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The Transformation Dynamics and Homogeneity of Different N Fractions in Compost following Glucose Addition

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Abstract: The application of compost to soil is a common fertilization practice for improving soil quality and crop growth. The isotopic labeling technique is mostly used to investigate the contribution of compost N to crop uptake. However, compost N includes various N fractions and labeling dissimilarity, which may cause bias when calculating the compost N contribution to plants. Therefore, the labeling dynamics of different N fractions in compost and the homogenous labeling time point should be clarified. Given the ^{15}N-labeling in chemical fertilizer and the carbon source, i.e., glucose, the compost N pools were divided into active N (mineral N, soluble organic N [SON], microbial biomass N [MBN]), stable N (hot-water extractable organic N [HWDON]), and recalcitrant N. The atom percentage excess (APE) of different N in compost notably varied at the beginning of incubation, ranging from 0–3.7%. After the addition of glucose, biological N immobilization was promoted (13.7% and 28.8% for MBN and HWDON, respectively) and promoted the transformation among available N pools. Adding distinct doses of glucose at three stages to ^{15}N-labeled compost resulted in diverse microbial responses, thereby redistributing exogenous N in each fraction ($^{15}NH_4^+$-N went into SO^{15}N from day 15 to day 30 and increased by 5.1%; SO^{15}N entered MB^{15}N and HWDO^{15}N during day 30 to day 45 and increased by 5.7% and 5.2%, respectively). On day 45, homogeneous ^{15}N-labeled compost was achieved, which was 2.4% for ^{15}N APE for all N fractions. Overall, the quantitative data for the transformation of N fractions in compost at distinct stages provides a scientific basis for compost labeling trials, in order to identify the time point at which compost N-labeling is homogeneous, which is necessary and meaningful to reduce the bias of the contribution rate of compost-N to plants.

Keywords: glucose addition; nitrogen fractions; ^{15}N-labeled compost; compost management

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1. Introduction

Numerous studies have shown that compost can, to some extent, replace chemical fertilizers, increase soil quality, and maintain plant growth [1–3]. However, the nitrogen fertilizer value, which denotes the contribution of compost-N to crop N uptake, is unclear, since various types of N are present and their conversions are dynamic in compost and after its application to soil. Employing ^{15}N stable isotope labeling can precisely track compost N transformation and its utilization by crops [2]. To evaluate the nitrogen fertilizer value of compost, a critical premise of applying labeled compost is that all N fractions in compost should be homogenously labeled (which means all N fractions have similar ^{15}N abundances), and the abundance of labeling should be determined.

In contrast with chemical fertilizer, the N types in compost are more abundant, including inorganic N (NH_4^+ and NO_3^-), soluble organic N (SON), and microbial biomass N (MBN). Thus, before fertilizer application to soil, the various N types in compost are

complex, relative to the simple and clear form of N in chemical fertilizer. The N mineralization processes include the transformation of macromolecular organic N into NH_4^+, NO_3^-, and some low-molecular weight organic N compounds (e.g., amino acids), depending on the compost size [4], which directly controls the bioavailability of compost-N [5]. Other factors also govern the transformation of N, such as the presence of labile carbon [6–8]. For example, researchers found that mineral N was rapidly immobilized by microorganisms and then gradually released, following glucose addition, while the immobilization rate of mineral N by the microbes was significantly reduced by the presence of cellulose [9]. In addition, it has been found that crop residue with a high C/N ratio (i.e., maize straw) incorporated into the soil immobilized more N than crop residue with a low C/N ratio [10]. Therefore, the effectiveness and molecular weight of labile carbon determined the microbial accessibility, thereby influencing the mineralization–immobilization turnover of N (N-MIT).

