Enhancing organic and inorganic carbon sequestration in calcareous soil by the combination of wheat straw and wood ash and/or lime

Huilil Zhao¹, Huijie Zhang¹, Abdul Ghaffar Shar¹, Jifei Liu¹, Yanlong Chen¹, Songjie Chu¹, Xiaohong Tian¹,²*

¹ College of Natural Resources and Environment/Key Lab of Plant Nutrition and the Agri-environment in Northwest China, Ministry of Agriculture, Northwest A&F University, Yangling, Shaanxi, China, ² Heyang Field Station of Agricultural Environment and Farmland Conservation, Ministry of Agriculture, Fuping, Shaanxi, China

*txhong@hotmail.com, txhong@nwsuaf.edu.cn

Abstract

Increasing organic carbon sequestration in agricultural soils is important for improving soil fertility and mitigating climate change. Wood ash is generally applied as a potassium fertilizer, but the effects of simultaneous incorporation of wood ash and crop straw on the turnover of soil organic carbon (SOC) and inorganic carbon (SIC) are not well understood. In this study, a 118-day lab incubation experiment was conducted using a calcareous soil (with 10 years of continuous maize cropping history) to study the effects of adding wheat straw, wood ash and lime. Our study showed that straw addition led to an increase in both SOC (19%) and SIC (3%). Wood ash and lime addition decreased CO₂ emission by 182 and 1210 mg kg⁻¹ and increased SIC by 125 and 1001 mg kg⁻¹ during the incubation, respectively, which was due to supply of CaO from wood ash and lime. The increase of SOC content was 2.4% due to the addition of lime. In addition to straw addition enhanced straw-derived OC content, the addition of lime also increased straw-derived OC content by 34.5%. This study demonstrated that lime was more effective in reducing CO₂ emission and enhancing SOC than wood ash. In conclusion, adding lime to calcareous soil might be an effective method of enhancing carbon sequestration and slowing climate change.

Introduction

Agricultural soils can mitigate the emission of greenhouse gases and enhance soil sustainability, so the potential for carbon sequestration in agricultural soils has received significant attention [1]. Soil organic carbon (SOC) and inorganic carbon (SIC) are important carbon reservoirs in arid and semi-arid regions that play an important role in the global carbon cycle and climate change [2]. However, soil organic carbon has attracted much attention, and SIC has received much less attention despite its potential for carbon sequestration. Wang et al. [3]
reported that the SOC and SIC stocks were greater in agricultural land than in non-agricultural land in arid and semi-arid regions. Many reports showed that long-term straw incorporation and manure application in arid cropland led to SOC enhancement and carbon sequestration in the form of carbonate [4–6]. These results suggest that there are many ways to increase SIC content in agricultural soils.

SOC content in soils can be enhanced by commonly used best management practices, including the utilization of minimum or no tillage, cover crops, organic amendments and balance fertilization [7, 8]. Soil organic carbon sequestration, which relies on traditional management practices, is not always effective because of the loss of applied organic C and native soil C [8]. For example, Zhao et al. [9] reported that the cumulative C input achieved by straw return was much higher than that observed without straw return; however, the corresponding increases in SOC stock under straw return were relatively minor, which indicated a substantial loss of C input. Therefore, research is required to determine how CO₂ released from straw can be fixed to form inorganic carbon in the context of widespread application of straw returning measures. In addition, Kirkby et al. [10] showed that the amount of straw converted into “new” fine fraction soil organic matter (SOM) was increased by up to three-fold by augmenting the residues with supplementary nutrients. Thus, the development of methods of fixing more carbon from straw into soil has significant potential to improve soil fertility and reduce CO₂ emissions. Jastrow et al. [11] reported that the application of soil amendments with a high specific area enhanced SOC content via physico-chemical protection of SOC by organo-mineral complexation. Previous studies suggest that fly ash increases SOC content because of its large specific area [11]. In addition, the presence of CaO, Ca(OH) and MgO in fly ash results in uptake of CO₂ by the soil as a consequence of carbonation reactions [12]. These studies suggest that the application of mineral amendments, such as wood ash, with its high specific surface area and metal oxide content, with organic residue could enhance soil C content [13]. In addition, revealing soil C dynamics under long-term straw return is essential to understanding changes in new soil C inputs and mineralization of old C. SOC sources can be determined because the δ¹³C values of C₃ (δ¹³C ca. −28‰) and C₄ (δ¹³C ca. −12‰) vegetation are different because of differences in C isotope utilization [14]. The relative contribution of new SOC and old SOC can be estimated based on the mass of each C isotope [14]. In the present study, new soil C formation was quantified by measuring δ¹³C abundance based on changes in decomposition after 10 years of continuous corn planting.

