relation to the size and placement of residues. Moreover, discrepancies can occur because of variation in the fertilizer N rate relative to the soil's N supplying capacity and can also reflect differences in the form of N applied, as microbial N utilization is greater with NH_4^+ than NO_3^- [11,12] but can be inhibited by acidifying N sources such as $(NH_4)_2SO_4$ or NH_4Cl. Another source of inconsistencies in the effect of exogenous N on residue decomposition is the method of

incubation, which can be compromised if aerobic conditions are not maintained throughout a study period with continuous collection of CO_2 [13].

The effect of N availability on C mineralization during residue decomposition can be clarified by investigating the impact on microbial biomass production and/or extracellular enzyme activities involved in microbial C and N cycling. Previous studies have shown that incorporation of N with residue or other carbonaceous substrates increases activities for cellulase and protease, two of the major enzymes responsible for C and N mineralization [14–17]; however, a negative effect is also possible when cellulase activity is limited by a low substrate concentration in ligneous materials [18] or when protease is repressed by a substantial concentration of NH_4^+ as the end product [16]. Microbial biomass content tends to follow changes in enzymatic activities and CO_2 production during residue decomposition [19] and can either be increased [20] or decreased [21] by the addition of N. These changes would necessarily affect the dynamics of soil and residue N through mineralization and immobilization [21–23].

Despite a massive input of residues when corn (*Zea mays* L.) is repeatedly grown with synthetic N fertilization in long-term cropping experiments, the usual trend over time is a decline in profile storage of soil organic C (SOC) [24–26]. Such findings motivated the laboratory incubation study reported herein, which utilized continuous CO_2 monitoring and relevant microbial indicators to test the null hypotheses that (1) exogenous or endogenous N increases C and N mineralization during corn residue decomposition, (2) the increased mineralization is due to stimulation of microbial biomass production and enzyme activities, and (3) the effectiveness of N for enhancing mineralization will be reduced by declining substrate availability.

2. Materials and Methods

2.1. Soil Studied

For use in comparing the decomposition of different residues, a bulk sample of surface (0–20 cm) soil was collected in early May 2019 from a Mollisol mapped as the Ipava series [Fine, smectitic, mesic Aquic Argiudolls (Chernozem)] near Farmer City (40°15′12.6″ N 88°34′59.4″ W) in central Illinois, USA. The sampling site had been cropped to a corn−soybean (*Glycine max* L. Merr) rotation for more than 40 years, during which the fertilizer N rate for corn was 180 kg ha^{-1}. The soil sample, collected in a 38 L polyethylene tote box, was sieved (2 mm screen) in the field-moist condition with removal of macro residues from the 2018 corn crop, thoroughly homogenized, and then returned to the tote box for no more than 2 weeks of storage in a refrigerator at 4 °C. A subsample was air-dried for triplicate analyses to determine the properties reported in Table 1.

Table 1. Physicochemical properties of the soil studied.

Property	Value	Reference
pH (soil:water ratio, 1:1)	6.6	[27]
Organic C (g kg^{-1})	21.5	[28]
Total N (g kg^{-1})	1.8	[29,30]
C:N ratio	12.2	
Potentially mineralizable N (mg kg^{-1})	258	[31]
Bioavailable P (mg kg^{-1})	22	[32]
Sand (g kg^{-1})	158	[33]
Silt (g kg^{-1})	595	[33]
Clay (g kg^{-1})	247	[33]
Water-holding capacity (WHC, mL kg^{-1})	635	[34]

2.2. Residues

Corn stover was collected within one day after harvest in late September from subplots of contrasting fertility under continuous corn and a corn−soybean rotation at the historic Morrow Plots in Urbana, Illinois, USA. The stover samples, consisting of leaves, stalks,

husks, and cobs, were allowed to dry for one week in a forced-air oven at 50 °C, subsequently ground to <2 mm using a Model 4 Wiley mill (Thomas Scientific, Swedesboro, NJ, USA), and then transferred to air-tight Mason jars for storage at room temperature. Before use, a composite mixture was prepared for each rotation by combining the four different residues according to the proportions given by Pordesimo et al. [35] for aboveground corn biomass. The two mixtures were characterized (Table 2) for organic C, total N, C:N ratio, and major organic fractions by proximate analysis to estimate the water-soluble fraction, lignin, and cellulose + hemicellulose [36].

Table 2. Characterization of corn residue mixtures used in incubation study. Data reported as a mean of duplicate or triplicate determinations. Values for total N and water-soluble fraction differ significantly at $p < 0.001$. Crude protein calculated as total N × 6.25 [37].

Residue Mixture	Cropping System	Organic C (g kg^{-1})	Total N (g kg^{-1})	Crude Protein (g kg^{-1})	C:N	Proximate Analysis (g kg^{-1})		
						Water-Soluble Fraction	Lignin	Cellulose + Hemicellulose
High N	Corn−corn (fertilized)	407	6.7	42	61	28.0	14.5	53.7
Low N	Corn−soybean (unfertilized)	404	4.3	27	94	18.0	13.8	52.9

2.3. Incubation Procedure

For preincubation, 252 samples of field-moist soil (50 g dry weight equivalent) were weighed into 120 mL polypropylene specimen containers and adjusted to 40% WHC by the addition of deionized water using a Metrohm 665 Dosimat (Metrohm, Herisau, Switzerland). To maintain moisture content and aerobic conditions, the containers were each sealed with Parafilm (Alcan Packaging, Neenah, WI, USA) that was punctured by forming 8–10 holes with a syringe needle. Preincubation was carried out for 7 d under dark conditions in a constant-temperature room maintained at 25 °C.

