



# Chemical Composition of High Organic Carbon Soil Amendments Affects Fertilizer-Derived N<sub>2</sub>O Emission and Nitrogen Immobilization in an Oxic Sandy Loam

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Nitrous oxide (N<sub>2</sub>O) emission is a negative side effect of modern agriculture and a serious issue for global climate change. The combined application of nitrogen (N) fertilizer and high organic carbon soil amendments (HCA) has been regarded as an alternative to promote fertilizer-related N immobilization and enhance nitrogen use efficiency. The effect of HCA on N<sub>2</sub>O emission and N immobilization highly depends on its chemical composition, as it controls carbon (C) supply to soil microbes and reactivity of lignin-derived phenols to fertilizer-derived N species. Here we present a 127-d laboratory incubation study to explore the N<sub>2</sub>O emission and N immobilization after combined application of N fertilizer and HCA (wheat straw, spruce sawdust, and commercial alkali lignin) differing in their chemical composition. The <sup>15</sup>N labeling technique was used to trace the transformation of fertilizer-N in ammonium (NH<sub>4</sub><sup>+</sup>), nitrate (NO<sub>3</sub><sup>-</sup>), soil organic nitrogen (SON), and N<sub>2</sub>O. The amendment of wheat straw and spruce sawdust greatly promoted N immobilization and N<sub>2</sub>O emission, while lignin amendment enhanced the immobilization of fertilizer N. The chemical composition of HCA explained 26% of the total variance of fertilizer-derived N<sub>2</sub>O emission and N retention via soil microbial biomass, composition of lignin-derived phenols, and nitrification. The holocellulose/lignin ratio of HCA could be used as an indicator for predicting HCA decomposition, microbial N immobilization and N<sub>2</sub>O emission. In addition, the composition of lignin-derived phenols was affected by HCA amendment and significantly related to N<sub>2</sub>O emission and N retention. The varying chemical composition of HCA could thus be a promising tool for controlling N<sub>2</sub>O emission and N immobilization in environment-friendly and climate-smart agriculture.

**Keywords:** holocellulose, lignin, nitrogen fertilizer, nitrous oxide, nitrogen immobilization

## INTRODUCTION

Since 1920s, the global consumption of industrial N fertilizers in agriculture has increased by 173 million tons in 2013 (FAO, 2015), which has caused serious environmental problems such as nitrous oxide (N<sub>2</sub>O) emission and nitrate (NO<sub>3</sub><sup>-</sup>) leaching (Zhu and Chen, 2002). Agriculture represents the largest worldwide anthropogenic N<sub>2</sub>O source (Ravishankara et al., 2009). N<sub>2</sub>O is not only the third-most important greenhouse gas, but also contributes the most to ozone-depletion in the stratosphere after the successful emission reduction of chlorine- and bromine-containing halocarbons (Ravishankara et al., 2009). It has been proposed that the combined application of N fertilizers and high organic carbon soil amendments (HCA) could strongly promote temporary N retention (Baggs et al., 2000; Millar and Baggs, 2004). Immobilized N can be gradually released into soil through ammonification and taken up by plants, hence, this process may finally improve nitrogen use efficiency of fertilizers and mitigate N<sub>2</sub>O emission (Burger and Jackson, 2003). Although decreasing N<sub>2</sub>O emissions after HCA application have been reported (Baggs et al., 2000; Gentile et al., 2008), increased emissions occurred in other cases (Frimpong and Baggs, 2010; García-Ruiz et al., 2012; Chen et al., 2013). Understanding the mechanisms involved in N<sub>2</sub>O emission and N immobilization upon HCA application is thus mandatory for developing and implementing environmentally friendly agricultural practices.

The quantity and quality of applied HCA play an important role in fertilizer-associated N<sub>2</sub>O emission and N immobilization in the soil. A C/N ratio of 30 is regarded as a threshold above which net N immobilization occurs (Mooshammer et al., 2014). Nonetheless, the application of HCA did not inhibit N<sub>2</sub>O emissions in highly anoxic soils even when the C/N ratio exceeded 30 (Chen et al., 2013). Some of this contrasting results can be explained by the different quality of organic matter in HCA. The introduction of labile carbon (C) into oxic soils enhanced N immobilization by promoting soil microbial activity, but simultaneously also promoted N<sub>2</sub>O emissions due to the activation of nitrifiers and denitrifiers (Chen et al., 2013). Nevertheless, several studies found that fertilizer-related N<sub>2</sub>O emissions were significantly negatively correlated with the lignin and polyphenol content as well as with the (lignin + polyphenol)-to-N ratio in HCA-amended soils (Millar and Baggs, 2004; Garcia-Ruiz and Baggs, 2007; Frimpong and Baggs, 2010). Hence, the chemical composition of HCA could play a decisive role in controlling N<sub>2</sub>O emission and N retention rates.

Holocellulose (including cellulose and hemicellulose) and lignin are the most abundant C components in plant residues (Kögel-Knaber and Amelung, 2014). Cellulose is a straight chain polymer derived from D-glucose units which are condensed through β(1→4)-glycosidic bonds, while hemicellulose is a polysaccharide containing many different sugar monomers (Kögel-Knaber and Amelung, 2014). Formed by radical polymerization of guaiacyl (G), syringyl (S), and p-hydroxyphenyl (H) units, lignin is a major component of plant cell walls accounting for 10–30% of lignocellulose

(Bugg et al., 2011). Due to the high degree of polymerization, the biological degradation of lignin by soil microorganisms can be very slow (Kögel-Knaber and Amelung, 2014). By contrast, holocellulose is more accessible to soil microbes, and its presence may promote microbial activity (Kiem and Kögel-Knabner, 2003; Chen et al., 2018). Thus, both lignin and holocellulose content affect the rate at which plant residues may be decomposed (Rahman et al., 2013), and thus very likely also the rate and degree of microbial N immobilization and N<sub>2</sub>O emission. Besides, lignin and its derivatives from the different G, S and H units show distinct reactivity to nitrite (NO<sub>2</sub><sup>-</sup>), suggesting that lignin could play a discriminating role in abiotic N<sub>2</sub>O emission and N immobilization (Wei et al., 2017a,b).

Based on the distinct microbial availability of holocellulose and lignin, we hypothesized that HCA with higher holocellulose content is more efficient in promoting microbial N immobilization and N<sub>2</sub>O emission than that containing higher lignin. To test this hypothesis, a laboratory incubation study was conducted with a sandy loam sampled from an agricultural site in Kiel, Germany. Wheat straw, sawdust and pure lignin, whose chemical composition strongly differed from each other, were selected as HCA. After the application of <sup>15</sup>N-labeled fertilizer, dynamics of fertilizer-derived N<sub>2</sub>O, ammonium (NH<sub>4</sub><sup>+</sup>), NO<sub>3</sub><sup>-</sup>, and soil organic nitrogen (SON) were monitored for 114 days. The role of chemical composition of HCA was interpreted based on HCA decomposition, N dynamics, and soil conditions. The present study was complementary to a previous study (Reichel et al., 2018), in which the influence of HCA on microbial N transformations was studied, with a common experimental set and shared samples.

## MATERIALS AND METHODS

### Materials

Soil (sandy loam, pH<sub>CaCl2</sub> = 6.0 ± 0.1, total C = 1.3 ± 0.1%, total N = 0.15 ± 0.01%) was sampled from a Cambic Luvisol of the Achterwehr field (54°19′05″N, 9°58′38″E), Hohenschulen experimental farm, Kiel, Germany in the summer of 2016 for the laboratory incubation experiment. After sampling, soil was air-dried, sieved at 2 mm and homogenized. Highly <sup>15</sup>N-enriched ammonium sulfate [(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 98 atom% <sup>15</sup>N, VWR, Germany] was diluted to 2.65 atom% <sup>15</sup>N by mixing it with (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> of natural <sup>15</sup>N abundance (0.367 atom% <sup>15</sup>N, VWR, Germany), and applied to the soil equivalent to 50 mg N kg<sup>-1</sup> soil.

Wheat straw (total C = 44.4 ± 0.1%, total N = 0.28 ± 0.02%, lignin content = 17%) was also obtained from the Hohenschulen experimental farm close to Kiel, Germany, and spruce sawdust (without bark, total C = 45.8 ± 0.2%, total N = 0.06 ± 0.01%, lignin content = 25%) was obtained in 2015 from Holz Ruser (Bornhöved, Germany). Both materials were ground to <1 mm in a rotary mill before usage. Alkali lignin (total C = 61.6 ± 0.1%, total N = 0.43 ± 0.01%, VWR, Germany) was suspended in deionized water and allowed to stand for 1 h. The soluble components and micro-particles in the suspension

were removed, and the precipitated lignin particles were freeze-dried for usage.

## Experimental Setup

For the experiment, as described in Reichel et al. (2018), round stainless steel soil columns were used (20 cm diameter × 30 cm height; workshop of Forschungszentrum Jülich, Jülich, Germany). Each soil column was equally divided into 6 equal sectors using polyvinyl chloride (PVC) boards to allow soil sampling at different time without disturbing the structure of the remaining soil. Three treatments with the combined application of N fertilizer and HCA were established: SWF, soil + wheat straw + fertilizer; SSF, soil + spruce sawdust + fertilizer; SLE, soil + alkali lignin + fertilizer. HCA was applied to the soil at a rate of 1.5 g C kg<sup>-1</sup> soil (equivalent to 4.5 t C ha<sup>-1</sup>). Before filling the soil columns, 90% of HCA had been homogeneously mixed with the soil, while the remaining 10% was buried in litter bags at a depth of 2 cm in the soil at the beginning of the pre-incubation period. The litter bags were made of 0.2 μm nylon mesh and their shape matched the sector surface of each column. Neither fertilizer nor HCA was applied to the blank control (S), while only N fertilizer was applied to the fertilizer control (SF). All treatments were prepared in triplicate.