The large quantities of wood ash generated by the wood industry and the increasing cost of wood ash storage have encouraged the search for alternative uses [15]. Recycling biomass ash in agriculture may solve the problem of disposal and reduce the doses of commercial fertilizer required for crops [16]. Wood ash is characterized by its high alkaline metal content and generally contains significant quantities of Ca, Mg and K [17]. Reductions in soil acidity and increased base saturation have been widely reported after the application of wood ash to forest mineral soils [18, 19]. Traditionally, the major use of wood ash in China has been as a potassium fertilizer [20, 21]. Although calcareous soils are common in arid and semi-arid climates and constitute more than 730×10⁶ ha of soil worldwide, information on the value of ash fertilizers in calcareous soils is scarce, especially when they are used in combination with straw [22]. In calcareous soils, carbonization of Ca and Mg present in wood ash fixes CO₂ produced from soil respiration and thus reduces CO₂ emissions while increasing SOC content [23]. However, Maljanen et al. [24] reported that wood ash resulted in greater CO₂ fluxes in acidified forest soils by stimulating the microbial population. These conflicting results may be due to differences in initial soil pH, ash application rates, ash types, soil types, and inputs of organic matter that occurred in these various studies. In addition, Kleber et al. [25] showed that the minerals and metals in wood ash can protect SOM from decomposition by interacting with it.
Conversely, Hansen et al. [26] reported that the liming effect and the input of nutrients in response to wood ash could provide more favorable conditions for soil microbes, which could expedite decay of SOM in acidic soil. However, the effect of the combination of wood ash and crop straw on SOC content has not been evaluated comprehensively in alkaline soil, and little is known regarding the effect of wood ash on straw-derived C sequestered in alkaline soil. Traditionally, lime materials, such as calcite (CaCO$_3$), burnt lime (CaO), and dolomite [CaMg(CO$_3$)$_2$] are used to neutralize acidic soils [27, 28]. In alkaline calcareous soils, CaCO$_3$ is added as a net sink of CO$_2$, whereas CaCO$_3$ functions as a net source of CO$_2$ in acidic soils [29]. Certainly the presence of CaO, Ca(OH)$_2$, and MgO will enhance uptake of CO$_2$ by the soil as a consequence of carbonation reactions [12]. A previous study conducted under controlled conditions (laboratory incubation experiments) suggested that wood ash reduced CO$_2$ emission [23] due to the formation of inorganic carbon. We believe that the presence of large amounts of oxides in wood ash will lead to carbonation of Ca and Mg as follows: (Ca$^{2+}$ or Mg$^{2+}$) + CO$_3^{2-}$ → CaCO$_3$ or MgCO$_3$. For the sake of verifying the function of wood ash materials for CO$_2$ capture, we selected the higher content of CaO in wood ash as a reference to test the mechanism, so we set up another lime treatment; moreover, the added lime amount was the same as the amount of CaO in the wood ash. The addition of lime to soil could increase the solubility of dissolved organic carbon (DOC) and enhance DOC leaching because of deprotonation or desorption [30]. Ahmad et al. [31] reported that liming-induced decreases in SOC were mainly attributed to enhanced C mineralization following an increase in C solubility. Therefore, liming could increase or decrease the DOC concentration in soil depending on which processes dominate. Andersson et al. [30] also demonstrated that the DOC concentration is more dependent on soil pH than on soil microbial activity, and a rapid increase in soil pH may increase the solubility of organic matter.

We hypothesized that adding wood ash and/or lime in the presence of straw would reduce CO$_2$ emission, enhance SIC formation through CaO hydration, directly increase SOC stocks by increasing the abundance of Ca$^{2+}$ ions (which function as soil aggregation agents), and indirectly affect SOC by changing soil properties. The combination of wood ash and lime significantly increased the amount of straw-derived C, so, we performed laboratory incubation experiments using calcareous soil to assess the effects of straw, wood ash and lime on CO$_2$ mineralization, SOC content, SIC content and soil chemical properties.

Materials and methods

Characterization of soil, wheat straw, wood ash and lime

We confirm that the owner of the land, Northwest A&F University, gave permission to conduct the study on this site. Our field studies did not involve endangered or protected species. The soil used in this experiment was taken from the topsoil (0–20 cm) of an agricultural field at the Changwu Agricultural and Ecological Experimental Station (35.14° N, 107.40° E; 1152 m a.s.l.) on the Loess Plateau in northwestern China. The soil samples were air dried and passed through a 2-mm sieve to remove rocks, coarse crop residues and roots. The soil samples were obtained from a monocrop planting area in which maize (C$_4$ crop) was grown for ten years. The soil at the site was a silt clay loam (according to the Chinese Soil Taxonomy) that contained 5.4% sand, 51.7% silt and 42.9% clay; the soil at the site was tentatively classified as Earth-cumuli-Orthic Anthrosol [32]. The soil samples had the following soil properties: pH 8.4, SOC 8.91 g kg$^{-1}$, total nitrogen 1.2 g kg$^{-1}$, microbial biomass C 163 mg kg$^{-1}$, DOC 32.4 mg kg$^{-1}$, available phosphorus 18.4 mg kg$^{-1}$, available potassium 152 mg kg$^{-1}$, $\delta^{13}$C -19.5‰, CaCO$_3$ 67 g kg$^{-1}$.
The wheat stem (internode) material used in this study was collected at harvest at the Doudou Experimental Station of Northwest A&F University, oven-dried at 70°C, and stored. The wheat straw had an average C content of 456 g kg\(^{-1}\) and a mean total N content of 6.8 g kg\(^{-1}\), which had a \(\delta^{13}C\) value of -27.5‰. The straw was cut into pieces approximately 2-cm long prior to mixing with the soil.