The ^{15}N stable isotope tracer technique has been widely used to quantify the migration and transformation of fertilizer N in the soil–plant system and to determine its fate and distribution in agricultural environments [11,12]. Nitrogen labeling methods can be divided into direct and indirect methods [13–16]. The direct methods include adding some high-abundance ^{15}N chemical fertilizer to compost [13], which is simple and time-saving. However, then the content of mineral N becomes high, which is very different from the original compost. Meanwhile, the other active pools of N (such as SON) are not labeled, causing serious bias in the calculation of the nitrogen recovery ratio. Indirect methods would first involve growing fodder crops with ^{15}N chemical fertilizer and feeding livestock and poultry with ^{15}N-labeled fodder. Next, the livestock and poultry excrement are collected to obtain ^{15}N-labeled compost. Because of the intricate composition of compost, almost all techniques amplify the deviations among different N fractions and incur the risk of inhomogeneous labeling [17,18], while the dynamics of N-labeling in different N fractions of compost and their potential differences are scarcely described. This may confound the actual N contribution from compost to plant uptake, since, in general, plants only prefer ammonium or nitrate, not other N fractions. Therefore, the potential difference in N-labeling in different N fractions needs to be clarified.

Available N pools in compost can be rapidly transformed into active N pools and stable N pools in soil, thereby regulating the N supply capacity of soil and N uptake by crops [19]. The ^{15}N-labeled manure can be used to investigate fertilizer–soil–crop N transformation, under the condition that the ^{15}N in each fraction is uniformly distributed. To eliminate heterogeneity between distinct compost fractions, based on the N-MIT theory [20–23], labile carbon sources were added to ^{15}N-labeled manure, in order to increase the immobilization and allocation efficiency of exogenous N and to achieve homogeneous N-labeling. Small molecule substrates, such as glucose, were used [24–26] and split additions of those substrates to soil were recommended [27,28], in order to maximize the bioactivity and N metabolic capability of microorganisms. However, to date, few studies have presented the dynamics of the heterogeneity N-labeling of N, i.e., different ^{15}N-labeling abundances in different N types (in compost to homogeneous labeling), following the addition of exogenous carbon.

The main objective of this study was to investigate and quantify the transformation and fate of the added inorganic N into the various fractions in compost after labile carbon addition. The ^{15}N-labeled $(NH_4)_2SO_4$ was used to track the N flow paths, and glucose was used as the labile carbon source. Furthermore, we hypothesized the following: (1) glucose addition would enhance microbial activity in the compost, thereby accelerating the process of N immobilization; (2) glucose split addition would promote the conversion of inorganic N into a more stable pool (i.e., hot-water extractable N); and (3) the heterogeneity of ^{15}N-labeling, from various compost N fractions, would decrease under glucose split additions, and homogeneous ^{15}N-labeled compost could be achieved. This research aimed to elucidate the mechanisms linking carbon availability and N pool transformation in compost and to inspire further research, regarding compost use in agriculture.

2. Materials and Methods

2.1. Experimental Materials and Design

Commercial compost (Organic Biotechnology Limited Company, Beijing, China) made from a mixture of cow manure and vegetable residues was dried and crushed until the particle size was <1 mm. Ammonium sulfate ($[^{15}NH_4]_2SO_4$, ^{15}N 50% atom) was used to label N. A mixed solution of 100 g glucose and 2 g ammonium sulfate was added to 1 kg compost (dry basis); the water content and exogenous (glucose and $(NH_4)_2SO_4$) C/N ratio were adjusted to 30% and >20, respectively, to produce net N immobilization. The 30% water content was kept constant during the study. To prepare enough ^{15}N-labeled organic fertilizer for the field trial, we set five replicates in total. The mixture was mixed thoroughly and then incubated in the dark at 25 °C for 45 days. During the incubation, 5 g glucose, with 2000 mg/kg C, was added at 15 and 30 days, respectively (Table S1).