About 8 kg of soil mixed with or without HCA was equally filled into all the six sectors of each soil column. The soil was compacted to field bulk density (1.5 g cm<sup>-3</sup>) with a custom-made PVC tool that had the same shape (segment of one sixth of a circle) as each single soil column sector. Afterward, the soil was rewetted to 40% of its water holding capacity (WHC, 35 g H<sub>2</sub>O/100 g dry soil in this study) and pre-incubated for 2 weeks to activate soil microorganisms. Then, N fertilizer dissolved in deionized water was applied at a rate of 50 mg N kg<sup>-1</sup> soil. The final soil moisture of all treatments was about 60% of WHC. Soil columns were incubated at room temperature for 114 days after the application of N fertilizer from 22nd November 2016 to 16th March 2017. The mean temperature during the whole experiment ranged from 20.3 to 22.3°C with mean value of 21 ± 1°C, and deionized water was supplied weekly to keep the soil moisture at about 50–60% of WHC.

## Gas Sampling and Analysis

Nitrous oxide flux, as well as N<sub>2</sub>O isotopic composition, was measured 2–3 times per week. For gas sampling, a gas-tight PVC chamber (20 cm diameter × 20 cm height) with one inlet and one outlet was installed on top of the soil column. The outlet was closed with a screwcap with septum, and the inlet was equipped with a rubber tube (50 mm diameter × 1 m length) closed with a clamp. The gas-tightness of the whole setup had been checked with a helium detector before the experiment. As soon as the PVC chamber was installed, 35 ml of headspace gas was sampled four times in 20–40 min intervals through the septum with a syringe and transferred to a 22.5 ml pre-evacuated gas chromatography vial, thereby creating an overpressure in the GC vial. During gas sampling, the clamp on the inlet tube was opened allowing ambient air to flow into the chamber for pressure equilibration. The N<sub>2</sub>O mixing ratio was measured with a gas chromatograph

equipped with an electron capture detector (GC-ECD, 8610C, SRI Instruments, CA, United States). At the end of each regular gas sampling, 120 ml of gas in the headspace was sampled into a 100-ml pre-evacuated glass bottle, and the <sup>15</sup>N enrichment of N<sub>2</sub>O was analyzed with an isotope ratio mass spectrometer (IRMS, IsoPrime 100, Elementar Analysensysteme, Hanau, Germany) coupled to an online pre-concentration unit (TraceGas, Elementar Analysensysteme) according to Heil et al. (2015).

## Soil Sampling and Analysis

Soil in each sector of the soil column was sampled destructively. The first soil sampling was performed 7 days before N fertilizer application as a reference, while the remaining sampling events occurred 8, 22, 50, 78, and 114 days after fertilizer application, respectively. After each sampling, emptied sectors were refilled with appropriately shaped PVC blocks to maintain the remaining soil structure and the air-filled headspace. After sampling, the soil was immediately freeze-dried, and then stored at room temperature until analysis.

Soil pH was determined according to the ISO 10390 method (ISO, 2005). In detail, 1 M potassium chloride (KCl, VWR, Germany) solution was mixed with freeze-dried soil at a ratio of 1:5 (w/v) for 2 h. The mixture was centrifuged at 3500 rpm for 20 min, then the suspension was measured with a pH meter (multi 340i, WTW GmbH, Germany). Soil NO<sub>2</sub><sup>-</sup> concentration was determined according to an improved colorimetric method (Colman, 2010; Homyak et al., 2015): 5 g of fresh soil was mixed with 25 ml of 0.012 M NaOH solution for 2 h to extract NO<sub>2</sub><sup>-</sup>; after filtered through 0.45 μm, NO<sub>2</sub><sup>-</sup> in the extract was reacted with sulfanilamide and N-(1-naphthyl)-ethylenediamine in diethylenetriaminepentaacetic acid buffer; finally the colored product was subsequently measured with a spectrophotometer (DU-800, Beckman Coulter, Fullerton, United States) at 560 nm. Dissolved organic carbon (DOC) and dissolved total nitrogen (DTN) were extracted with 0.01 M CaCl<sub>2</sub> solution (Gonet and Debska, 2006), while soil moisture was measured with the classical thermo-gravimetric technique (Lekshmi et al., 2014). Soil microbial biomass was determined with the chloroform fumigation-extraction method as described in Reichel et al. (2018).

The contents of NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup>, as well as their <sup>15</sup>N-enrichment, were sequentially measured through micro-diffusion and liquid-liquid extraction methods (Mulvaney et al., 1997; Huber et al., 2012): 80 ml of 1 M KCl solution (pre-treated by heating for 16 h at 550°C to minimize background NH<sub>4</sub><sup>+</sup>) was mixed with 8–9 g of freeze-dried soil. The mixture was shaken for 1 h at 200 rpm, then centrifuged for 20 min at 3000 rpm and filtered through 2–3 μm filter paper (grade 305, VWR Germany). A 60 ml aliquot of KCl extract was transferred to 100-ml polypropylene bottles and adjusted to pH ~12 with 1 M NaOH solution to deprotonate NH<sub>4</sub><sup>+</sup> to NH<sub>3</sub>, which was evaporated and collected from the headspace using an oxalic acid (2 × 15 μl) disk trap (quartz glass, VWR, Germany) for 7 d at room temperature. Afterward, the filter disks were dried in a desiccator over silica gel for 24 h, then packed in Sn capsules (10 × 10 mm) and analyzed using an elemental analyzer coupled

to an isotope ratio mass spectrometer (EA-IRMS, Flash EA 2000 and Delta V Plus; Thermo Fisher Scientific, Bremen, Germany). After micro-diffusion, the remaining KCl extract was dried at around 65°C for about 3 d, re-dissolved in 3 ml of 1 M NaOH, and then mixed with 37 ml of acetone for 30 s. The mixture was centrifuged for 20 min at 3000 rpm, and the supernatant acetone phase containing NO<sub>3</sub><sup>-</sup> was transferred into a glass beaker and dried at 30–40°C overnight. The dry residue was re-dissolved in 5 ml of deionized water, freeze-dried, packed in Sn capsules (5 × 9 mm), and analyzed with EA-IRMS. To analyze the immobilized SON, water-soluble N substances, such as NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup>, and dissolved organic nitrogen (DON), were removed by washing the soil samples with 1 M KCl solution at a ratio of 1:5 (w/v) for three times. The water-soluble N free soil was collected by centrifugation, freeze-dried, packed in Sn capsules (5 × 9 mm), and analyzed using EA-IRMS. The calculated recovery of <sup>15</sup>N in the soil ranged from 71 to 101%. Fertilizer-derived DON was estimated by subtracting fertilizer-derived SON, NH<sub>4</sub><sup>+</sup>, and NO<sub>3</sub><sup>-</sup> from total fertilizer-N in the soil.

The composition of lignin-derived phenols in the soil was characterized according to the cupric oxide (CuO) oxidation method (Hedges and Ertel, 1982; Amelung et al., 1997): aryl ether bonds of lignin were cleaved using CuO under alkaline pH, and phenolic monomers released were separated on a GC (HP 6890, Agilent, CA, United States) equipped with an HP-5 capillary column (30 m × 0.32 mm × 0.25 μm film thickness) and quantified with a flame ionization detector (FID). Lignin G units were calculated as the sum of vanillin, acetovanillone, and vanillic acid, while lignin S units were represented by the sum of acetosyringone, syringaldehyde, and syringic acid. Finally, *p*-cumaric acid and ferulic acid represented the lignin H units.

## HCA Sampling and Analysis

High organic carbon soil amendments in litter bags buried in each soil sector was sampled and freeze-dried together with the soil. Freeze-dried litter bags were carefully cleaned with a brush to remove the soil dust. The remaining organic material was weighed to calculate the decomposition rate. Total lignin in wheat straw and spruce sawdust was extracted according to the Klason method (Schwanninger and Hinterstoisser, 2002; Nicholson et al., 2014): 20–30 mg of HCA was weighed into 50-ml centrifuge tubes, rinsed with acetone, and then stepwise hydrolyzed with 300 μl of 72% H<sub>2</sub>SO<sub>4</sub> for 16–20 h at 5°C and 7.2 ml of 3% H<sub>2</sub>SO<sub>4</sub> for 4 h at 105°C. The lignin precipitate was centrifuged at 3500 rpm for 25 min, washed with hot water (80°C) twice and then freeze-dried for analysis. Holocellulose was extracted according to Wissel et al. (2008): 20–30 mg of HCA was weighed into 50-ml centrifuge tubes, bleached in 7% NaClO<sub>2</sub> at pH 3 (adjusted with 100% acetic acid) at 60°C for 10 h, and centrifuged at 3500 rpm for 25 min. Then, the precipitated holocellulose was freeze-dried after having been washed twice with hot water (80°C). The immobilization of fertilizer-N in HCA was determined with EA-IRMS after washing the HCA with 1 M KCl solution at a ratio of 1:5 (w/v) three times. The holocellulose/lignin ratio of HCA was calculated by dividing the holocellulose content with total lignin content.