Dust-like wood ash was produced from the branch of a kiwi fruit tree (*Actinidia*) by combustion in a home fireplace. The wood ash was dried at 50°C and sieved through a 0.25-mm mesh screen prior to use and analysis. The wood ash had a \(\delta^{13}C\) value of -26.4‰. The chemical properties of the wood ash are given in Table 1.

The lime used in the experiment contained an amount of CaO equivalent to that contained in the wood ash.

### Experimental design

An incubation experiment was conducted using a completely randomized design. A \(2 \times 2 \times 2\) factorial experiment corresponding to two levels of wood ash addition (no wood ash, \(W_{0}\); 12 g wood ash kg\(^{-1}\) soil, \(W_{12}\)), two levels of lime addition (no lime, \(L_{0}\); 5 g lime kg\(^{-1}\) soil, \(L_{5}\)) and two levels of straw addition (no straw, \(S_{0}\); 15 g straw kg\(^{-1}\) soil, \(S_{15}\)) was established. Each treatment had three replicates.

Fresh soil samples (250 g dried weight) were placed in 1-L plastic jars and pre-incubated for 7 days. The wood, lime and/or straw were mixed thoroughly with the soil samples in each jar before a solution containing N and P was added. Urea and diammonium phosphate were dissolved in deionized water and added in the form of a solution (4.4 g L\(^{-1}\) N, 2.1 g L\(^{-1}\) P\(_2\)O\(_5\), 5 mL to each glass jar), and the soil moisture content was adjusted to 70% of the soil water holding capacity using deionized water. During the 118-day incubation period, the temperature was maintained at 25 ± 1°C. The weight of each sample was recorded at the beginning of the treatment, and the water lost by evaporation was replaced with deionized water every five days. Three replicates per treatment group were destructively sampled to determine the soil DOC.

### Table 1. Chemical properties of wood ash used in the experiment.

All data are expressed on a dry weight basis.

| Property                  | Value  |
|---------------------------|--------|
| pH                        | 12.44  |
| EC (dS/m)                 | 24.75  |
| Organic carbon (g kg\(^{-1}\)) | 3.2    |
| Total carbon\(^a\) (g kg\(^{-1}\)) | 39     |
| Total nitrogen (g kg\(^{-1}\)) | 0.75   |
| SOC:TN\(^\dagger\)        | 4.3    |
| CaO (g kg\(^{-1}\))      | 434.0  |
| K\(_2\)O (g kg\(^{-1}\)) | 150.9  |
| SiO\(_2\) (g kg\(^{-1}\)) | 123.4  |
| MgO (g kg\(^{-1}\))      | 98.4   |
| P\(_2\)O\(_5\) (g kg\(^{-1}\)) | 58.7  |
| Al\(_2\)O\(_3\) (g kg\(^{-1}\)) | 44.9  |
| Fe\(_2\)O\(_3\) (g kg\(^{-1}\)) | 24.5  |
| Na\(_2\)O (g kg\(^{-1}\)) | 10.4   |
| Cl (g kg\(^{-1}\))       | 7.1    |

\(^a\)represents soil organic carbon plus soil inorganic carbon
\(^\dagger\)represents the ratio of soil organic carbon to total nitrogen

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and pH at days 2, 10, 45, and 118. In order to allow destructive measurements of different experimental units on each sampling date [33], we prepared 96 experimental units (8 treatments × 4 sampling dates × 3 replicates).

**Measurement of soil CO₂ effluxes and analysis**

An open plastic vial containing 20 mL of 1 M sodium hydroxide (NaOH) was placed in the jar containing soil to absorb CO₂ resired during the subsequent incubation. Four blank jars containing only water and NaOH were also set up as above. Soil CO₂ efflux was assessed on days 2, 3, 4, 5, 7, 10, 15, 20, 25, 35, 45, 65, 95, and 118. The NaOH vials were replaced with fresh vials on each test day. The jars were opened at each measurement of CO₂ emission to allow air exchange. The trapped CO₂ was determined as total CO₂ evolved by titrating alkali to a phenolphthalein end point with 0.5 M HCl [34] and expressed as mg C kg⁻¹ soil.

At the completion of the incubation period, all of the samples were ground through a 2-mm sieve. After mixing thoroughly, one subsample of the sieved soil was air-dried for soil analysis, while another subsample was stored at 4°C for DOC analysis. The DOC was determined using a glass electrode meter in soil/water suspensions (1:2.5 soil:water). At the completion of the 118-day incubation period, any C remaining in the samples was again assumed to be SOC (determined by the wet oxidation method) after any partially degraded wheat straw remaining in the jars was removed by the dry-sieving winnowing method [36]. In order to measure the amount of C in the remaining straw, three replicates of each treatment were prepared in another 12 jars (4 treatments and 3 replicates). Briefly, each mixture of soil and straw was poured into water and then sufficiently stirred so that the straw floated in the water, after which the undecomposed straw was filtered using a sieve, and the process was repeated again with fresh water. Finally, the partially degraded straw was dried and weighed. The C content of the remaining straw was calculated as the residual straw weight multiplied by the residual straw carbon content.