After preincubation, the following treatments were randomized among nine sets of replicate soil samples: (1) no amendment (control), (2) high N residue (HNR), (3) HNR + KNO_3, (4) HNR + $(NH_4)_2SO_4$, (5) low N residue (LNR), (6) LNR + KNO_3, (7) LNR + $(NH_4)_2SO_4$, (8) KNO_3, and (9) $(NH_4)_2SO_4$. Residues were added at the rate of 10 mg dry weight g^{-1} soil, which was selected to roughly represent modern corn production in the Midwestern USA, followed by thorough mixing for uniform incorporation. Nitrogen as NO_3^- or NH_4^+ was applied as uniformly as possible using the Dosimat to dispense a solution that supplied 0.1 mg N g^{-1} soil. All soil samples were treated with sufficient deionized water to bring the moisture content to 50% WHC by using the Dosimat, three specimen containers from each treatment (total of 27) were weighed for monitoring soil moisture content, and every specimen container was transferred to a 1.9 L wide-mouth Mason jar. Three jars from each treatment-specific set of samples, and three additional jars with no soil, were sealed using lids equipped with a pair of ball valves for atmospheric sampling, and the remainder were sealed using standard jar lids. All jars were returned to the constant-temperature room for 60 d of incubation in the dark at 25 °C.

After incubation for 7, 14, 30, 45, and 60 d, five moist soil samples per treatment were thoroughly mixed, and the composite sample was analyzed for soil pH, active biomass, microbial biomass C and N, cellulase and protease activities, and gross N mineralization and immobilization.

2.4. Atmospheric Analyses

At 1 d intervals during the first week of incubation, and at 2 or 3 d intervals thereafter, the Mason jars equipped with gas sampling lids (Figure 1) were each connected to a sampling tube and circulating pump for atmospheric sample collection following the

technique described by Horgan et al. [38]. After sampling, lids were removed from all jars in use for incubation and also from the three used for background atmospheric sampling, and the jars were left open for one hour of aeration. If necessary, deionized water was added to replace evaporative losses before reattaching jar lids, and incubation was resumed in darkness at 25 °C.

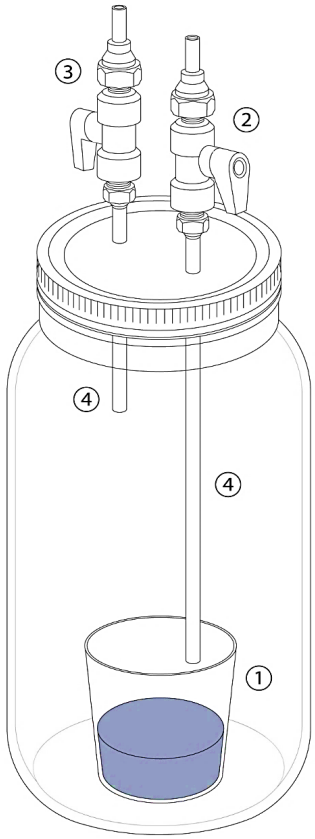


Figure 1. Unit used for incubation with atmospheric sampling by the system of Horgan et al. [38], consisting of a specimen container with soil (1) in a 1.9 L Mason jar equipped with a lid having inlet (2) and outlet (3) ball valves (item # 38EF92, Grainger, Lake Forest, IL, USA) connected to 6.4 mm O.D. brass tubing (4).

Analyses for CO_2 and O_2 were performed using a Hewlett-Packard Model 5790A gas chromatograph (GC) (Agilent Technologies, Santa Clara, CA, USA) equipped with an eight-port sampling valve (Valco Instruments Co., Houston, TX, USA) employing dual 0.5 mL sample loops, a Tracor U-90 ultrasonic detector (Tracor, Austin, TX, USA), and a Hewlett-Packard Model 3390A reporting integrator. This instrument used ultra-high purity He as the carrier gas, Porapak Q for separation of CO_2 at 50 °C, and molecular sieve 5A for separation of O_2 + Ar at 25 °C. Calibration was carried out for every set of analyses using certified mixtures of CO_2 in He (Matheson, Joliet, IL, USA) and of O_2 in N_2 (Airgas, Radnor, PA, USA).

2.5. Soil pH

Duplicate 5 g (dry weight) samples from each treatment were mixed with sufficient deionized water to obtain a 1:1 soil:water suspension, and pH was measured using a glass electrode [27].

2.6. Microbial Biomass Parameters

The active component of microbial biomass was estimated by a modified version of the biokinetics method described by Van de Werf and Verstraete [39]. In the modified method,

5 g (dry weight) of soil was adjusted to 60% WHC with or without the addition of glucose medium and then incubated (25 °C, 6.67 h) in a 250 mL straight-sided glass jar equipped with a gas-tight lid having a ball valve for CO_2 analysis using the GC system previously described. Active biomass was calculated using the equation given by Van de Werf and Verstraete [39] from the increase in CO_2 production observed for glucose-treated samples.

The chloroform fumigation/extraction procedure described by Vance et al. [40] was utilized to measure soil microbial biomass C (MBC) and N (MBN). Following K_2SO_4 extraction, organic C was determined by dichromate oxidation [28] for calculation of MBC (B_c) using the equation proposed by Vance et al. [40], $B_c = F_c/0.45$, where F_c = [(organic C extracted by K_2SO_4 from fumigated soil) − (organic C extracted by K_2SO_4 from non-fumigated soil)], and the value in the denominator represents the proportion of biomass C mineralized to CO_2.