## Calculations and Statistical Analysis

The N<sub>2</sub>O flux was calculated from the increase of their mixing ratios during the total sampling time and related to the total mass of soil according to the following equation:

$$E = \frac{\text{slope}(C_1 : C_4, t_1 : t_4) \times V \times 273.15 \times 28}{m \times (273.15 + T) \times 22.4} \quad (1)$$

$E$ , N<sub>2</sub>O flux (ng N kg<sup>-1</sup> soil h<sup>-1</sup>);  $t_1:t_4$ , sampling time (h);  $C_1:C_4$ , N<sub>2</sub>O mixing ratio in the headspace at each sampling time (ppm);  $V$ , the volume of the headspace (L);  $m$ , the dry weight of the remaining soil (kg);  $T$ , air temperature (°C); 22.4, molar volume of ideal gas at 0°C and 101.325 kPa (L); 28, the molecular weight of N in N<sub>2</sub>O.

Fertilizer-derived N<sub>2</sub>O, NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup>, and SON were calculated according to a two-end-member mixing model based on their <sup>15</sup>N-enrichment:

$$M_{fer} \times {}^{15}N_{fer} + M_{nat} \times {}^{15}N_{loc} = M_{tot} \times {}^{15}N_{tot} \quad (2)$$

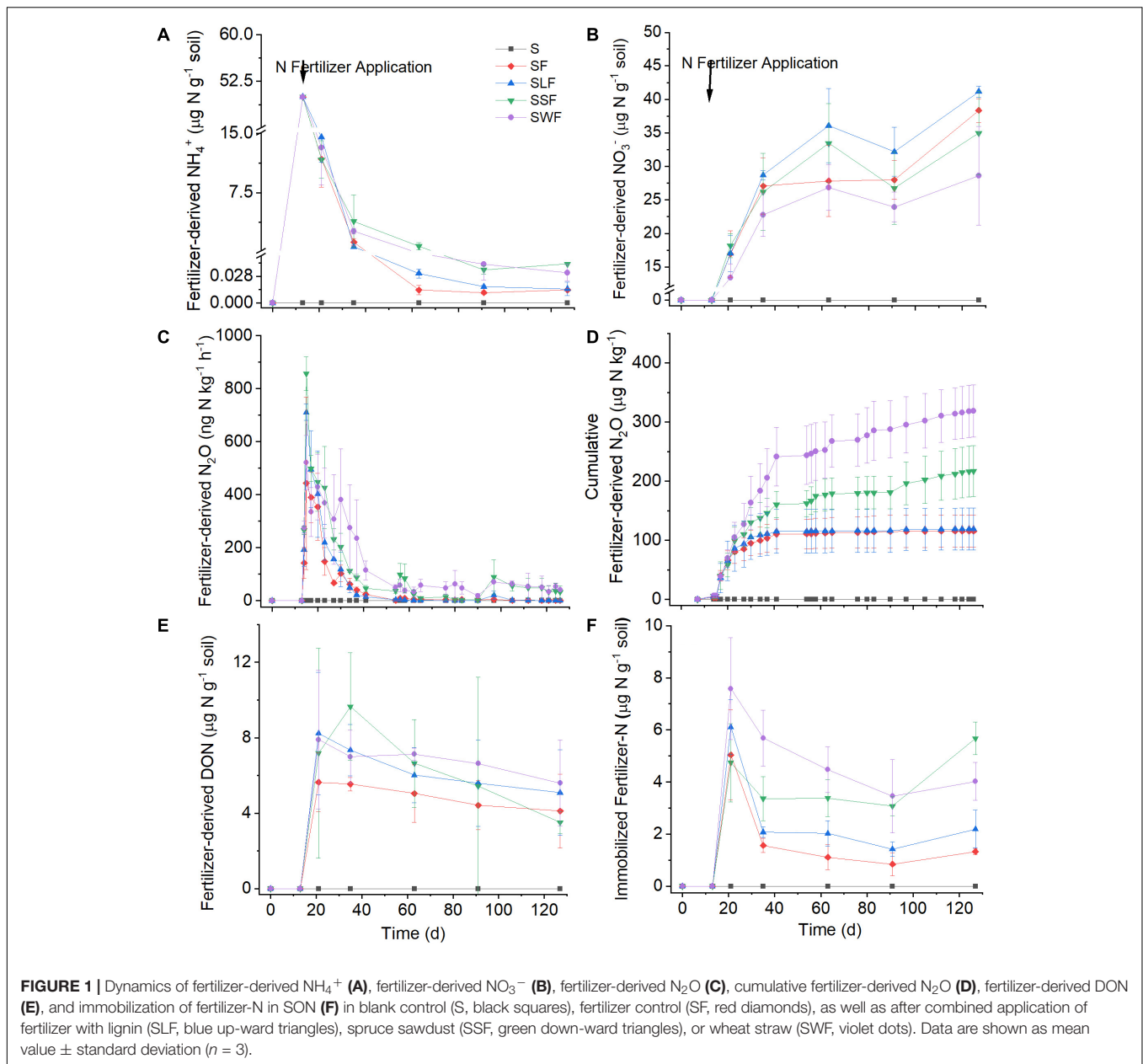
$M_{fer}$ ,  $M_{nat}$ , and  $M_{tot}$  denoted the amount of fertilizer-derived, native, and total N<sub>2</sub>O (or NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup>, SON), respectively;  ${}^{15}N_{fer}$ ,  ${}^{15}N_{loc}$ , and  ${}^{15}N_{tot}$  were the <sup>15</sup>N enrichment of fertilizer-derived, native, and total N<sub>2</sub>O (or NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup>, SON), respectively. The  $\delta^{15}N$  value in the blank control served as the value of native <sup>15</sup>N enrichment.

Shapiro-Wilk tests showed that data were normally distributed ( $p < 0.05$ ), therefore, one-way analysis of variance (ANOVA) with Tukey *post hoc* test at  $p = 0.05$  was used to analyze the significance of difference between treatments. One-way ANOVA, as well as Pearson correlation between N species and HCA parameters, were performed using Origin 8.0 (Originlab Corporation, Wellesley Hills, MA, United States). Redundancy analysis (RDA)-based variation partitioning analysis (VPA) was used to test the contribution of HCA chemical composition to the total variance of fertilizer-derived N<sub>2</sub>O emission and N immobilization, and the relative response factors of HCA decomposition, holocellulose, and lignin were evaluated by path analysis. Path analysis and VPA were completed using RStudio (Version 1.1.463, R Development Core Team, Vienna, Austria) with packages *lavaan* 0.6–3, *semPlot* 1.1, *OpenMx* 2.12.1, *tidyverse* 1.2.1, *knitr* 1.21, *kableExtra* 1.0.1, *GGally* 1.4.0, and *Vegan* 2.5–3. Data were not transformed prior to statistical testing.

## RESULTS

### Fate of Fertilizer N

Fertilizer-derived NH<sub>4</sub><sup>+</sup> was quickly converted to NO<sub>3</sub><sup>-</sup>, N<sub>2</sub>O, or SON after application to the soil; only approximately 23–29% of the initial <sup>15</sup>NH<sub>4</sub><sup>+</sup> was left at day 8, and almost all of the applied <sup>15</sup>NH<sub>4</sub><sup>+</sup> had disappeared 11 weeks later (Figure 1A). Correspondingly, the net NH<sub>4</sub><sup>+</sup> consumption rate decreased from 5.0–5.5 μg N g<sup>-1</sup> soil d<sup>-1</sup> to 0.2–0.3 μg N g<sup>-1</sup> soil d<sup>-1</sup>, while there was no significant difference of NH<sub>4</sub><sup>+</sup> consumption between the four treatments with fertilizer application. Fertilizer-derived NH<sub>4</sub><sup>+</sup> accounted for more than 90% of the total NH<sub>4</sub><sup>+</sup> at