Soil inorganic carbon was measured as described by Bao et al. [37]. The δ¹³C values of wheat straw, wood ash and SOC were determined using a dry combustion analyzer with an attached isotope ratio mass spectrometer (Europa Scientific Model 20–20). 1 g soil was pretreated with 10 mL 1 M HCl for 12 hours to remove carbonate. The precision for δ¹³C was ± 0.10‰ based on repeated measurements of a working standard. The δ¹³C of the samples was expressed as following:

\[
\delta^{13}C(\text{‰}) = \left( \frac{R_{\text{sample}}}{R_{\text{PDB}}} - 1 \right) \times 1000
\]

where \(R_{\text{sample}}\) is the \(^{13}\text{C}/^{12}\text{C}\) ratio of the sample and \(R_{\text{PDB}}\) is the \(^{13}\text{C}/^{12}\text{C}\) ratio of the Pee Dee Belemnite (PDB) standard [38].

**Data calculation**

The ratio of straw-derived C to SOC (\(f_{\text{new}}\)) from the incubated jars over 118 days was calculated using a two-component isotopic mixing model [39].

\[
f_{\text{new}}(\%) = \left( \delta^{13}C_{\text{soc-a}} - \delta^{13}C_{\text{soc-b}} \right) / \left( \delta^{13}C_{\text{material}} - \delta^{13}C_{\text{soc-b}} \right)
\]

where \(\delta^{13}C_{\text{soc-a}}\) is the δ¹³C of SOC in straw/lime/wood ash-amended soils after the incubation, \(\delta^{13}C_{\text{soc-b}}\) is the δ¹³C of SOC in non-amended soil before the incubation, and \(\delta^{13}C_{\text{material}}\) is the
Statistical analysis

All reported values are the means of 3 replicate jars. Data Processing System (DPS) version 7.05 statistical software (Ruifeng Information Technology Co., Ltd., Hangzhou, China) was used for the statistical analysis. The significance of the effects of the straw, wood ash and lime treatments, as well as their interactions on the reported traits, were evaluated by analysis of variance (ANOVA). All statistical differences were calculated by ANOVA, and the means were segregated by the LSD multiple comparison test at $P < 0.05$.

Results

Soil CO$_2$ emission

Unsurprisingly, in this study, the CO$_2$ emission rate from wheat straw-amended soil was much higher than that of soil with no straw added during the entire incubation (Fig 1A). Cumulative CO$_2$ emission was significantly affected by main effects of wood ash and lime addition (Table 2). With no wood ash or lime addition ($S_0W_0L_0$, $S_0W_0L_5$), the soil CO$_2$ emission rates peaked on the second day of the incubation, and decreased with time. Incorporation of wood ash or lime into the soil postponed the peak occurrence of the CO$_2$ emission rate whether or not straw was added (Fig 1A). Incorporation of wood ash or lime into the soil decreased the CO$_2$ emission rate. However, in soils with no straw addition, adding only wood ash decreased the CO$_2$ emission rate mainly during the first 5 days of the incubation relative to that of the soil with no amendment, whereas adding lime reduced the CO$_2$ emission rate during the entire incubation period (118 days). In the soil with straw amendment, adding only wood ash reduced the CO$_2$ emission rate only on the first day of the incubation, whereas the reduction in the CO$_2$ emission rate produced by adding lime persisted for the first ten days (Fig 1A). Soil amended with straw had much greater cumulative CO$_2$ emission than that of soil with no addition ($p < 0.05$, Fig 1B). Whether or not straw was added, the addition of wood ash and/or lime decreased cumulative CO$_2$ emission compared with that of soil with no amendment. The cumulative reduction in CO$_2$ emission from soil with lime was 5.4 times that of soil with wood ash ($p < 0.05$, Table 3).

SOC, straw-derived new OC, and SIC

In soil with no straw amendment, the net SOC content at the end of the incubation was reduced compared to the initial value because of mineralization (9317 mg C kg$^{-1}$ soil, Table 4). The reductions in net SOC content produced by the $S_0W_0L_0$, $S_0W_0L_5$, $S_0W_12L_0$, and $S_0W_12L_5$ treatments were 9.6%, 9.8%, 10.4%, and 10.5%, respectively; In soil with straw amendment, the net SOC content was increased compared to the initial SOC (by 3.7%, 9.1%, 5.6%, and 9.9% by $S_{15}W_0L_0$, $S_{15}W_0L_5$, $S_{15}W_12L_0$, and $S_{15}W_12L_5$, respectively). On average, straw addition increased net SOC content by 19% compared to that of soil with no amendment. In addition, lime addition increased the average net SOC content by 2.4% compared to that of soil with no amendment ($p < 0.05$, Table 3). At the end of the incubation, in the case of straw addition, the organic carbon of residual straw mixed in the soil was reduced by 47.4% in soil with lime amendment ($S_{15}W_0L_0$, $S_{15}W_0L_5$, $S_{15}W_12L_0$, and $S_{15}W_12L_5$) relative to that of soil with no lime amendment ($S_{15}W_0L_0$, $S_{15}W_0L_5$, $S_{15}W_12L_0$, $S_{15}W_12L_5$) ($p < 0.05$, Table 4).
Fig 1. CO$_2$-C emission rate (a) and cumulative CO$_2$-C emission (b) during straw decomposition under different treatments. (S$_0$W$_0$L$_0$, only soil; S$_0$W$_{12}$L$_0$, only wood ash; S$_0$W$_{12}$L$_5$, wood ash plus lime; S$_{15}$W$_0$L$_0$, only wheat straw; S$_{15}$W$_{12}$L$_0$, wheat straw plus wood ash; S$_{15}$W$_{12}$L$_5$, wheat straw plus lime; S$_{15}$W$_{12}$L$_5$, wheat straw and wood ash plus lime). The error bars outside the curve represent LSD.