2.2. Sample Collection and Measurements

During the incubation, 50 g samples (n = 5) were collected on days 0, 15, 30, and 45 for analysis. Samples were sequentially extracted, following the modified Bremner procedure [29,30], to determine the N content and ^{15}N abundance of the different N fractions (Figure S1). According to the above Bremner method, the N pools were divided into active N (mineral N, soluble organic N [SON], microbial biomass N [MBN]), stable N (hot-water extractable organic N [(HWDON]), and recalcitrant N [31–33]. Briefly, 20 g of ^{15}N-labeled compost and 80 mL of 2 M potassium chloride (KCl) were mixed and shaken for 1 h at 200 rpm. Then, the suspension was centrifuged at 3000× g for 15 min, and the supernatant was collected to determine mineral N (NH_4^+ and NO_3^-) and SON. The SON content was obtained by subtracting the mineral N from the potassium chloride extractable total nitrogen, KEN (i.e., SON = KEN − NH_4^+ − NO_3^-). The residue was fumigated with chloroform for 24 h and extracted with 80 mL of 0.5 M potassium sulfate (K_2SO_4), shaken for 30 min at 150 rpm, and centrifuged at 3000× g for 15 min to measure the microbial biomass nitrogen (MBN). Then, the residue was hydrolyzed in hot water (80 °C) for 4 h, shaken, and centrifuged to measure HWDON. Mineral N was determined using a continuous flow analyzer (AA3, SEAL, Norderstedt, Germany). Other N fractions (KEN, MBN, HWDON) were determined using an elemental analyzer (Vario TOC Cube, Elementar, Germany). The ^{15}N abundance was determined using a stable isotope ratio mass spectrometer (Isoprime 100, Elementar, Langenselbold, Germany). The total C, N, and ^{15}N abundances of the compost were measured using a stable isotope ratio mass spectrometer with a C/N ratio analyzer (EA-IRMS, Vario Pyro Cubeand Isoprime 100, Elementar, Langenselbold, Germany) (Table 1).

Table 1. Basic physical and chemical properties of ^{15}N-labeled compost at different incubation times. TC, total carbon; TN, total nitrogen; C/N, total carbon content/total nitrogen; APE, atom percent excess. Data showing mean ± stand error (n = 5).

Incubation Time (Days)	TC (%)	TN (%)	C/N	APE (%)
0 †	14.3 ± 0.3b	0.93 ± 0.17a	15.4 ± 0.2b	0.0 ± 0.1a
15	14.9 ± 0.1b	0.96 ± 0.13a	15.5 ± 0.2b	2.3 ± 0.1a
30	14.7 ± 0.3b	0.95 ± 0.08a	15.4 ± 0.3b	2.4 ± 0.2a
45	16.0 ± 0.3a	0.98 ± 0.14a	16.4 ± 0.2a	2.4 ± 0.1a

† indicates glucose and $(NH_4)_2SO_4$ have been added at this time. Different letters indicate the significant difference (p < 0.05) among different incubation days.

2.3. Data Analysis

Data were analyzed by one-way analysis of variance to test for significant differences (p < 0.05) at different sampling times using SPSS (IBM SPSS 19.0, Amonk, NY, USA). Multiple comparisons were performed by Duncan analysis. In Equation (3), mineralization rate of each N fraction (in Table S2)

$$\text{Proportion of exogenous N (\%)} = [\text{atom percent excess (APE) in each N fraction/APE of ammonium sulfate}] \times 100. \quad (1)$$

$$\text{Content of exogenous N (mg/kg)} = \text{content of each N fraction} \times \text{Proportion of exogenous N.} \quad (2)$$

$$\text{Supply of exogenous N (mg/kg)} = \text{mineralization rate of each N fraction} \times \text{Content of exogenous N.} \quad (3)$$

$$\text{Exogenous N distribution content (mg/kg) of each nitrogen fraction} = \text{APE of each N fraction} \times \text{N content of each N fraction.} \quad (4)$$

$$\text{Exogenous N distribution rate (\%) of each nitrogen} = \text{exogenous N distributioncontent of each nitrogen fraction/exogenous N distribution content of the available nitrogen fraction.} \quad (5)$$