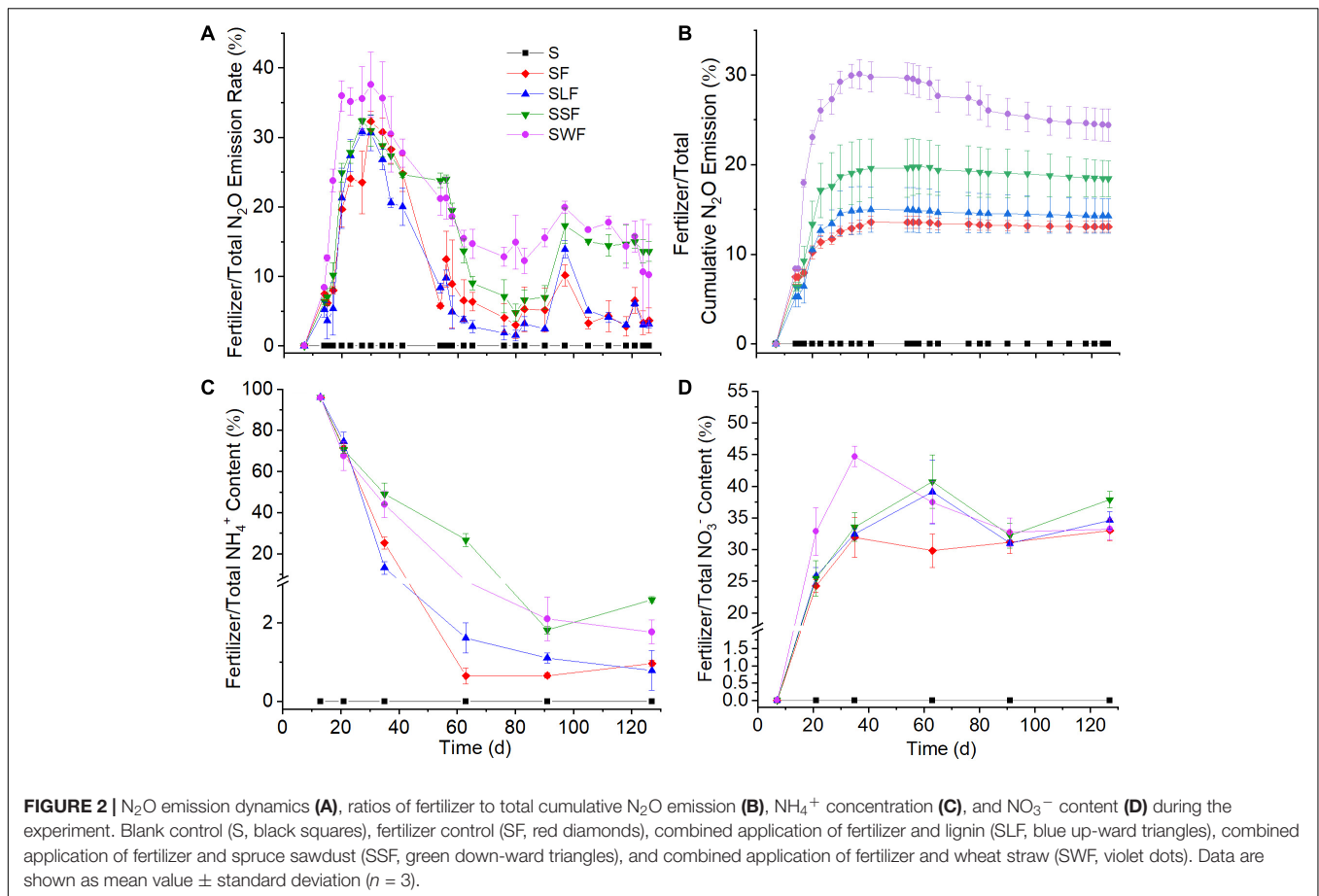


the beginning of the experiment, but for less than 3% at the end of the incubation period (Figure 2C). In line with the oxidation of NH<sub>4</sub><sup>+</sup>, the concentrations of total NH<sub>4</sub><sup>+</sup> (the sum of fertilizer-derived and local NH<sub>4</sub><sup>+</sup>) decreased from around 53 μg N g<sup>-1</sup> soil to about 2 μg N g<sup>-1</sup> soil (Supplementary Figure S1E).

### Fertilizer-Derived N<sub>2</sub>O Emission

As soon as <sup>15</sup>N-NH<sub>4</sub><sup>+</sup> had been applied, a part of the <sup>15</sup>N was immediately emitted to the atmosphere as N<sub>2</sub>O (Figure 1C). The highest fertilizer-derived N<sub>2</sub>O emission rate (more than 800 ng N kg<sup>-1</sup> h<sup>-1</sup>) occurred one day after fertilizer application, it then decreased quickly to less than 100 ng N kg<sup>-1</sup> h<sup>-1</sup> in the following 26 days. The application of wheat straw and spruce sawdust greatly enhanced fertilizer-derived N<sub>2</sub>O emission compared with

the fertilizer control, while there was no significant difference (*p* > 0.05) of fertilizer-derived N<sub>2</sub>O emission between SLF and SF treatments. Around 60 days later, the cumulative N<sub>2</sub>O emission in SWF and SSF treatments were significantly higher compared with SF and SLF treatments (Figure 1D). At the end of the experiment, cumulative fertilizer-derived N<sub>2</sub>O amounted to 126, 157, 275, and 341 μg N kg<sup>-1</sup> in SF, SLF, SSF, and SWF treatments, respectively, accounting for 0.2–0.7% of applied fertilizer-N (Figure 1D). Even though fertilizer-derived N<sub>2</sub>O peaked one day after fertilizer application, the ratio of fertilizer/total N<sub>2</sub>O emission rate reached the maximum 16 days later (Figure 2A). Among all the treatments, the ratio of fertilizer-to-total cumulative N<sub>2</sub>O emission rate was the highest in SWF treatment followed by SSF, and SLF (Figure 2B). Cumulative



fertilizer-derived N<sub>2</sub>O accounted for 12.0% of the total N<sub>2</sub>O emission in SF, for 15.1% in SSF, for 8.8% in SLF and for 23.6% in SWF treatments at the end of the experiment (Figure 2B).

### Immobilization of Fertilizer-N in the Soil

A substantial amount (9.5–15.2%, corresponding to 4.7–7.6 μg N g<sup>-1</sup> soil) of fertilizer-N was immobilized in SON within 7 days after the application of N fertilizer, while immobilized fertilizer-N decreased slowly to 0.8–3.5 μg N g<sup>-1</sup> soil in the following 9 weeks (Figure 1F). There was no significant difference of N immobilization in SON between different treatments in the first week after fertilizer application. However, the release of immobilized N was much slower in HCA amended soils, resulting in significantly ( $p < 0.05$ ) larger fertilizer-derived SON in SWF, SSF, and SLF treatments compared with the fertilizer-only control. By the end of the experiment, N immobilization in SON was 2–9% larger in HCA-amended soil than in the fertilizer control.

The retention of fertilizer-N (7.0–19.3% of applied fertilizer-N) in DON was comparable with that of SON, while no significant difference ( $p > 0.05$ ) between the treatments was observed (Figure 1E). Considering the N retention in both SON and DON, the highest N immobilization (21.7–31.0% of applied fertilizer-N) in the soil occurred 1 week after fertilizer application, followed by a slow release of immobilized N afterward. In

addition, N immobilization was most enhanced after wheat straw additions, followed by those of spruce sawdust and lignin.

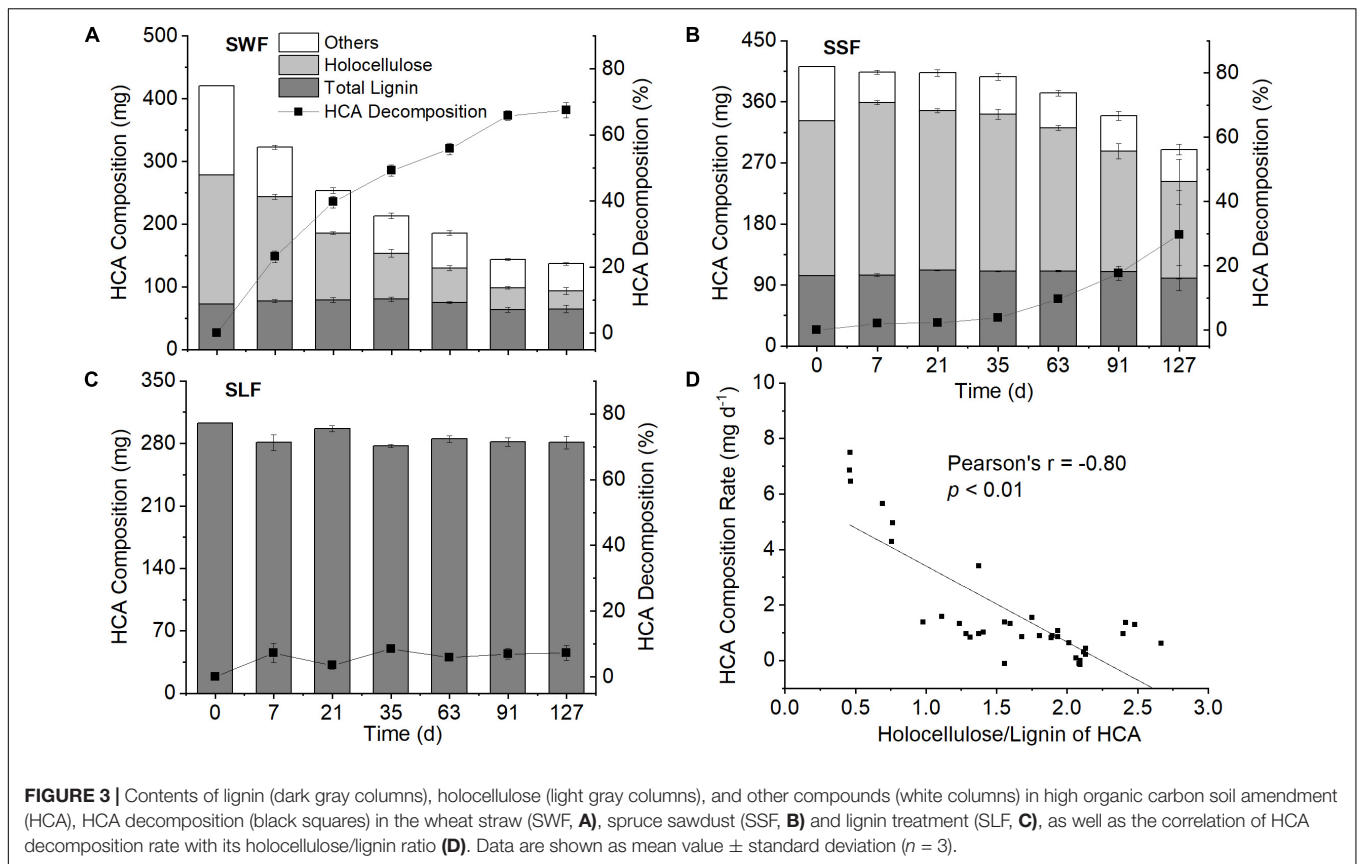
### Fertilizer-Derived NO<sub>3</sub><sup>-</sup>