Microbial biomass N was estimated from the difference between fumigated and unfumigated extracts when analyzed for total N by Kjeldahl digestion [29] and diffusion [30]. A value of 0.54 was assumed as the correction factor for calculating biomass N (B_N) by an equation that follows the same form as the one given in the previous paragraph for biomass C.

2.7. Enzyme Activities

Two of the major enzymes involved in C and N mineralization—cellulase and protease—were assayed to determine how their activities changed over the incubation period. Cellulase activity was determined by a *para*-nitrophenol (*p*NP) method [41] using *p*NP-β-D-cellobioside substrate prepared in modified universal buffer (MUB, pH 6.5). For this purpose, triplicate 1 g soil samples (dry weight equivalent) were treated with 5 mL of 12 mM substrate solution, and the mixture was briefly vortexed and then incubated for 2 h at 37 °C. Hydrolysis was terminated immediately following incubation by adding 4 mL of 0.1 M tris buffer (pH 12) and 1 mL of 2 M $CaCl_2$, a 1 mL aliquot of the supernatant was centrifuged to remove sediment, and *p*NP was quantified from absorbance measurements at 405 nm.

Protease assays were performed using the casein-based technique of Ladd and Butler [42] with modifications described by Jesmin et al. [43]. Briefly, 1 g of soil (dry weight equivalent) was treated with 2.5 mL of tris buffer (pH 8) and 2.5 mL of sodium caseinate solution (50 mg casein g^{-1} soil) and incubated for 2 h at 50 °C on a shaking sand bath. After incubation, trichloroacetic acid was added to terminate hydrolytic activity, the supernatant obtained by centrifugation was treated with Na_2CO_3 solution and Folin reagent, and the tyrosine released was determined spectrophotometrically at 650 nm.

2.8. Gross N Mineralization and Immobilization

For comparing treatment effects on N mineralization and immobilization, pool-dilution measurements were performed using quadruplicate 10 g (dry weight) samples of soil from each composite mixture. Two of these samples were treated with 100 mL of 2 M KCl followed by 0.64 mL of deionized water containing 40 μg of N as $(^{15}NH_4)_2SO_4$ (30 atom % ^{15}N), and the remaining samples were incubated at 25 °C for 3 d after the same addition of $(^{15}NH_4)_2SO_4$. In both cases, mineral N was extracted by shaking soil samples with 2 M KCl for 1 h and filtering the resulting soil suspension through Whatman no. 42 filter paper (GE Healthcare, Maidstone, UK) in a Büchner funnel under vacuum. The extracts were analyzed for exchangeable NH_4^+-N by accelerated diffusion methods of Khan et al. [44], followed by ^{15}N analysis using an automated Rittenberg system [45]. Gross rates of mineralization and immobilization were calculated using the zero-order equations derived by Kirkham and Bartholomew [46].

2.9. Statistical Analyses

Replicate data were characterized by computing means and standard deviations. PAST version 3.22 [47] was used to test for homogeneity (Levene's test) and normality (Shapiro−Wilk's

test), to evaluate the significance of treatment effects by a one-way analysis of variance (ANOVA), and to carry out mean comparisons by Tukey's procedure ($p < 0.05$ to 0.001).