3. Results

3.1. Conversion Dynamics of Available Nitrogen Fractions, as Affected by Glucose Addition

Glucose addition significantly promoted the transformation of mineral N (Figure 1). During the first 15 days, the NH_4^+-N content significantly decreased by 39.1% and was more pronounced for NO_3^--N, whose content rapidly decreased by 98.1%. On day 45, the contents of NH_4^+-N and NO_3^--N were only 41.9% and 1.0% of TN on day 0, respectively. The SON content decreased quickly at first and then slowly and was 44.1% lower ($p < 0.05$), while MBN and HWDON were 13.7% and 28.8% higher on day 45 than on day 0, respectively. The content of different N pools exhibited the following trend on day 0: NH_4^+-N > HWDON > SON > MBN > NO_3^--N (Figure 2). During the early stage (the first 15 days), the active N pools were transformed into the more stable HWDON pool. During days 15–45, the mineral N and SON converted into the MBN pool. Of the available N, approximately 35% was converted into the recalcitrant pool (i.e., residue) at the end of the incubation (Figure 1).

3.2. Transformation of the Exogenous Nitrogen

The N-labeling analysis showed that exogenous N primarily entered the NH_4^+-N pool (7.7%) during the first 15 days (Table 2). Thereafter, it was immobilized by microorganisms forming HWDON (6.6%, Table 2). After 30 days of incubation, the conversion of exogenous N to MBN occurred. Meanwhile, exogenous N was redistributed under glucose stimulation (Figure 3). From days 15–30, microbes transferred ^{15}N from NO_3^--N to the organic N pool through assimilation (i.e., $SO^{15}N$, $MB^{15}N$, and $HWDO^{15}N$ increased by 5.1%, 2.7%, and 1.4%, respectively), and exogenous N was transferred into the "biological immobilization pool" ($MB^{15}N$ and $HWDO^{15}N$) during the late stage (days 30–45). Considering days 15–45, exogenous N into NH_4^+-N and MBN was significantly decreased and enhanced, respectively (Figure 3).

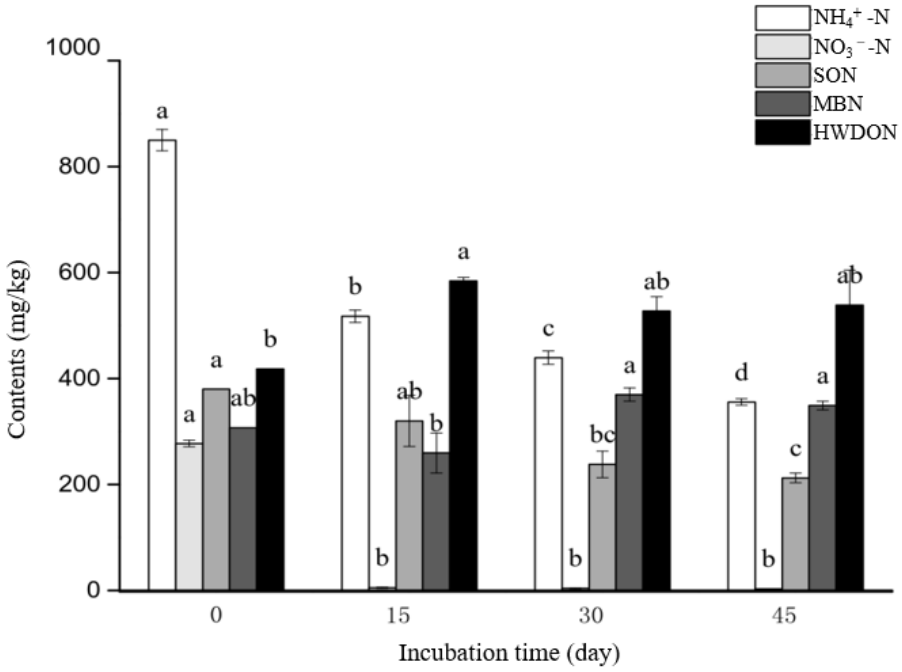

Figure 1. Contents of the available nitrogen fractions in the compost at different incubation times. SON, MBN, and HWDON indicate soluble organic nitrogen, microbial biomass nitrogen, and hot-water extractable organic N, respectively. Different letters indicate significant differences in a certain N fraction content between different stages ($p < 0.05$). Data shown as the mean ± standard error ($n = 5$).