Concomitant with the quick consumption of <sup>15</sup>NH<sub>4</sub><sup>+</sup>, the concentration of fertilizer-derived NO<sub>3</sub><sup>-</sup> increased substantially from 0 to 28.6–41.1 μg N g<sup>-1</sup> soil toward the end of the incubation period with the average net NO<sub>3</sub><sup>-</sup> production rate ranging from 1.0 to 1.3 μg N g<sup>-1</sup> soil d<sup>-1</sup> (Figure 1B). At the end of the incubation experiment, 57–82% of fertilizer-N had been transformed into fertilizer-derived NO<sub>3</sub><sup>-</sup>, which accounted for 33–37% of the total NO<sub>3</sub><sup>-</sup> concentration in soil (Figure 2D). Compared with the fertilizer control, fertilizer-derived NO<sub>3</sub><sup>-</sup> exhibited much lower concentrations in wheat straw amended soils, but higher ones in the lignin amended soils. Pearson correlation analyses revealed that fertilizer-derived NH<sub>4</sub><sup>+</sup>, N<sub>2</sub>O, and SON were significantly ( $p < 0.01$ ) positively correlated with each other, but significantly ( $p < 0.01$ ) negatively correlated with fertilizer-derived NO<sub>3</sub><sup>-</sup> (Supplementary Table S1).

## Decomposition and N Immobilization of HCA

### Decomposition of HCA

The contents of holocellulose and Klason lignin in spruce sawdust were about 55 and 25%, respectively, with the remaining 20%



being other compounds, such as ash, pigment, and proteins (Figure 3B and Supplementary Figure S2B). After 127 days of incubation, 29.7  $\pm$  13.8% of the spruce sawdust had decomposed, most of which was attributed to holocellulose (Figure 3B). Compared with spruce sawdust, wheat straw contained around 8% less Klason lignin and 6% less holocellulose, while about 67% of wheat straw had decomposed at the end of the experiment (Figure 3A and Supplementary Figure S2A). The average decomposition rate of wheat straw (16.7 mg d<sup>-1</sup> kg<sup>-1</sup> soil) was significantly ( $p < 0.01$ ) larger than that of spruce sawdust (7.2 mg d<sup>-1</sup> kg<sup>-1</sup> soil) and lignin (1.3 mg d<sup>-1</sup> kg<sup>-1</sup> soil), with holocellulose accounting for most of the HCA decomposition (Figure 3). In 127 days, 91.8  $\pm$  1.8% and 39.4  $\pm$  17.0% of the holocellulose in wheat straw and spruce sawdust, respectively, had been decomposed. Consequently, the ratio of holocellulose/lignin in wheat straw and spruce sawdust decreased from 2.2 to 0.4 and from 2.4 to 1.4, respectively (Supplementary Figure S2). Interestingly, the holocellulose/lignin ratio of HCA was significantly ( $p < 0.01$ ) negatively correlated with its decomposition rate (Figure 3D), but positively ( $p < 0.01$ ) with the cumulative fertilizer-derived N<sub>2</sub>O emission (Figure 4D).

### N Immobilization in HCA

The immobilization of fertilizer-N in HCA was several magnitudes larger than that in the soil (Table 1). The highest N immobilization was found in wheat straw, which was more than four times larger than that in spruce sawdust and about eight

**TABLE 1 |** N immobilization in HCA ( $\mu\text{g N g}^{-1}$  HCA), data are displayed as mean value  $\pm$  standard deviation ( $n = 3$ ).

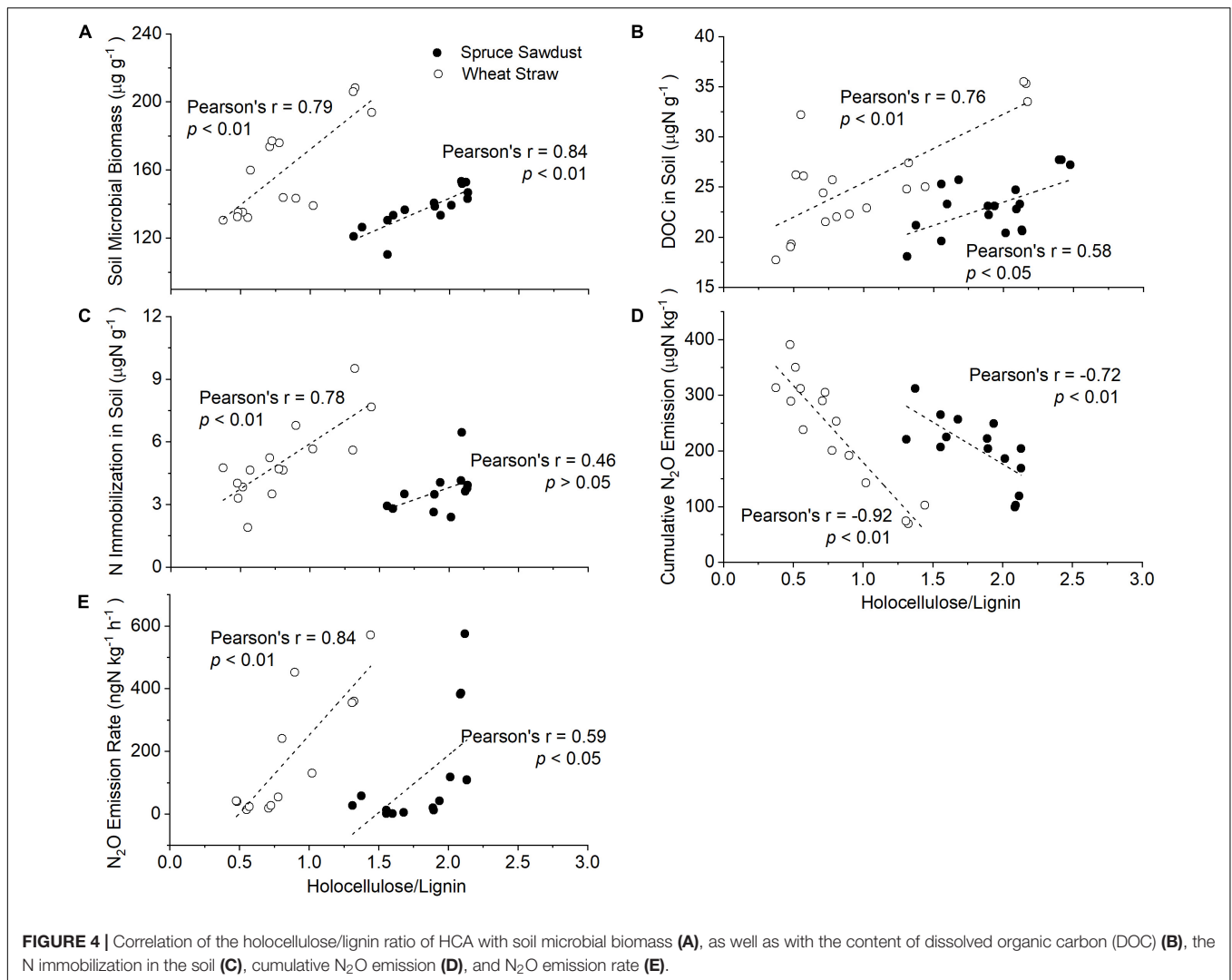
Incubation time (d)	Lignin	Wheat straw	Spruce sawdust
14	94.9 $\pm$ 13.1 Aa	2668.9 $\pm$ 281.2 Ac	289.4 $\pm$ 45.4 Ab
21	134.5 $\pm$ 13.1 Aa	4864.3 $\pm$ 564.4 Bc	442.5 $\pm$ 60.8 Ab
63	146.9 $\pm$ 34.6 Aa	5576.3 $\pm$ 162.4 Bc	848.1 $\pm$ 109.3 Ab
91	117.9 $\pm$ 7.3 Aa	6337.8 $\pm$ 1017.8 Bc	1405.8 $\pm$ 304.9 Bb
127	106.0 $\pm$ 14.1 Aa	7818.5 $\pm$ 635.7 Cc	1741.7 $\pm$ 510.2 Bb

Different small letters represent significant ( $p < 0.05$ ) difference between treatments at the same sampling time, while different capital letters represent significant ( $p < 0.05$ ) difference at different sampling time in the same treatment.

times larger than that in lignin. N immobilization continued to increase in both wheat straw and spruce sawdust during the experiment, however, it started to decrease in lignin 63 days later. At the end of the experiment, 7818  $\mu\text{g N g}^{-1}$  HCA was immobilized in the wheat straw treatment, 1742  $\mu\text{g N g}^{-1}$  HCA in the spruce sawdust treatment, and 106  $\mu\text{g N g}^{-1}$  HCA in the lignin treatment. In addition, a positive correlation between N immobilization and holocellulose/lignin ratio of HCA was observed (Figure 4C).

### Soil Conditions

The content of lignin-derived phenols was increased by around 61-100% in the SLF treatment, but only by maximum 33%



in the treatments of SWF and SSF compared to the blank control (Figure 5). HCA amendment also largely changed the composition of lignin-derived phenols in the soil. Soil amended with wheat straw was characterized by the highest proportion of lignin S (43-45%) and H (6.8-8.0%) units, but lowest proportion of lignin G (47-49%) units (Table 2). In contrast, lignin-amended soil was characterized by the highest content of lignin G (64-67%) units and lowest content of lignin S (28-31%) and H (3.9-5.9%) units (Table 2). Neither the content nor composition of lignin-derived phenols changed significantly along time (Figure 5). The content of lignin G unit was positively correlated to fertilizer-derived NO<sub>3</sub><sup>-</sup>, it was negatively correlated with fertilizer-derived N<sub>2</sub>O and SON, and the opposite was found for the lignin S and H units (Supplementary Table S2 and Supplementary Figures S3, S4).