3. Results and Discussion

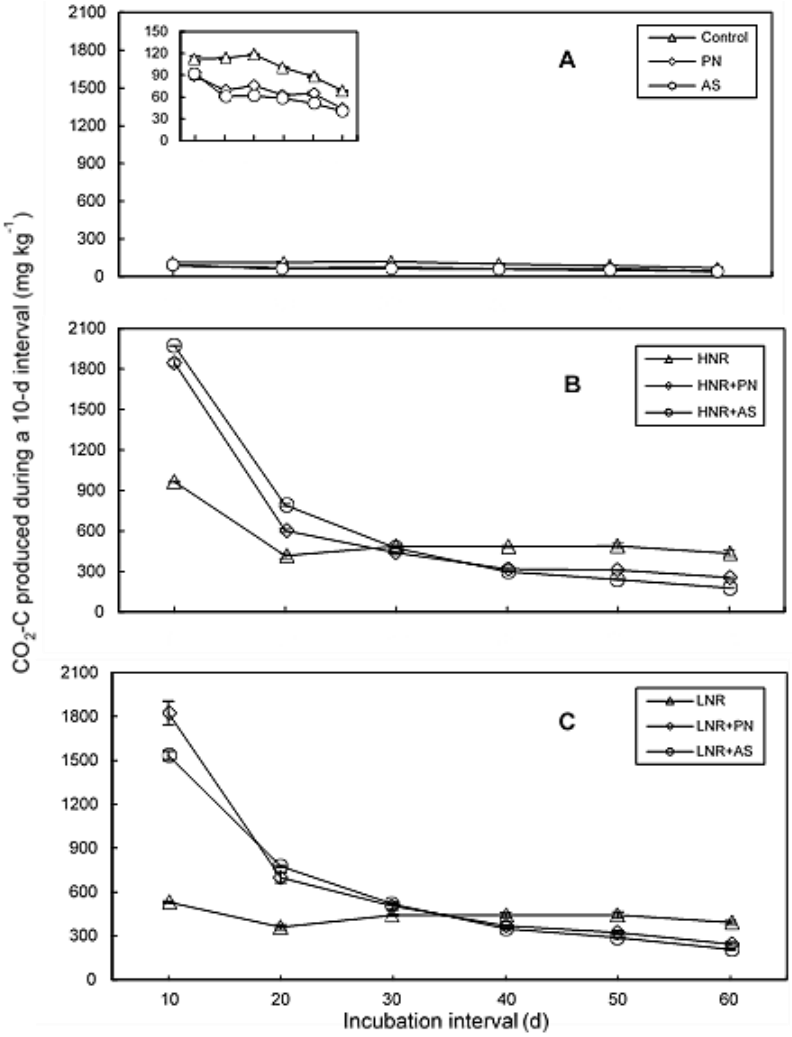
3.1. Residue Quality

Besides increasing grain yields and the quantity of above- and below-ground residue inputs, N fertilization has an intrinsic effect on residue quality by increasing biomass content of total N or crude protein [48–50], thereby lowering the C:N ratio [51,52]. These changes are apparent from Table 2, which shows that total N was significantly greater for the high N residue (HNR) collected after using fertilizer N, which caused a substantial decline in C:N ratio. Table 2 also shows that fertilization led to a significant increase in the water-soluble fraction obtained by proximate analysis. This fraction, consisting largely of monomeric sugars (predominately glucose and fructose) along with related alditols, aliphatic acids, and inorganic ions [53], enriches the soil in labile organic C when leached from residue and is readily utilized during microbial decomposition [54–56]. The proximate analyses reported by Table 1 are consistent with previous reports that N fertilization is more effective for increasing soluble than structural carbohydrates in corn residues [57–59].

3.2. C Mineralization

The effects of endogenous and exogenous N on microbial decomposition during the 60 d incubation period are documented by temporal (Figure 2) and cumulative (Figure 3) data for CO_2-C production. As expected, the amount of CO_2-C collected was always greater for incubations with rather than without residue (Figure 2), the cumulative effect being at least a four-fold difference (Figure 3) with O_2 concentrations that always exceeded 0.20 kPa O_2 kPa^{-1} to ensure aerobic conditions. Examination of the inset panel in Figure 2A reveals that CO_2 production without residue was significantly ($p < 0.01$) reduced by the presence of KNO_3 (PN) or $(NH_4)_2SO_4$ (AS), although neither effect was significant when evaluated using the entire dataset (Figure 3). Such findings can be explained by 'microbial N mining', whereby N limitation stimulates microbial attack on indigenous organic matter [60–62], the result being greater C mineralization than would occur when microbial N demand is alleviated by the input of mineral N. Moreover, the high salt index of KNO_3 [63] would have limited microbial activity and biomass due to plasmolysis caused by osmotic stress [64–66], whereas the acidifying effect of $(NH_4)_2SO_4$ [67], which is documented by Figure 4, would have been more important than salinization for inhibiting heterotrophic C oxidation [68–70]. The findings in Figures 2 and 3 are consistent with previous reports that, in the absence of residue inputs, N fertilization usually decreases soil respiration [22,71,72].

Given that the two residue mixtures studied differed considerably in their N contents, an increase in CO_2 production was expected in comparing the HNR with the LNR treatment. This was indeed observed, as collection of CO_2 was significantly greater for HNR than LNR during the first 10 d of incubation but not thereafter (Figure 2B,C). Due to the initial enhancement, a significant increase also occurred in the cumulative emission of CO_2 (Figure 3), which in terms of the C applied was equivalent to 66% for HNR and 50% for LNR. The latter finding is in line with previous studies showing that a higher N content promotes microbial decomposition when crop residues incubate following their incorporation in soil [73–76], but also reflects the fact that HNR was substantially greater in the water-soluble fraction (Table 2). According to Shi and Marschner [55], this fraction serves as a key source of energy to support active growth by the heterotrophic microflora, which promotes residue decomposition during the early stage of incubation.



Control	e	e	c	e	d	d
PN	e	e	cd	e	d	d
AS	e	e	d	e	d	d
HNR	c	d	a	a	a	a
HNR + PN	a	c	b	cd	b	b
HNR + AS	a	a	ab	d	c	c
LNR	d	d	ab	a	a	a
LNR + PN	a	b	a	b	b	b
LNR + AS	b	ab	a	bc	b	bc

Figure 2. Total quantity of CO_2-C produced by soil in 10 d intervals during a 60 d aerobic incubation involving the following nine treatments: (**A**) unamended control, potassium nitrate (PN), ammonium sulfate (AS); (**B**) high N residue (HNR) with or without PN (HNR + PN) or AS (HNR + AS); and (**C**) low N residue (LNR) with or without PN (LNR + PN) or AS (LNR + AS). Data shown as a mean from triplicate incubations with standard error bars and a table for mean comparisons. Within a given incubation interval, treatments followed by the same letter do not differ significantly at $p < 0.05$. When compared at a smaller scale (**A**), CO_2-C was significantly greater ($p < 0.01$) for the control than for the PN or AS treatment.

Regardless of which residue mixture was incorporated prior to incubation, cumulative CO_2 production, ranging from 82 to 88% of the residue C applied, was significantly increased by the presence of exogenous NH_4^+ or NO_3^- relative to the HNR and LNR treatments. This is evident from Figure 3, which also shows that the two N sources did not

differ in their effects on cumulative CO_2 production, presumably because NO_3^- utilization is promoted by the presence of carbonaceous residues. The finding that addition of mineral N promoted liberation of CO_2 during decomposition of corn residue is consistent with results previously obtained in many relevant incubation studies [2,61,73,76–79] and can presumably be attributed to microbial N utilization for cellular synthesis and metabolism. In some cases, exogenous N has had no significant effect on soil respiration in the presence of corn residue [9,21,80], which may reflect variations in incubation procedure, the type of soil studied, and/or the relative rates of residue and N addition. Reports that decomposition is unaffected or even inhibited by the addition of mineral N are more common from studies with more ligneous plant materials such as wheat (*Triticum aestivum* L.) or rice (*Oryza sativa* L.) straw, tree bark, or sawdust [4,81–83].