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The addition of straw reduced the $\delta^{13}C$ of SOC value by 5.4% relative to that of soil with no amendment. Moreover, adding lime reduced the $\delta^{13}C$ of SOC value by 1.9% relative to that of soil with no amendment ($p < 0.05$, Table 3). Accordingly, the average new straw-derived OC

### Table 2. Cumulative CO$_2$ emission, final SOC, final SIC, soil DOC and pH at each sampling time.

| Time (days) | Variation  | CO$_2$ emission$^*$ | Final CO$_2$ source | Final SOC | Final SIC | Final DOC* | pH |
|-------------|------------|---------------------|---------------------|---------|----------|----------|-----|
| 10          | Straw (a)  | $<0.001$            | -                   | -       | $<0.001$ | $<0.001$ |     |
|             | Wood ash (b) | NS               | -                   | -       | NS       | NS       |     |
|             | Lime (c)   | $<0.001$            | -                   | -       | $<0.001$ | $<0.001$ |     |
|             | a × b      | NS                  | -                   | -       | NS       | NS       |     |
|             | b × c      | $<0.001$            | -                   | -       | NS       | NS       |     |
|             | a × c      | $<0.001$            | -                   | -       | 0.029    | $<0.001$ |     |
|             | a × b × c  | NS                  | -                   | -       | NS       | NS       |     |
| 45          | Straw (a)  | $<0.001$            | -                   | -       | NS       | $<0.001$ |     |
|             | Wood ash (b) | 0.008         | -                   | -       | NS       | $<0.001$ |     |
|             | Lime (c)   | $<0.001$            | -                   | -       | $<0.001$ | $<0.001$ |     |
|             | a × b      | NS                  | -                   | -       | NS       | NS       |     |
|             | b × c      | $<0.001$            | -                   | -       | NS       | NS       |     |
|             | a × c      | $<0.001$            | -                   | -       | NS       | $<0.001$ |     |
|             | a × b × c  | NS                  | -                   | -       | NS       | NS       |     |
| 118         | Straw (a)  | $<0.001$            | $<0.001$            | 0.004   | $<0.001$ | $<0.001$ |     |
|             | Wood ash (b) | $<0.001$        | NS                  | 0.008   | 0.002    | $<0.001$ |     |
|             | Lime (c)   | $<0.001$            | 0.004               | $<0.001$ | $<0.001$ | $<0.001$ |     |
|             | a × b      | NS                  | 0.002               | NS      | 0.031    | NS       |     |
|             | b × c      | 0.004               | NS                  | NS      | 0.044    | NS       |     |
|             | a × c      | $<0.001$            | NS                  | 0.005   | $<0.001$ | $<0.001$ |     |
|             | a × b × c  | NS                  | NS                  | NS      | 0.019    | NS       |     |

NS, not significant
$^*$represents cumulative CO$_2$ emission at each sampling time.
* DOC, dissolved organic carbon

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### Table 3. Effects of straw, wood ash and lime amendment on cumulative CO$_2$ emission, final SOC, final SIC and $\delta^{13}C$ after the incubation.

| Treatment | CO$_2$ emission (mg kg$^{-1}$) | Increase amount (mg kg$^{-1}$) | Increase rate (%) | Final net SOC | Increase amount (mg kg$^{-1}$) | Increase rate (%) | Final SIC | Increase amount (mg kg$^{-1}$) | Increase rate (%) | $\delta^{13}C$ (%) | Increase rate (%) |
|-----------|--------------------------------|-------------------------------|-------------------|---------------|--------------------------------|-------------------|----------|--------------------------------|-------------------|-----------------|------------------|
| Straw     | $S_0$                          | 428b                          | -                 | -             | 8377b                         | -                 | 8852b    | -                              | -                 | -20.5a          | -                |
|           | $S_{15}$                       | 3978a                         | 3550              | 829%          | 9974a                         | 1597              | 9119a    | 267                            | 3.0%              | -21.6b          | -5.4%            |
| Wood ash (W) | $W_0$                        | 2294a                         | -                 | -             | 9161a                         | -                 | 8923b    | -                              | -                 | -21.0a          | -                |
|           | $W_{12}$                      | 2112b                         | -182              | -7.9%         | 9189a                         | 28                | 9048a    | 125                            | 1.4%              | -21.1a          | 0.5%             |
| Lime (L)  | $L_0$                         | 2808a                         | -                 | -             | 9066b                         | -                 | 8485b    | -                              | -                 | -20.9a          | -                |
|           | $L_5$                         | 1598b                         | -1210             | -43%          | 9284a                         | 218               | 9486a    | 1001                           | 11.8%             | -21.3b          | -1.9%            |

Mean values having different lower-case letters are significantly different among straw rate, wood ash rate or lime rate ($P \leq 0.05$).