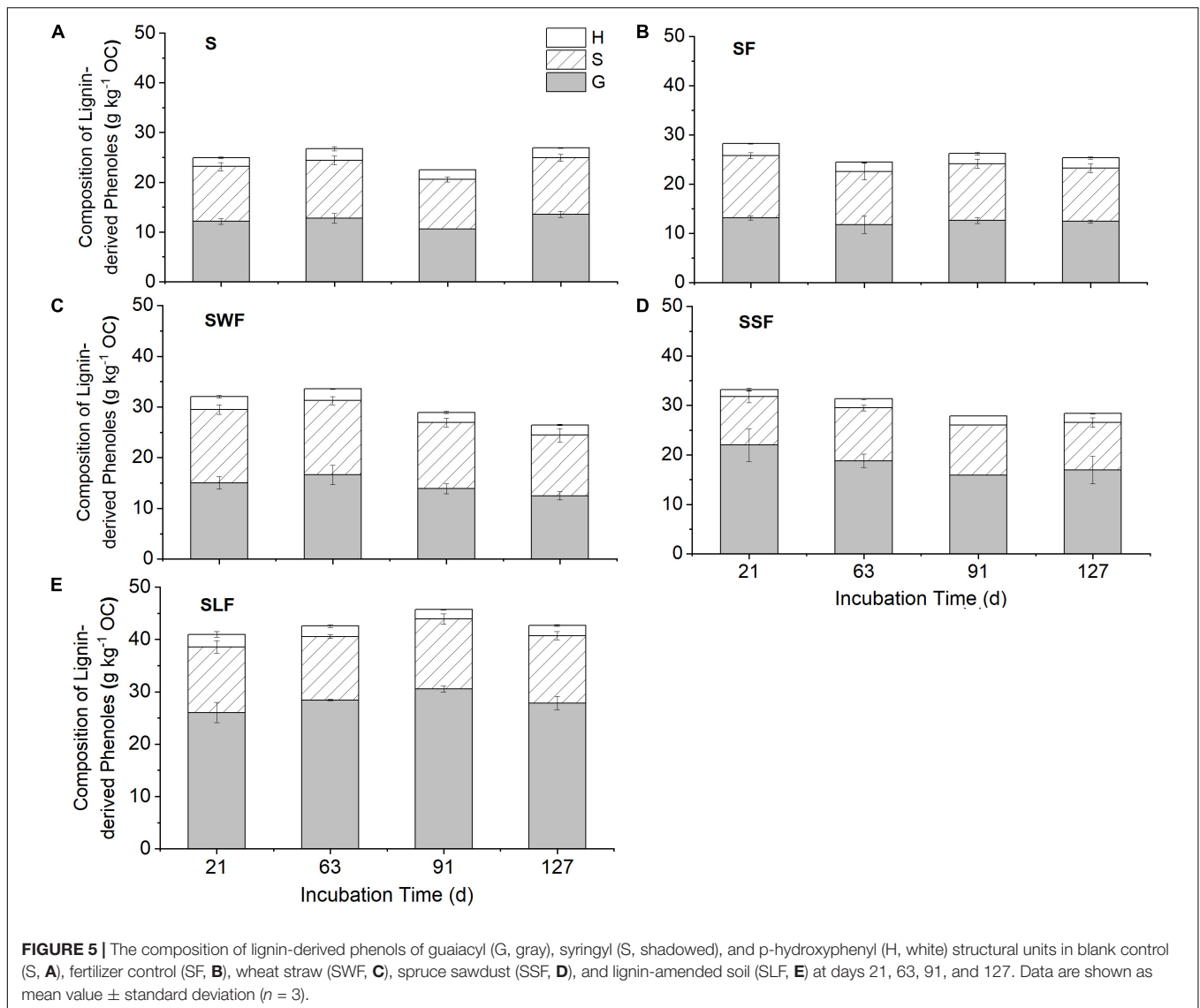
Fertilizer and HCA amendment introduced less than 4% of native N and 10.5% of native C into the soil, resulting in negligible changes of total soil N and C contents. Therefore, no significant ( $p > 0.05$ ) difference of soil N and C contents between the treatments were observed during the whole

experiment, and the soil C/N ratio remained relatively stable at approximately 9. On the other hand, soil pH decreased by about 0.2 units after fertilizer application, accompanied by a decrease of 20-26 µg DOC g<sup>-1</sup> dry soil, an increase of 20-70 µg DTN g<sup>-1</sup> dry soil, and a decrease of 5-80 µg C g<sup>-1</sup> of soil microbial biomass (Supplementary Figure S1). Compared with SF treatment, the amendments of wheat straw and spruce sawdust largely enhanced soil microbial biomass and DOC but decreased total NO<sub>3</sub><sup>-</sup>. By contrast, no significant changes of soil microbial biomass, DOC, nor total NO<sub>3</sub><sup>-</sup> by lignin amendment was observed. In addition, holocellulose/lignin ratio of HCA was significantly ( $p < 0.01$ ) negatively correlated with soil pH, soil microbial biomass, DOC, and soil nitrite (Figure 4).

### Influence of HCA and Soil Conditions on the Fate of Fertilizer-N

Variation partitioning analysis and path analysis were used for sorting the effects of soil-related parameters (soil pH,





soil microbial biomass, soil moisture, soil lignin G/S ratio), HCA-related (HCA decomposition rate, holocellulose/lignin ratio, and N immobilization in HCA), and fertilizer-related parameters (fertilizer-derived  $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) on fertilizer-derived  $\text{N}_2\text{O}$  emission and N retention (**Figure 6**). Fertilizer and HCA alone contributed 8% and 4% to the total variation of fertilizer-derived  $\text{N}_2\text{O}$  emission and N retention, respectively (**Figure 6A**). The combined effect of soil parameters and fertilizer explained 43% of the total variation, while the combined effect of soil parameters and HCA explained 26% of the total variation.

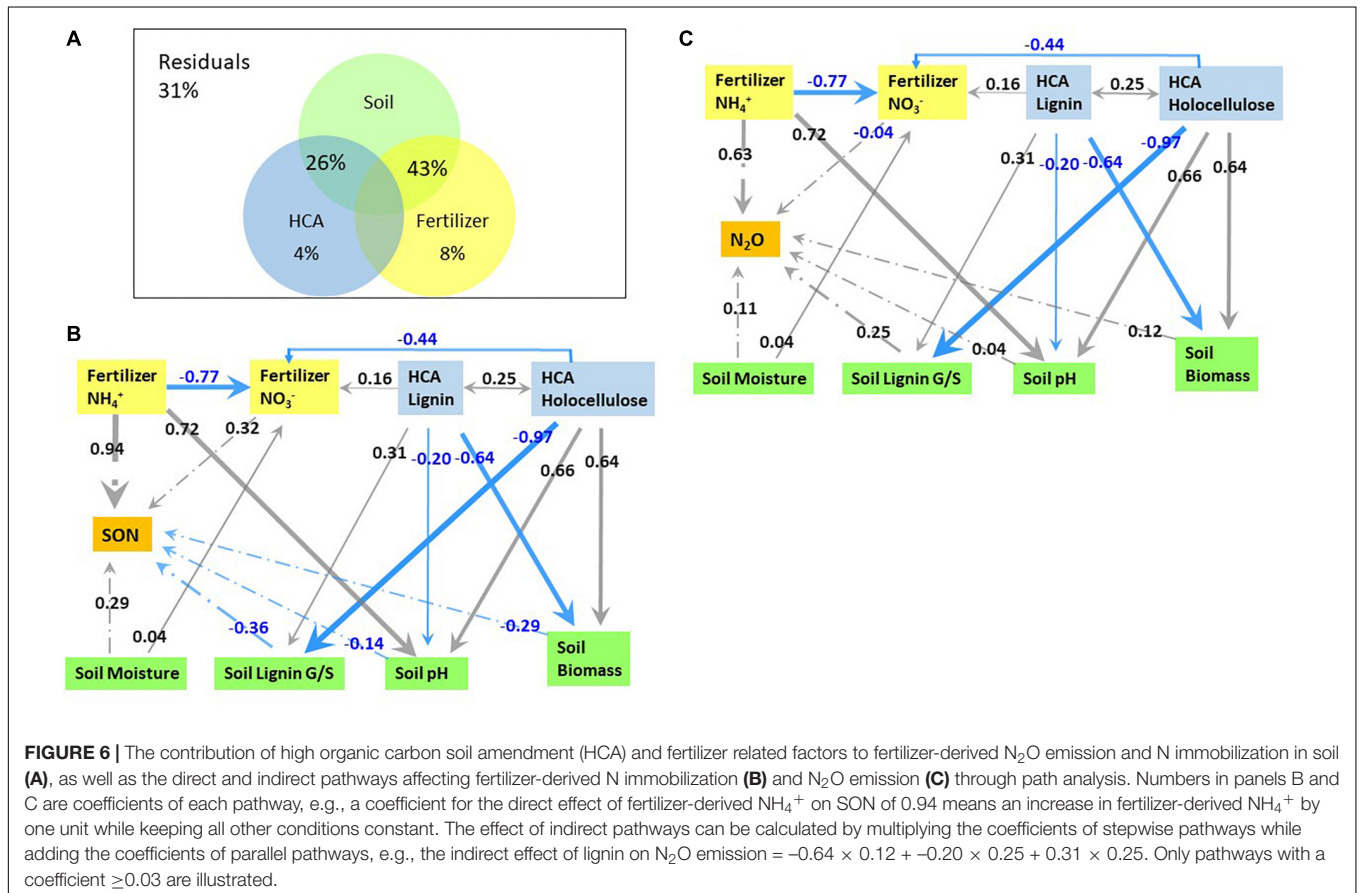
Factors directly affecting fertilizer-derived  $\text{N}_2\text{O}$  emission included fertilizer- $\text{NH}_4^+$ , soil pH, the composition of lignin-derived phenols, soil microbial biomass, soil moisture, and fertilizer-derived  $\text{NO}_3^-$ , with a partial coefficient of 0.63, 0.25, 0.25, 0.12, 0.11, and  $-0.04$ , respectively (**Figure 6C**). The contents of both lignin and holocellulose in HCA acted indirectly on  $\text{N}_2\text{O}$