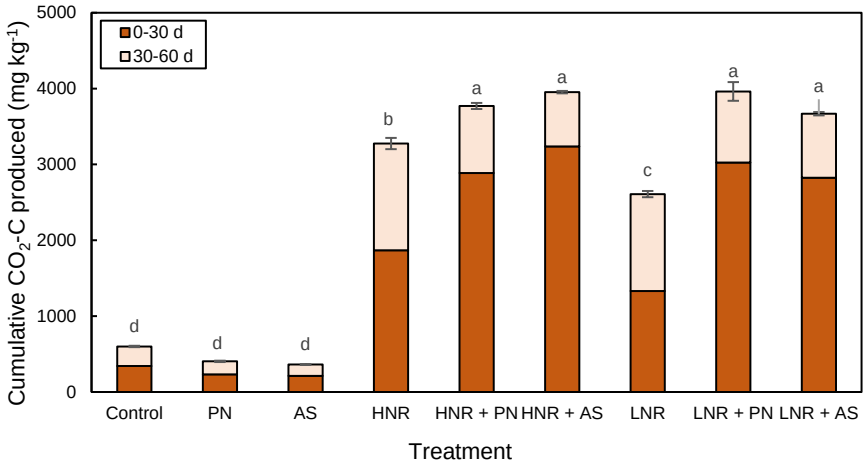


Figure 3. Cumulative CO_2-C produced by soil during half or all of a 60 d aerobic incubation involving an unamended control and the following eight treatments: potassium nitrate (PN), ammonium sulfate (AS), high N residue (HNR) with or without PN (HNR + PN) or AS (HNR + AS), and low N residue (LNR) with or without PN (LNR + PN) or AS (LNR + AS). Data shown as a mean from triplicate incubations with standard error bars obtained for the total amount of CO_2 collected. Treatments do not differ significantly ($p < 0.05$) for the entire 60 d incubation period when bars are accompanied by the same letter.

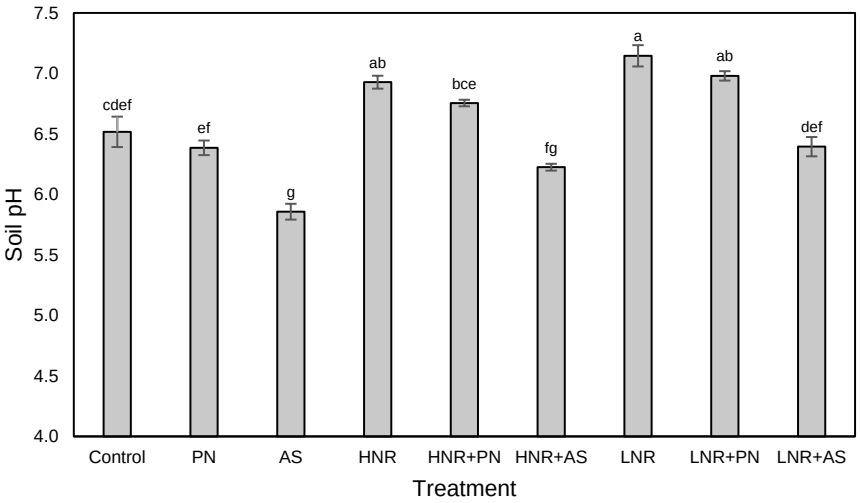


Figure 4. Soil pH during a 60 d aerobic incubation involving an unamended control and the following eight treatments: potassium nitrate (PN), ammonium sulfate (AS), high N residue (HNR) with or without PN (HNR + PN) or AS (HNR + AS), and low N residue (LNR) with or without PN (LNR + PN) or AS (LNR + AS). Data shown as a mean from duplicate incubations for 7 and 60 d with standard error bars. Treatments do not differ significantly ($p < 0.05$) when bars are accompanied by the same letter.

Besides increasing the cumulative production of CO_2 from residue-treated soil, exogenous N shifted the temporal pattern of decomposition, such that 76 to 82% of the CO_2 collected was liberated in the first month of incubation, as compared to 51% for the LNR and 57% for the HNR treatment (Figure 3). A substantial decline subsequently occurred for the fertilized but not the unfertilized treatments with residue, and the difference was usually significant (Figure 2). This shift has previously been observed in numerous incubation studies [2,4,76,80] and can be explained by microbial utilization and subsequent depletion of labile constituents released by residue decomposition.

3.3. Microbial Parameters

3.3.1. Active Biomass

The response of microbial communities to a readily available supply of substrate can be estimated by the assay of active biomass, which in the present project involved a short-term measurement of respiration following the addition of glucose. The results (Figure 5) show that, when averaged over the entire incubation period, active biomass for residue-treated soil was higher with than without exogenous N, although the difference was not necessarily significant due to limited replication. The importance of an active microbial fraction to soil respiration is also evident from the finding that active biomass was highly correlated ($r = 0.94$, $p < 0.001$) with cumulative CO_2 production in the incubation study, as in previous work by Alvarez and Alvarez [84]. A decline in active biomass was expected with the loss of residue C over time, and this was indeed observed when a 2 to 34% decrease occurred during the second month of incubations with residue, as opposed to a corresponding increase of 19 to 119% for treatments without residue (Figure 5). The latter increase, which was most pronounced for the AS treatment, reflects a drastic stimulation of glucose-responsive microbial growth and respiration following an intensifying level of starvation as substrate depletion was exacerbated by aerobic incubation. Under such conditions, the introduction of substrate triggered a microbial shift from the potentially active fraction to an active physiological state, thereby increasing the active biomass pool [85,86] that represented 13 to 35% of the total biomass C.