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was 2832 mg C kg$^{-1}$ soil. In particular, adding lime increased new OC by 34.6% compared to that of soil with no amendment ($p < 0.05$, Table 5).

The final SIC content was affected by adding straw, wood ash or lime ($p < 0.05$, Table 2). Lime increased SIC by 11.8% in comparison with that of soil without lime, whereas wood ash increased SIC by 1.4% in comparison with that of soil without wood ash ($p < 0.05$, Table 3). In soil with no straw amendment, the net SIC content at the end of the incubation was increased compared to its initial value. In soil with straw amendment, the net SIC content at the end of the incubation was further increased compared to its initial value, with the exception of soil with straw only ($p < 0.05$, Table 6).

### Soil pH and DOC

Irrespective of whether straw was added, the addition of lime greatly increased the DOC content in comparison with that of soil with no amendment after different periods of incubation ($p < 0.05$, Fig 2). In contrast, the addition of wood ash significantly increased DOC content on

Table 4. Change in organic C content (mg C kg$^{-1}$ soil) caused by the treatments before and after the incubation.

| Treatment | FinalSOC increase relative to control | Input | Lost | Cumulative CO$_2$-C emission | CO$_2$ emission reduction relative to control | Organic C in remaining straw |
|-----------|-------------------------------------|-------|------|-----------------------------|---------------------------------------------|-----------------------------|
|           |                                     | SOC in straw | SOC in wood ash |                             |                                             |                             |
| S0        | W0                                 | 8422c | -    | 927d                         |                                             |                             |
|           | L0                                 | 8399c | -23c | 85f                          | -842b                                       | -                           |
| W12       | L0                                 | 8350c | -72c | 680e                         | -247a                                       | -                           |
|           | L5                                 | 8337c | -85c | 20f                          | -907b                                       | -                           |
| S15       | W0                                 | 9660b | -    | 4991a                        |                                             | 1378a                       |
|           | L5                                 | 10166a| 506a | 3173c                        | -1818b                                      | 677b                        |
| W12       | L0                                 | 9836b | 176b | 4637b                        | -354a                                       | 1442a                       |
|           | L5                                 | 10237a| 577a | 3111c                        | -1880b                                      | 805b                        |

Initial SOC content is 9317 mg C kg$^{-1}$ soil.

$^a$Soils with any partially degraded straw removed.

$^b$Represents SOC formation in soil amended with exogenous substances relative to that of the S$_0$W$_0$L$_0$ and S$_{15}$W$_0$L$_0$ soils.

$^c$Represents reduced CO$_2$ emission relative to that in the S$_0$W$_0$L$_0$ and S$_{15}$W$_0$L$_0$ soils.

Different lower-case letters indicate significant differences among the treatments ($P < 0.05$).

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Table 5. Effects of straw, wood ash and lime amendment rate on the $\delta^{13}$C signature of soil organic carbon ratio of straw-derived OC to SOC ($f_{\text{new}}$, %), ratio of native soil-derived OC to SOC ($f_{\text{native}}$, %), straw-derived OC amount (New OC) and native soil-derived OC (Native SOC) with wheat straw addition.

| Treatment | $\delta^{13}$C (%) | $f_{\text{new}}$ (%) | New OC (mg kg$^{-1}$) | $f_{\text{native}}$ (%) | Native SOC (mg kg$^{-1}$) |
|-----------|-------------------|----------------------|----------------------|------------------------|--------------------------|
| S0        | W0                | -20.38               | 0                    | 100                    | 8423                     |
|           | L0                | -20.70               | 0                    | 100                    | 8151                     |
|           | L5                | -20.58               | 0                    | 100                    | 8217                     |
| W12       | L0                | -20.35               | 0                    | 100                    | 8355                     |
|           | L5                | -20.58               | 0                    | 100                    | 8217                     |
| S15       | W0                | -21.37a              | 23.9b                | 76.1                   | 7351a                    |
|           | L5                | -21.64b              | 27.3b                | 72.7                   | 7392a                    |
| W12       | L0                | -21.36a              | 25.6b                | 74.4                   | 7321a                    |
|           | L5                | -22.18c              | 36.4a                | 63.6                   | 6489b                    |

Mean values with different lower-case letters are significantly different among the straw addition treatments ($P < 0.05$).

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day 118 by 21.6% compared to that of soil with no addition, whereas the soil DOC content was unchanged on the other test days (p < 0.05, Table 7).

During the entire incubation, straw decreased soil pH, but lime increased soil pH. In contrast, wood ash increased soil pH only during the late period of incubation (p < 0.05, Table 7). When no straw was added, lime amendment caused the largest increase in soil pH among the tested treatments (0.68 on average in comparison with soil without lime), whereas the addition of straw increased the soil pH by only 0.28 in comparison with that of soil without straw (Fig 2B).