emission by affecting soil pH, fertilizer-derived  $\text{NO}_3^-$ , and the soil lignin G/S ratio. Holocellulose affected positively on soil microbial biomass (0.64) and soil pH (0.66), but negatively on soil lignin G/S ratio ( $-0.97$ ) and fertilizer-derived  $\text{NO}_3^-$  ( $-0.44$ ), resulting in a total correlation coefficient of 0.017 for fertilizer-derived  $\text{N}_2\text{O}$  emission. By contrast, lignin influenced negatively on soil microbial biomass ( $-0.64$ ) and soil pH ( $-0.20$ ), but positively on the soil lignin G/S ratio (0.31) and fertilizer-derived  $\text{NO}_3^-$  (0.16), leading to a total coefficient of  $-0.056$  for fertilizer-derived  $\text{N}_2\text{O}$  emission. In addition, fertilizer-derived  $\text{NH}_4^+$  indirectly influenced  $\text{N}_2\text{O}$  emission through fertilizer-derived  $\text{NO}_3^-$  with an indirect coefficient of 0.03. In summary, the influence of each factor on  $\text{N}_2\text{O}$  emission decreased in the order: fertilizer-derived  $\text{NH}_4^+$  (0.66), soil pH (0.25), soil lignin G/S ratio (0.25), soil moisture (0.11), decomposition of HCA lignin ( $-0.056$ ), fertilizer-derived  $\text{NO}_3^-$  ( $-0.04$ ), and decomposition of HCA holocellulose (0.017).

**TABLE 2** | Proportions of lignin guaiacyl (G), syringyl (S), and p-hydroxyphenyl (H) units in soils, data are displayed as mean value ± standard deviation (n = 3).

Treatment	Incubation time (d)	Lignin G unit (%)	Lignin S unit (%)	Lignin H unit (%)
Blank control	21	48.6 ± 1.1 Aa	44.3 ± 1.3 Ab	7.1 ± 0.4 Ab
	63	47.8 ± 0.3 Aa	43.6 ± 1.0 Ac	8.6 ± 1.1 Ab
	91	47.2 ± 1.3 Aa	44.2 ± 1.3 Ac	8.5 ± 0.0 Ac
	127	50.2 ± 1.0 Aa	42.4 ± 1.0 Ac	7.5 ± 0.3 Ab
Fertilizer control	21	46.5 ± 0.8 Aa	44.7 ± 0.7 Ab	8.80 ± 0.3 Ab
	63	48.0 ± 0.8 Aa	44.2 ± 0.9 Ac	7.83 ± 0.8 Ab
	91	48.2 ± 1.3 Aa	44.0 ± 0.7 Ac	7.71 ± 0.7 Ac
	127	49.2 ± 1.9 Aa	42.5 ± 1.5 Ac	8.23 ± 0.5 Ab
Spruce sawdust + Fertilizer	21	66.2 ± 1.5 Ac	29.6 ± 1.7 Aa	4.23 ± 0.4 Aa
	63	60.0 ± 2.3 Bb	34.1 ± 1.8 Ab	5.88 ± 0.7 Aa
	91	57.3 ± 0.0 Bb	36.1 ± 0.0 Bb	6.59 ± 0.0 Bb
	127	59.5 ± 2.0 Bb	34.1 ± 1.4 Ab	6.39 ± 0.7 Bb
Wheat straw + Fertilizer	21	46.9 ± 0.2 Aa	45.1 ± 0.5 Ab	8.03 ± 0.3 Ab
	63	49.3 ± 1.5 Aa	43.8 ± 1.2 Ac	6.83 ± 0.4 Bb
	91	48.0 ± 0.1 Aa	45.1 ± 0.3 Ac	6.86 ± 0.2 Bb
	127	47.2 ± 1.0 Aa	45.2 ± 1.2 Ac	7.59 ± 0.2 Abb
Lignin + Fertilizer	21	63.6 ± 3.0 Ab	30.6 ± 1.8 Aa	5.86 ± 1.2 Aa
	63	66.8 ± 1.1 Ac	28.4 ± 0.5 Aa	4.76 ± 0.6 Aa
	91	66.9 ± 1.7 Ac	29.2 ± 1.8 Aa	3.92 ± 0.2 Aa
	127	65.2 ± 0.8 Ac	30.1 ± 0.8 Aa	4.64 ± 0.2 Aa

Different letters represent significant ( $p < 0.05$ ) difference between treatments at the same sampling time, while different capital letters represent significant ( $p < 0.05$ ) difference at different sampling time in the same treatment.



Fertilizer-derived NH<sub>4</sub><sup>+</sup> acted on N retention in the soil directly (0.94) and indirectly by affecting fertilizer-derived NO<sub>3</sub><sup>-</sup> (-0.25), resulting in a final coefficient of 0.69 (Figure 6B). By contrast, lignin and holocellulose of HCA affected N immobilization in SON indirectly through soil pH, lignin composition in the soil, fertilizer-derived NO<sub>3</sub><sup>-</sup>, and soil microbial biomass with a coefficient of -0.22 and 0.30, respectively. Soil moisture affected N retention both directly (0.29) and indirectly (0.13), leading to a final coefficient of 0.42. As a result, the immobilization of fertilizer-N was affected mostly by fertilizer-derived NH<sub>4</sub><sup>+</sup> (0.69), followed by soil moisture (0.42), soil lignin composition (-0.36), fertilizer-derived NO<sub>3</sub><sup>-</sup> (0.32), holocellulose decomposition of HCA (0.30), soil microbial biomass (0.29), lignin decomposition of HCA (-0.22), and soil pH (-0.14).

## DISCUSSION

### N<sub>2</sub>O Emission in the Soil

To understand how HCA and fertilizer parameters affect N<sub>2</sub>O emission and N retention, it is necessary to understand the main pathways involved in N transformation. When applied to the soil, NH<sub>4</sub><sup>+</sup> is oxidized to NO<sub>3</sub><sup>-</sup> through autotrophic nitrification with N<sub>2</sub>O as a main side product (Bol et al., 2003), at the meantime, NO<sub>3</sub><sup>-</sup> can be reduced to N<sub>2</sub>O through denitrification (Russow et al., 2009). It has been reported that N<sub>2</sub>O emission increased with increasing soil moisture, where 60% of WHC was found to be a threshold below which nitrification acted as the dominant N<sub>2</sub>O producing process, whereas denitrification would gradually take over as the dominant N<sub>2</sub>O source above this threshold (Schindlbacher et al., 2004; Bateman and Baggs, 2005). Soil moisture of 50-60% WHC used in the current study is an optimal condition for both autotrophic and heterotrophic nitrification with unrestricted diffusion of N substrates and O<sub>2</sub>, while autotrophic and heterotrophic nitrification compete for O<sub>2</sub> depending on the availability of organic sources and soil pH (Bateman and Baggs, 2005). In this experiment, the fast consumption of fertilizer-NH<sub>4</sub><sup>+</sup> and accumulation of fertilizer-derived NO<sub>3</sub><sup>-</sup> is a typical nitrification phenomenon (Figure 1), indicating that nitrification dominated N<sub>2</sub>O emission (Figure 6B). Moreover, the production rate of fertilizer-NO<sub>3</sub><sup>-</sup> was larger than the consumption rate of fertilizer-NH<sub>4</sub><sup>+</sup>, and a decrease of fertilizer-SON in the last 100 days was observed (Figure 1). This decline likely resulted from the oxidation of immobilized SON to NO<sub>3</sub><sup>-</sup> through heterotrophic nitrification (Zhang et al., 2015).

The effects of HCA on fertilizer-derived N<sub>2</sub>O emission and N retention accounted for 30% of the total variance, mostly via the influence of nitrification, soil microbial biomass, and soil lignin G/S ratio (Figure 6A). Since holocellulose contributed the most to HCA decomposition, while lignin contributed the least (Figure 3), holocellulose is regarded here as the representative of a C source easily accessible to soil microbes and lignin as the representative of microbial resistant C from the HCA. The lignin-containing complex in plant cell walls acts as a natural barrier preventing microbial access to C source in the cell, and

biological decomposition of plant residues is in general negatively correlated with their lignin content (Pauly and Keegstra, 2008). A negative effect of HCA lignin content, but positive effect of HCA holocellulose content on soil microbial biomass was found in our study (Figure 6B). Therefore, the holocellulose/lignin ratio is regarded as a factor controlling both C input into the soil and subsequent microbial N<sub>2</sub>O emission and N immobilization rates. In our study, the HCA decomposition rate largely decreased along decreasing holocellulose/lignin ratio (Figure 3D), resulting in less C input into soil and less soil microbial biomass (Figure 4A). The much larger N<sub>2</sub>O emission and N retention in SSF and SWF treatments compared with those in the SF treatment can be explained by promoted microbial N<sub>2</sub>O production and N uptake (Figure 1).