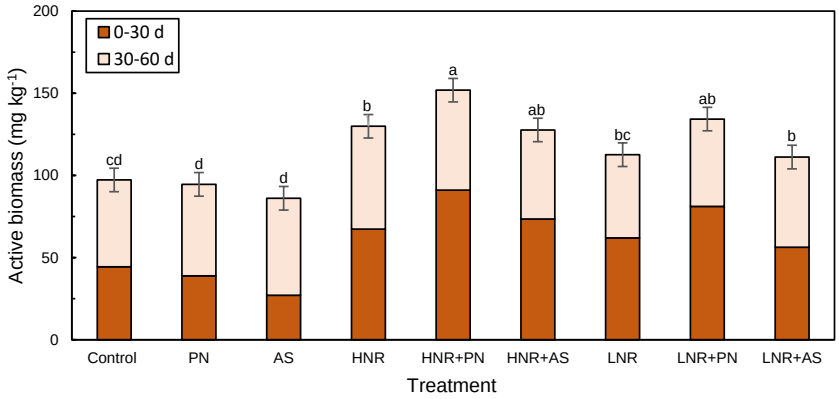


Figure 5. Active biomass measured at five intervals (7, 14, 30, 45, and 60 d) during a 60 d aerobic incubation involving an unamended control and the following eight treatments: potassium nitrate (PN), ammonium sulfate (AS), high N residue (HNR) with or without PN (HNR + PN) or AS (HNR + AS), and low N residue (LNR) with or without PN (LNR + PN) or AS (LNR + AS). Data for each incubation interval shown as a mean from duplicate subsamples with standard error bars. Statistical analyses were performed after averaging data for all five intervals, and treatments do not differ significantly ($p < 0.05$) when bars are accompanied by the same letter.

3.3.2. Microbial Biomass C and N

Unlike active biomass, MBC was unaffected by the presence or absence of exogenous N, with significant differences only being observed in comparing some but not all averages for treatments with and without residue (Figure 6A). Similar findings have previously

been reported in relevant incubation studies by Muhammad et al. [21], Song et al. [87], and Li et al. [88], and reflect the contrasting effects of residue and mineral N inputs on microbial growth and activity. Organic substrates supply C essential to microbial biomass synthesis, while inorganic N predominantly contributes to extracellular enzyme activities [89]. The view that N can be more important for promoting microbial activities than growth is relevant to the present study because residue treatments with and without inorganic N differed significantly in active biomass and cumulative CO_2 production (Figures 3 and 5) but not in MBC (Figure 6A), despite a significant correlation of the latter two parameters ($r = 0.81$, $p < 0.01$).

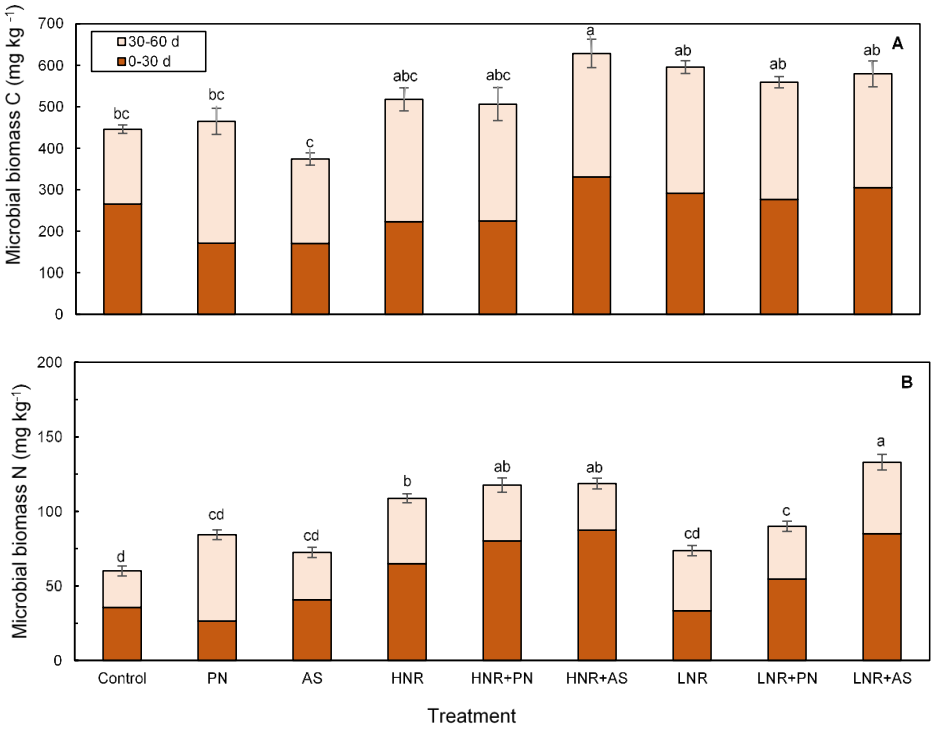


Figure 6. Microbial biomass C (**A**) and microbial biomass N (**B**) measured at five intervals (7, 14, 30, 45, and 60 d) during a 60 d aerobic incubation involving an unamended control and the following eight treatments: potassium nitrate (PN), ammonium sulfate (AS), high N residue (HNR) with or without PN (HNR + PN) or AS (HNR + AS), and low N residue (LNR) with or without PN (LNR + PN) or AS (LNR + AS). Data for each incubation interval shown as a mean from duplicate subsamples with standard error bars. Statistical analyses were performed after averaging data for all five intervals, and treatments do not differ significantly ($p < 0.05$) when bars are accompanied by the same letter.