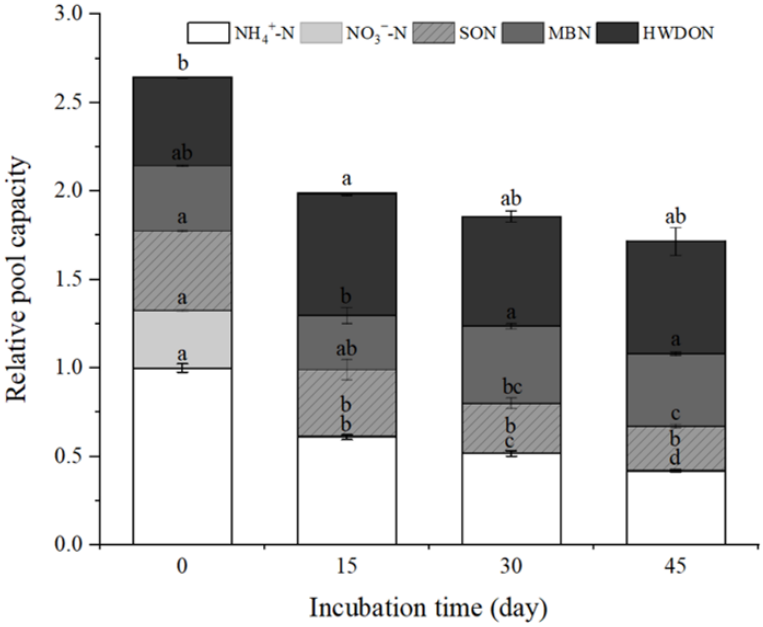

Figure 2. Relative pool capacity of the available nitrogen fractions in the compost. Assuming that the capacity of the NH_4^+-N pool at day 0 was 1, the capacities of the remaining measured N were obtained by dividing their contents by the content of NH_4^+-N at day 0. SON, MBN, and HWDON indicate soil organic nitrogen, microbial biomass nitrogen, and hot-water extractable organic N, respectively. Different letters indicate significant differences in a certain N fraction content between different stages ($p < 0.05$). Data are shown as the mean ± standard error ($n = 5$).

Table 2. Proportion of the available N fractions derived from exogenous N. On day 0, exogenous N was just added; it was regarded as no exogenous N having entered the available N fractions of the compost; SON, soluble organic nitrogen, MBN, and microbial biomass nitrogen; HWDON and hot-water extractable organic N.

Incubation Time (Days)	NH_4^+-N (%)	NO_3^--N (%)	SON (%)	MBN (%)	HWDON (%)	Sum (%)
0	/	/	/	/	/	/
15	7.7a	0.1a	3.7b	3.8a	5.1b	20.5b
30	7.9a	0.01b	7.7a	3.7a	6.6a	26.0a
45	5.1a	0.02b	6.3ab	4.2a	5.6b	21.1b

Different letters indicate the significant difference ($p < 0.05$) among different incubation days.