Despite the lower soil microbial biomass (Supplementary Figure S1), N<sub>2</sub>O emission was slightly larger in the SLF treatment than in the fertilizer control (Figure 1D). We attribute this finding to chemodenitrification (Zhu-Barker et al., 2015; Ostrom et al., 2016; Wankel et al., 2017; Wei et al., 2017a). Phenolic compounds released from lignin decomposition have a strong capability of retaining N substances and chemically reducing NO<sub>2</sub><sup>-</sup> to N<sub>2</sub>O (Smolander et al., 2012). We found that fertilizer-derived N<sub>2</sub>O emission was significantly (*p* < 0.05) related to the composition of lignin-derived phenols in the soil (Supplementary Table S2 and Supplementary Figure S3), which was in accordance with previous studies (Millar and Baggs, 2004; Garcia-Ruiz and Baggs, 2007; Frimpong and Baggs, 2010; Wei et al., 2017a,b). Above all, nitrification, denitrification, and chemodenitrification contributed to N<sub>2</sub>O emissions, with nitrification dominating the N<sub>2</sub>O source especially at the beginning of the experiment.

### N Immobilization in the Soil

As N is an essential element for the growth of soil microbes, biotic N retention, namely the uptake of mineral N by soil microbes, is a spontaneous way of fertilizer-N incorporation into SON. Dissolved organic carbon, which can be quickly and easily consumed by soil microbes, is very efficient in reducing mineral N content in the short term by promoting soil microbial biomass and biological N uptake (Burke et al., 2013). However, the microbial immobilization of fertilizer-N is a short- to medium-term effect (Burke et al., 2013), as immobilized N will eventually be mineralized or undergo heterotrophic nitrification and released into the soil again (Figure 1). As high holocellulose/lignin ratios correlated with larger soil microbial biomass and high N<sub>2</sub>O emission rates, they also related to more efficient N retention in the soil (Figure 4). Gentile et al. (2008) reported that mineral N content was lowered by the combined application of crop residues and urea in different soils, most likely by promoting microbial N uptake. In agreement with this study, we also found that the amendment of both wheat straw and spruce sawdust greatly enhanced soil microbial biomass and N immobilization but decreased DTN content compared with fertilizer control (Figure 1 and Supplementary Figure S1).

In addition to microbial N immobilization, abiotic N fixation by DOC as a function of soil pH, redox potential, and ferric

iron content has also been reported (Corre et al., 2007; Torres-Canabate et al., 2008). Both NH<sub>4</sub><sup>+</sup> and NO<sub>2</sub><sup>-</sup> are chemically reactive to various soil organic compounds, such as humic and fulvic acid-like structures as well as lignin derivatives, leading to the formation of SON and to abiotic N retention (Thorn and Mikita, 2000; Fitzhugh et al., 2003). Moreover, it has been proven that NO<sub>3</sub><sup>-</sup> can be incorporated into SON through the so-called ferrous wheel mechanism under various conditions (Davidson et al., 2003; Fitzhugh et al., 2003; Corre et al., 2007; Torres-Canabate et al., 2008). Regardless of the similarity in the contents of soil microbial biomass and DOC in SF and SLF treatments (**Supplementary Figure S1**), the N retention in the SLF treatment was significantly larger than that in the SF treatment (**Figure 1F**), which could be explained by abiotic N immobilization. According to Schmidt-Rohr et al. (2004), lignin-derived phenols may chemically react with NH<sub>4</sub><sup>+</sup>, leading to chemical immobilization of fertilizer-N in the soil. The abiotic formation of organic N compounds, e.g., oximes, amides, pyrroles, and nitrophenols, has been found in various chemical reactions of NO<sub>2</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> with SOM (Nommik and Nilsson, 1963; Azhar et al., 1989; Rousseau and Rosazza, 1998; Thorn and Mikita, 2000).

## Application of HCA in Agricultural Management

Organic amendments based on locally available plant residues or organic waste is regarded as an alternative that could be an economic way to improve soil quality and fertilizer use efficiency in agricultural management (Gentile et al., 2008). The application of HCA to soil promotes short-term N immobilization by increasing microbial N uptake and chemical N fixation (Baggs et al., 2000; Frimpong and Baggs, 2010; Cucu et al., 2014). Compared with the fertilizer control, the retention of fertilizer-N in HCA-amended soil increased by 50-200% with decreasing lignin content of the HCA in our study (**Figure 1F**). However, it should be noted that only less than 15% of fertilizer-N was immobilized as SON, and most of the rest prevailed in the form of NO<sub>3</sub><sup>-</sup> (**Figure 1B**), which could be used by crops, or leached into groundwater or surface water in case of heavy rains or flooding (Schnebelen et al., 2004). Soil moisture was maintained at 50-60% WHC in this study, which favors nitrification instead of denitrification. Therefore, further work is needed to explore the consumption of fertilizer-derived NO<sub>3</sub><sup>-</sup> by crops, as well as N<sub>2</sub>O emissions under denitrification-favored conditions, before one can state unambiguously if the combined application of HCA and N fertilizer could enhance the nitrogen fertilizer use efficiency in agriculture.

No obvious mitigation of fertilizer-derived N<sub>2</sub>O emission by HCA was found in our study, instead, the application of wheat straw and spruce sawdust promoted fertilizer-derived N<sub>2</sub>O emission in the sandy soil by 170 and 120%, respectively (**Figure 1D**). In agreement with our results, Frimpong and Baggs (2010) reported that the application of leguminous residues (*Vigna*, *Mucuna*, and *Leucaena*) enhanced N<sub>2</sub>O emission by up to 200% in a reddish brown sandy loam. High availability of amended C can strongly support the growth of microbial

communities favoring fast C cycling and N reducing, which finally results in an increase of soil N<sub>2</sub>O emissions (Rummel et al., 2019). The holocellulose/lignin ratio, acted as an important indicator for soil microbial biomass development, microbial N<sub>2</sub>O production and N retention. Both N<sub>2</sub>O emission and N immobilization increased with increasing holocellulose/lignin ratio of HCA. However, no significant increase of N<sub>2</sub>O emission was observed in lignin-amended soil, whereas N immobilization increased by 50% compared with the fertilizer control (**Figure 1**). Chemical composition of HCA controls the C availability and quality for N transformation processes, subsequently, acts as the driving force of sustainable agriculture (Rummel et al., 2019). Successive amendment of organics in two olive cultivars for 2 years increased the soil cation exchange capacity by about 2.5 meq 100 g<sup>-1</sup> and promoted C assimilation rate of plants by 3.5-3.8 μmol m<sup>2</sup> s<sup>-1</sup>, which finally lead to an increase of plant yield (Roussos et al., 2017). The chemical composition of HCA, especially its holocellulose/lignin ratio, should thus be considered when using HCA amendments for improving climate-smart agriculture practice (Millar and Baggs, 2004; García-Ruiz et al., 2012; Chen et al., 2013; Cucu et al., 2014).

## CONCLUSION

Here, we studied the fate of fertilizer-N after combined application of fertilizer and HCA to an agricultural sandy loam. The chemical composition of HCA, as reflected by different holocellulose/lignin ratio and lignin content and composition, affected largely the fertilizer-derived N<sub>2</sub>O emission and N immobilization in the soil. The holocellulose/lignin ratio proved to be a useful indicator for C input into the soil and subsequent N<sub>2</sub>O emission and N immobilization by soil microorganisms. Particularly the amendments with wheat straw and spruce sawdust, both exhibiting elevated holocellulose/lignin ratio, significantly increased the contents of soil microbial biomass and thereby promoted both microbial N<sub>2</sub>O emission and N immobilization. By contrast, a significant increase of N immobilization and slight increase of N<sub>2</sub>O emission were found in lignin-amended soil. Biotic pathways dominated during the whole experiment: nitrification together with less pronounced denitrification for N<sub>2</sub>O emission and microbial N uptake for N immobilization. The composition of lignin-derived phenols also significantly correlated with N<sub>2</sub>O emissions and N immobilization, indicating lignin-related chemodenitrification and N retention. Lignin-related N immobilization was less pronounced than that of holocellulose-related microbial N uptake, but at the same time caused less climate-relevant N<sub>2</sub>O emission to an agricultural sandy loam soil.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher on request.

## AUTHOR CONTRIBUTIONS

JW, NB, and RR designed the experiment. RR, MI, and JW performed the sampling. JW, MI, and HW performed the  $\delta^{15}\text{N}$  measurements of N<sub>2</sub>O, NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup>, and SON. RR performed the analysis of soil microbial biomass. MI and JW performed the analysis of NO<sub>2</sub><sup>-</sup> and soil pH. HW analyzed the decomposition of holocellulose and lignin of HCA. JW and WA conducted the lignin analysis of soil samples. JW performed the statistical analysis. JW and NB wrote the manuscript. JW, RR, MI, WA, HW, and NB revised the manuscript.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fenvs.2020.00015/full#supplementary-material>

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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