To better clarify the interacting effects of C and N on total microbial biomass, the MBN fraction was measured at the same incubation intervals utilized in characterizing MBC. The results (Figure 6B) reveal that MBN was significantly greater for the HNR, HNR + PN, and HNR + AS treatments as compared to soils incubated without residue, and with the exception of LNR + AS, significantly lower for the LNR than the HNR treatments. Both findings can be explained by differences in the supply of assimilable N, following similar reports in several previous studies [90–92]. A significant correlation was obtained between MBN and cumulative CO_2 production ($r = 0.78$, $p < 0.05$), indicating that organic C mineralization released C and N substrates for microbial biomass synthesis [93]. For the HNR + PN and LNR + PN treatments that supplied ample C and N, MBC was 2 to 25% greater in the second than in the first month of incubation, whereas the corresponding change for the HNR + AS and LNR + AS treatments was a 10% decrease due to soil acidification. Relative to the same temporal periods, a 35 to 64% decrease in MBN was observed for these four treatments, reflecting a decline in microbial activity due to substrate limitation.

3.3.3. Enzymes Involved in C and N Mineralization

Given the fundamental role of C-degrading enzymes in residue decomposition [16], assays of cellulase activity were performed at periodic intervals throughout the current study. As shown by Figure 7A, cellulase activities were significantly greater for treatments with rather than without residue regardless of whether LNR or HNR was used, and significant increases were also observed upon incubating residue-treated soils with exogenous N. A positive effect of residue addition would be expected for an inducible enzyme such as cellulase [94], while a stimulatory effect of mineral N is documented for cellulosic substrates by several previous studies [15,17,94–96], presumably because increased N availability enhances the activities of the wide variety of cellulolytic soil fungi and bacteria. In line with previous reports by Geisseler and Horwath [16] and Luo et al. [17], cellulase activity was strongly correlated ($r = 0.90$, $p < 0.001$) with cumulative CO_2 production.

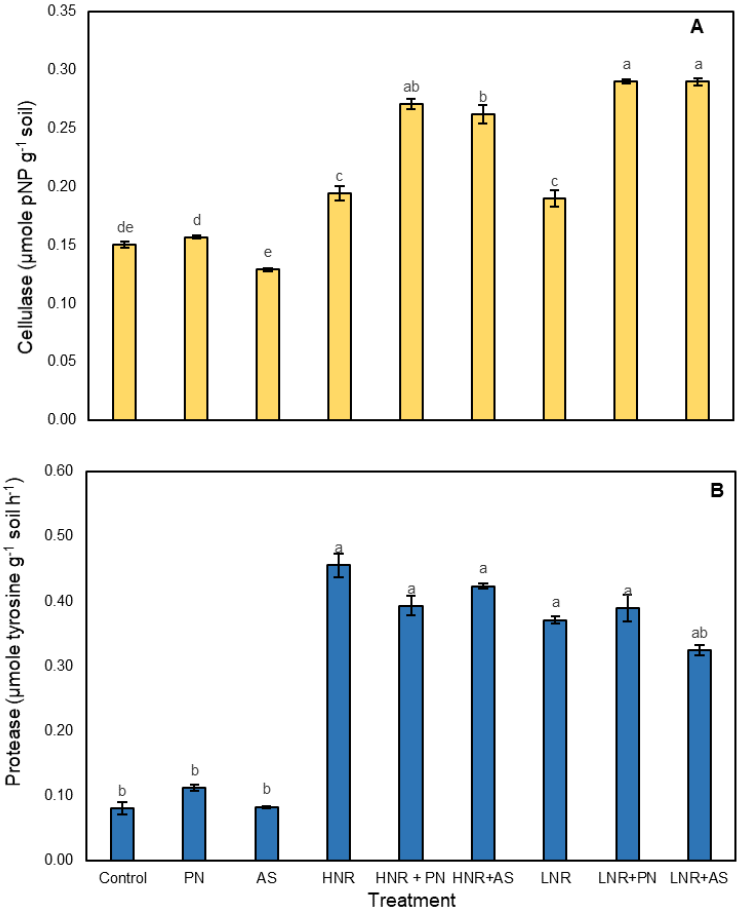


Figure 7. Mean cellulase (**A**) and protease (**B**) activities measured at five intervals (7, 14, 30, 45, and 60 d) during a 60 d aerobic incubation involving an unamended control and the following eight treatments: potassium nitrate (PN), ammonium sulfate (AS), high N residue (HNR) with or without PN (HNR + PN) or AS (HNR + AS), and low N residue (LNR) with or without PN (LNR + PN) or AS (LNR + AS). Data shown as a mean from triplicate incubations with standard error bars. Treatments do not differ significantly ($p < 0.05$) when bars are accompanied by the same letter.