Discussion

Potential mechanism of the reduction in CO$_2$ emission caused by wood ash and lime

According to a previous study conducted in our laboratory, the simultaneous addition of straw and wood ash decreased cumulative CO$_2$ emission by approximately 6.2% compared to the addition of straw only. In the present study, the extent of the reduction of cumulative CO$_2$ emission due to wood ash amendment (7.9%, Table 3) was very similar to that from our previous study, but the addition of wood ash had no obvious effect on SOC sequestration. We surmised that the high content of CaO in wood ash (434 g kg$^{-1}$) might be the main reason for the lack of an observable effect of wood ash amendment on SOC sequestration [23]. Therefore, in the present study, experiments were conducted using lime (CaO) to determine whether CaCO$_3$ formed from CaO and CO$_2$ emitted during the incubation was responsible for the reduction in CO$_2$ emission. Moreover, the rate of CaO added to the soil using lime was equivalent to that added by wood ash amendment.

In the present study, lime amendment led to much less accumulative CO$_2$ emission than did wood ash (Fig 1; Table 3). When no straw was added to the soil, the period of CO$_2$ emission reduction caused by wood ash (5 d) was much shorter than that caused by lime (118 d). When straw was added to the soil, the periods of CO$_2$ emission reduction caused by wood ash (1 d) and lime (10 d) were both shortened. Several factors could be responsible for this phenomenon. First, when CO$_2$ emission was low (no straw added), the CaO contained in wood ash and lime was depleted for a relatively long period; however, when CO$_2$ emission was high (after straw amendment), CaO from lime and wood ash was exhausted more rapidly. Second, although the CaO content of wood ash was as high as 43%, and the rate of CaO addition from

### Table 6. Change in inorganic C content (mg C kg$^{-1}$ soil) caused by the treatments before and after the incubation.

| Treatment | SIC Final | Inorganic carbon in wood ash | Net $\Delta$SIC | SIC increase relative to control |
|-----------|-----------|-------------------------------|-----------------|---------------------------------|
| S$_0$     | W$_0$     | L$_0$                         | -30d            | -                               |
|           | L$_0$     | -                             | 822b            | 852a                            |
| W$_{12}$  | L$_0$     | 9039c                         | 217c            | 247b                            |
|           | L$_5$     | 9660b                         | 838b            | 868a                            |
| S$_{15}$  | W$_0$     | 8377d                         | -13d            | -†                              |
|           | L$_0$     | 9743b                         | 1353a           | 1353a                           |
| W$_{12}$  | L$_0$     | 9029c                         | 207c            | 220b                            |
|           | L$_5$     | 10192a                        | 1370a           | 1383a                           |

Initial SIC content is 8390 mg C kg$^{-1}$ soil.

$\Delta$ Represents the final SIC after the incubation minus the initial SIC content and inorganic carbon from wood ash itself.

† Represents SIC formation in soil amended with an exogenous substance relative to that in the S$_0$W$_0$L$_0$ and S$_{15}$W$_0$L$_0$ soils. Different lower-case letters indicate significant differences among the treatments (P < 0.05).

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lime was kept at 43% to attempt to match the amount of CaO added by the wood ash amendment, the actual amount of CaO in wood ash may have been much lower than this value. This difference in the assumed and actual CaO content of wood ash may have been the primary reason why the reduction in CO$_2$ emission caused by wood ash was much shorter than that caused by lime. Another finding supporting this notion is the measurement of inorganic carbon, present in the form of CaCO$_3$; the increase in net SIC following the addition of wood ash was much smaller than that observed following the addition of lime (Table 3). Future studies should determine the true proportions of CaO and other forms of Ca in wood ash. The findings described above were all obtained from experiments using calcareous soils. However, Pugliese et al. [40] and Ohlsson [41] found similar results using acidic forest soil, whereas Zimmermann et al. [42] showed that wood ash amendment in acidic forest soil resulted in significant increases in the rate of CO$_2$ evolution and microbial biomass C due to an increase in microbial activity, which was related to increases in the pH value and quantity of nutrients over the 460-day experiment. These differences in the findings from different studies are likely due to differences in initial soil pH, ash types, ash application rate, and other experimental parameters.

This study showed that lime and wood ash amendment significantly increased SIC content (Table 3). The major reason for the reduction in CO$_2$ emission reduction due to wood ash amendment was probably CaCO$_3$ formed from CO$_2$ and CaO. The reductions in CO$_2$ caused by wood ash and lime were very close in magnitude to the change in SIC; therefore, we postulate that the mechanism for CO$_2$ emission reduction by lime and wood ash is the reaction of CO$_2$ produced during the incubation process with CaO to produces CaCO$_3$. The possibility of direct microbial/enzymatic involvement in the reaction of CaO and CO$_2$ to produce CaCO$_3$ is relatively small.

**Wood ash or lime amendment affects the formation of straw-derived new OC/SIC and SOC content**

In this study, lime amendment caused a significant increase in SOC, but wood ash did not; moreover, while lime greatly promoted the formation of straw-derived new OC (46%), it only increased net SOC by 2.4% compared to that of soil with no amendment (Tables 3 and 4). This

| Incubation time (days) | Treatment | DOC (mg kg$^{-1}$) | Soil pH |
|------------------------|-----------|-------------------|---------|
| 2                      | W$_0$     | 432a              | 8.65b   |
|                        | W$_{12}$  | 433a              | 8.86a   |
| 10                     | W$_0$     | 317a              | 8.62a   |
|                        | W$_{12}$  | 336a              | 8.70a   |
| 45                     | W$_0$     | 262a              | 8.35b   |
|                        | W$_{12}$  | 233a              | 8.56a   |
| 118                    | W$_0$     | 171b              | 8.32b   |
|                        | W$_{12}$  | 208a              | 8.50a   |

Mean values with different lower-case letters are significantly different (P < 0.05).