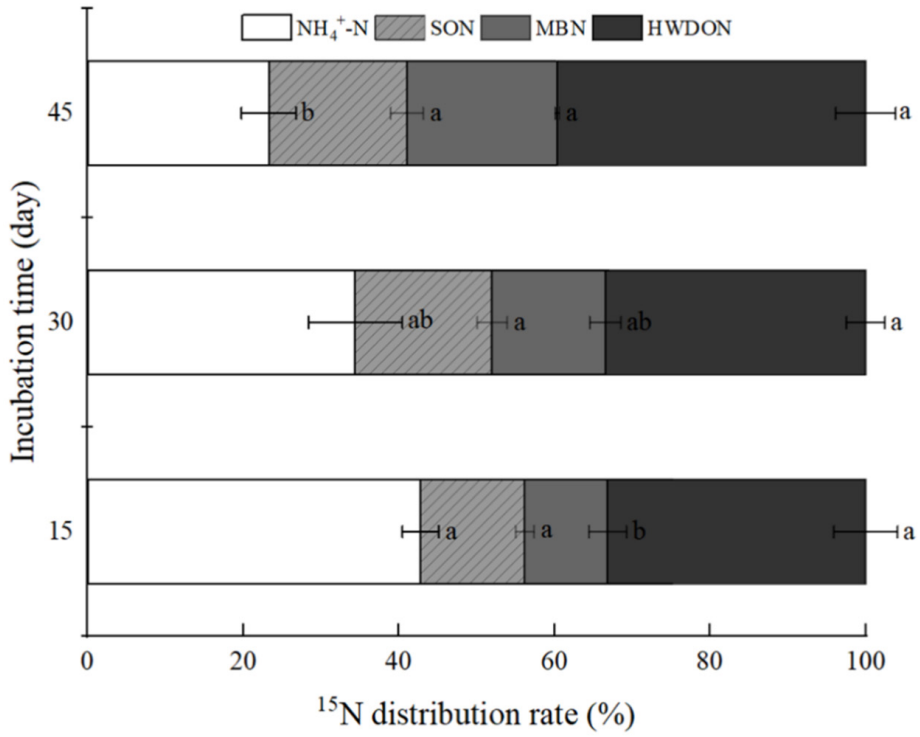

Figure 3. Exogenous nitrogen (^{15}N) distribution ratios of the available nitrogen fractions in the compost (NO_3^-−N in all treatments was not shown here since all close to 0 and no significant difference among the treatments). SON, MBN, and HWDON indicate soil organic nitrogen, microbial biomass nitrogen, and hot-water extractable organic N, respectively. Different letters indicate significant differences in a certain N fraction content between different stages ($p < 0.05$). Data were shown as the mean ± standard error ($n = 5$).

3.3. Distribution of Labeled ^{15}N

The APE of $^{15}NO_3^-$-N decreased from day 0 to day 15, whereas the other N fractions increased rapidly in this phase (Figure 4). During days 15–30, the increasing APE of $^{15}NH_4^+$-N and HWDO^{15}N decelerated, and the peak value during the study was 3.4% on day 30. The APE of SO^{15}N first increased and then decreased and reached a peak of 4.1% on day 35. In contrast, the APE of MB^{15}N varied slightly and stabilized at 1.9%. The APE of all N fractions ranged from 2–3% on day 45. The homogeneity of ^{15}N-labeling was obtained by fitting a polynomial (the position is indicated by an arrow in Figure 4). The average APE was 2.4% on day 48.