To further evaluate the effect of substrate availability on the microbial turnover of C and N, assays were carried out concurrently for protease as well as cellulase. Proteolytic enzymes play a key role in soil N mineralization, comprising a group of extracellular hydrolases that convert proteins to peptides and/or amino acids [97]. Figure 7B reveals that soil protease activities were much lower when residues were absent with the control, PN, and AS treatments, owing to the lack of C sources for heterotrophic microbial metabolism and extracellular enzyme synthesis. Protease activities increased markedly for the remaining

treatments that all involved residue addition. Such increases were induced in response to ample availability of organic C and N, but there is no evidence of any effect due to the input of mineral N. Similar findings from previous research concerning soil protease activities have been linked to substrate C:N ratios and the repressive effect of NH_4^+ or NO_3^- [16,98–100]. In the present study, protease activity was significantly correlated with cumulative CO_2 ($r = 0.94$, $p < 0.001$), active biomass ($r = 0.89$, $p < 0.001$), MBC ($r = 0.76$, $p < 0.05$), and cellulase activity ($r = 0.71$, $p < 0.05$), which is consistent with previous findings by Geisseler and Horwath [16,98], Geisseler et al. [99], and Mishra et al. [101].

3.4. N Mineralization

Because soil C and N cycling is closely coupled, ^{15}N pool dilution was used to evaluate the treatments under investigation with respect to gross N mineralization and immobilization. The results are summarized by Figure 8A, which shows that immobilization always exceeded mineralization, even when there was no residue addition with the control, PN, and AS treatments. Both findings can be attributed to the use of a soil that contained carbonaceous residues when collected from a field previously cropped to corn [102]. Despite limited replication that minimized significant differences, net immobilization (Figure 8B) tended to be higher for treatments involving the addition of NH_4^+ than NO_3^-, as would be expected from the microbial preference previously documented for N utilization [11,12,22]. Gross mineralization and immobilization were highly correlated ($r = 0.91$, $p < 0.001$), as would be expected given their concurrent occurrence as the central processes in soil N cycling [22].

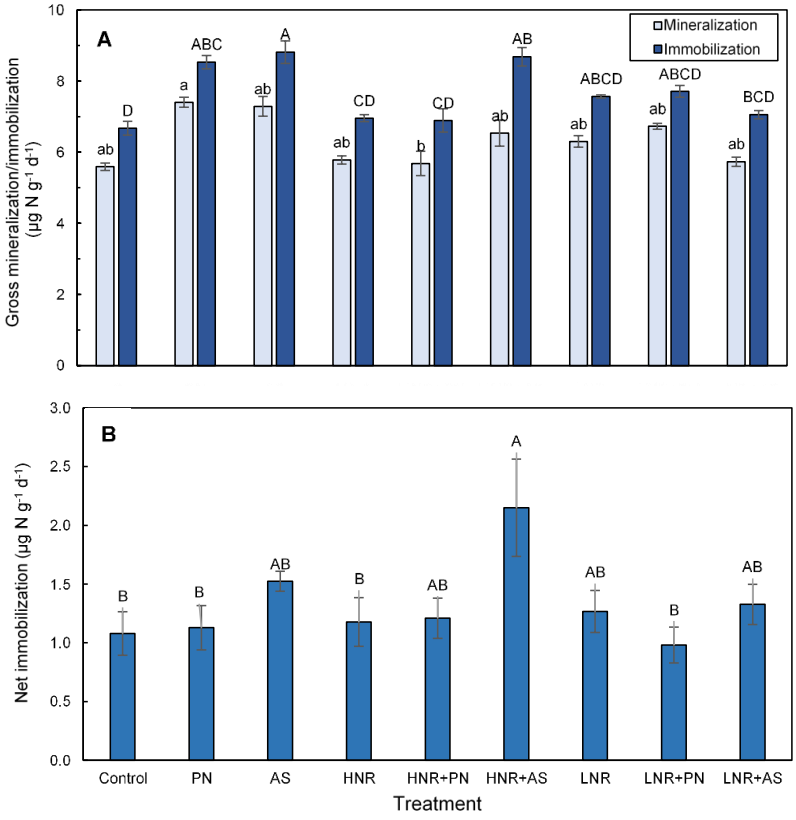


Figure 8. Gross mineralization/immobilization (**A**) and net immobilization (**B**) measured at five intervals (7, 14, 30, 45, and 60 d) during a 60 d aerobic incubation involving an unamended control and the following eight treatments: potassium nitrate (PN), ammonium sulfate (AS), high N residue (HNR) with or without PN (HNR + PN) or AS (HNR + AS), and low N residue (LNR) with or without PN (LNR + PN) or AS (HNR + AS). Values reported as a mean with standard error bars representing duplicate data collected before and after a 3 d incubation with $(^{15}NH_4)_2SO_4$. Treatments do not differ significantly ($p < 0.05$) when bars are accompanied by the same lowercase (mineralization) or uppercase (immobilization) letter.

4. Conclusions

The soil incubation study reported herein supports the view that N availability is an important factor in the decomposition of corn stover, as cumulative CO_2 production after 60 d was directly affected by residue N content and a substantial increase occurred from the application of mineral N, although there was no significant impact on gross N mineralization. A stimulatory effect of exogenous N was observed for active biomass and cellulase activity but not for MBC, MBN, or protease. With respect to C mineralization, this effect was much more pronounced in the first than in the second month of incubation, as would be expected due to a greater decline in substrate availability.

These findings have practical implications for modern corn production that relies on high planting rates and intensive N fertilization to increase grain yield and is often assumed to promote SOC storage by enhancing residue inputs. On the contrary, five decades of synthetic N fertilization led to a net decline in profile storage of SOC, relative to treatment-specific baseline data collected for the historic Morrow Plots [26], which is consistent with similar evidence from numerous other long-term cropping experiments throughout the world. Such findings would be expected if fertilizer and residue N promote heterotrophic C utilization during microbial decomposition, as demonstrated by the short-term incubation approach adopted for the present project.

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Sample Availability: Samples of the compounds are not available from the authors.

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