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finding indicates that the simultaneous addition of straw and lime had a positive priming effect on native SOC (data not shown). Therefore, while adding lime (CaO 5 g kg\(^{-1}\) soil, CaCO\(_3\) content of the tested calcareous soil was 6.7%) can promote the conversion of straw C to new OC, it may also cause a loss of native OC (priming effect). Of course, the potential microbial mechanism underlying the priming effect of straw and lime amendment on native SOC merits exploration in future research.

The maize crop was planted in the tested soil over 10 years, so the soil was regarded as C\(_4\) soil. The \(\delta^{13}\)C of SOC value of the tested soil (from which SIC was removed) showed a distinct decrease after the addition of straw (C\(_3\) crop residue) compared to that of soil with no straw amendment (Table 4). Wood ash amendment had no effect on \(\delta^{13}\)C of SOC, but lime significantly reduced \(\delta^{13}\)C of SOC in comparison with that of soil with no lime amendment. It is possible that lime increased the soil pH, which increased the negative charge on molecules of organic matter and eventually increased organic matter solubility [43]. In different studies, liming has been reported to decrease SOC content [44], increase SOC content [45], and leave SOC content unchanged [46]. In this study, the simultaneous addition of straw with lime significantly enhanced net SOC and new OC formation in comparison with soil with no amendment (Tables 3 and 4). The rate and direction of changes in SOC following lime amendment depend on the balance between SOC gains and losses. In our study, it appears that increased C input as a result of lime addition promoted straw degradation, which greatly increased the DOC concentration in the soil treated with straw and lime; this effect may be one explanation for the simultaneous increase in SOC and reduction in C in the remaining straw (Table 5). Moreover, Ca\(^{2+}\)-mediated formation of soil particle aggregates creates a favorable environment for C accumulation [47]. Lime-induced changes in pH initially increase SOC solubility [30] and may subsequently prevent microbial decomposition by stabilizing SOC via Ca\(^{2+}\) bridging.

In this study, with no amendment with exogenous substances no obvious changes in net SIC content occurred before or after the incubation. As mentioned, above, the addition of lime or wood ash increased the net SIC content, which indicated that CaO reacted with CO\(_2\) to form CaCO\(_3\) (Table 6). This finding is in agreement with those reported by Zhao et al. [23] and Lee et al. [48].

**Wood ash and lime have significant effects on soil DOC and pH**

The high effective CaO content of lime is likely responsible for the greater magnitude of the increase in soil pH produced by lime in comparison with that produced by wood ash. The high soil pH following lime amendment increased the solubility of organic matter in comparison with that of organic matter in soil with no amendment, which resulted in higher DOC content in the lime-amended soil.

**Conclusions**

This study demonstrated that simultaneously adding wood ash or lime with returned crop straw delayed the CO\(_2\) emission peak and reduced the amount of total CO\(_2\) emitted into the atmosphere. The reduction in cumulative CO\(_2\) emission was much less with adding wood ash than with lime. The extent of CO\(_2\) emission reduction caused by wood ash or lime depends on the actual CaO content in each substance. Accordingly, the addition of wood ash or lime enhanced SIC content by 1.4% and 11.8%, respectively. As expected, straw could enhance straw-derived OC, and the lime addition also increased straw-derived OC by 34.5% compared to no addition. In addition, straw addition or lime addition increased the net SOC content by 19% and 2.4%, respectively. Irrespective of straw addition or not, lime addition greatly
increased the DOC content compared to no addition at the different period of incubation. During the entire incubation, straw addition decreased soil pH, and lime addition increased soil pH; whereas, wood ash increased soil pH only at the late period of incubation. A possible mechanism for the observed reduction in CO$_2$ emission from the tested calcareous soil was CaCO$_3$ formation, i.e., the reaction between CaO supplied by wood ash or lime and CO$_2$ emitted from the soil. The addition of lime in an appropriate manner can reduce CO$_2$ emission into the atmosphere and promote SOC sequestration.

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Author Contributions

Data curation: Huili Zhao, Abdul Ghaffar Shar, Yanlong Chen, Songjie Chu.
Formal analysis: Huili Zhao, Huijie Zhang, Jifei Liu.
Funding acquisition: Xiaohong Tian.
Investigation: Huili Zhao, Huijie Zhang, Jifei Liu, Songjie Chu, Xiaohong Tian.
Methodology: Huili Zhao, Huijie Zhang, Xiaohong Tian.
Project administration: Xiaohong Tian.
Resources: Huili Zhao.
Software: Huili Zhao, Huijie Zhang, Jifei Liu.
Supervision: Huili Zhao, Xiaohong Tian.
Validation: Huili Zhao.
Visualization: Huili Zhao.
Writing – original draft: Huili Zhao, Yanlong Chen, Xiaohong Tian.
Writing – review & editing: Huili Zhao, Abdul Ghaffar Shar, Yanlong Chen, Xiaohong Tian.

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Wood ash and lime addition on soil carbon sequestration

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