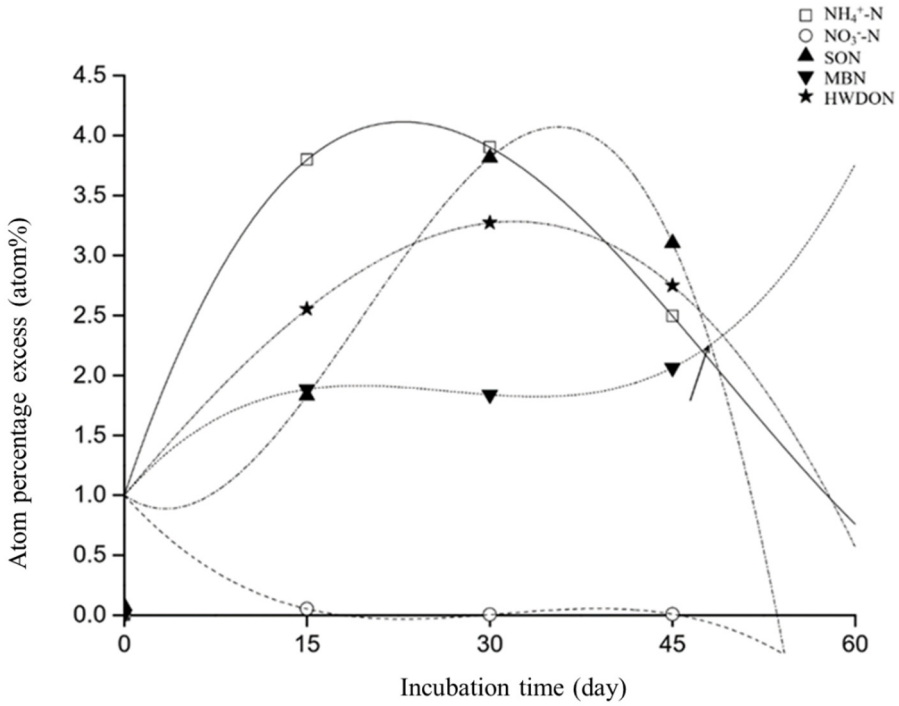

Figure 4. Nitrogen atom percent excess (APE) of available nitrogen fractions in compost. The arrow in the figure indicates that all available nitrogen fractions of the compost were labeled homogeneously, and the average APE was 2.36% at day 48.

4. Discussion

4.1. Changes in Microbial-Derived Nitrogen Fractions Due to Glucose Addition

In this study, the MBN and HWDON contents were increased by 13.7% and 28.8% by the end of the experiment (Figures 1 and 2). A previous study reported that the release of nutrients from decomposed organic amendment was relatively stable, and the microorganisms were in a physiologically inactive state [34]. These results indicate that glucose addition provided a sufficient source of carbon and energy for the microbes, stimulated the recovery of their activity, and improved their metabolic rate, thereby promoting the immobilization of mineral N [35–37].

Large doses of glucose (40,000 mg/kg C), when added to ^{15}N-labeled compost, increased only HWDON but not the other N fractions (Figure 1). These results are likely attributable to the enhanced osmotic pressure in the compost, caused by the addition of labile substrates and the fact that high osmotic pressure is not conducive to bacterial growth and reproduction [38,39]. Our results are consistent with the previous research [40], who found that when the glucose loading rate was <4000 mg/kg C, bacterial growth was accelerated, and that when the rate was >4000 mg/kg C, fungal growth was promoted. When the total glucose loading rate reached 32,000 mg/kg C, the growth and metabolism of fungi increased rapidly, whereas those of bacteria were inhibited. However, because of their favorable physiological structure, fungi are better able to adapt to changes in the environment [41–43]. HWDON contains mainly chitin, proteins, and other macromolecular compounds, which are crucial components of the fungal cell wall [44,45].

Instead of entirely metabolizing the added glucose, the microorganisms quickly absorb it into their bodies and stored it temporarily [46]. When a small amount of glucose (2000 mg/kg C) was added on day 15, bacteria had a competitive advantage and used labile C to form their cellular structures [47]. Therefore, the content of MBN increased (Figure 1), which includes peptidoglycan and small peptides and was derived primarily from the cell wall of bacteria [48,49]. After 30 days of incubation, glucose (2000 mg/kg C) was added for the third time; HWDON increased by only 10.76 mg/kg and MBN even started to decline (Figure 1). In an enclosed environment, microorganisms produce abundant

secondary metabolites and toxic substances; the long-term, high-carbon environment sharply increases osmotic pressure, thereby inhibiting the growth and reproduction of microorganisms [43]. Therefore, a large amount of N was transferred into the residue and weakened the bioavailability of the compost-derived N.

4.2. Distribution of Labeled ^{15}N for N Fractions in Compost

In this study, the total supply of exogenous N and the exogenous contribution rate of each fraction under actual (day 45) conditions exhibited no significant differences (Table 3). The results showed that the target of the same abundant ^{15}N-labeling for a different N fraction of the compost was achieved after approximately 45 days of incubation. At other incubation times, there was a dramatic difference in the APEs of the different N fractions, ranging from approximately 0–3.7%. Meanwhile, the APEs of the whole compost were 2.3% during the incubation. These results highlight that dissimilarities in different N fractions could generate bias in the contribution rate of the compost to plant N uptake, since we generally consider the APEs in different N fractions of compost to be homogenous and identical. In addition, we found that the time achieving the same ^{15}N concentration in different N fractions was transient. Therefore, our results indicate that homogenous ^{15}N-labeling in compost using exogenous N has a specific equilibrium time, and land-application should only be done when ^{15}N concentrations reach equilibrium in different N pools.

Table 3. Supply of exogenous N and contribution rates of available N fractions; SON, soluble organic nitrogen, MBN, and microbial biomass nitrogen; HWDON and hot-water extractable organic N.

Homogeneity of ^{15}N Labeling	Supply of Exogenous N (mg/kg)	Contribution Ratios of Available N Fractions (%)				
		NH_4^+-N	NO_3^--N	SON	MBN	HWDON
Actual (2–3% APE, day 45)	38.9	47.0	0.0	13.7	17.0	22.3
Theoretical (2.4% APE, day 48)	34.9	47.5	0.0	11.2	20.7	20.6

In addition, the major N supply from compost was NH_4^+-N (47.3%), followed by HWDON (21.4%) and MBN (18.9%); N derived from microbial structures is highly effective for plants, since soil microorganisms are in places where exogenous organic matter is converted into soil organic matter. The higher contribution rate of HWDON illustrated its larger relative pool capacity of compost, but that does not mean that it was easily decomposed (Table 3) (Figure 2). It has been found that HWDON accounted for 2.6–8.7% of total soil N; however, approximately three-quarters of HWDON was relatively recalcitrant [50]. Exogenous N did not nitrify because microorganisms would consume substantial energy for this process. Therefore, the contribution rate of NO_3^--N was very low (Table 3).

5. Conclusions

Our study clarified that the transformation of N fractions in the compost changed, e.g., NH_4^+; they first transformed into HWDON and then into microbial biomass nitrogen or other recalcitrant nitrogen. The NH_4^+ content continuously decreased with the incubation time, independent of the glucose addition time. A high dose of glucose (40,000 mg/kg C) input caused the available N to enter the recalcitrant pool, but it did not dramatically change the microbial biomass nitrogen. A low dose of glucose (2000 mg/kg C) tended to increase the microbial biomass nitrogen and decrease SON and NH_4^+. Importantly, we clarified that the N-labeling effectiveness for different N fractions was not the same, and a considerable difference existed in the labeling abundance of each N fraction (0% to 3.7%), compared with the total nitrogen (2.4%). Furthermore, we found that an equal labeling time existed for the different N fractions, approximately 48 days after incubation in our study, based on the simulation. These findings indicate that the N fractions of compost, especially

for organic N, could be labeled with the same ^{15}N concentrations, under the regulation of labile carbon. More importantly, the finding of an equal labeling time provides a reference for future compost labeling traits, which are essential for evaluating the real contribution rate from exogenous N to plants and other possible soil functions.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agriculture11100971/s1, Figure S1: Sequentially extracting nitrogen fractions from ^{15}N-labeled compost, Table S1: Frequency and glucose addition, Table S2: Mineralization rates of available N fractions. SON, soluble organic nitrogen, MBN, microbial biomass nitrogen, HWDON, hot water extractable organic N.

Author Contributions: Conceptualization, G.L. and Z.S.; methodology, S.D.; formal analysis, C.L. and Z.M.; investigation, C.L. and S.D.; data curation, C.D. and Y.H.; writing—original draft preparation, C.L. and S.D.; writing—review and editing, S.D.; supervision, Z.S.; project administration, G.L. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The data presented in this study are available on demand from the corresponding author at Zhencai_Sun@cau.edu.cn.

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

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