



Original article

Carbon budget by priming in a biochar-amended soil

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ABSTRACT

Understanding the balance between the priming effect and the retention of added organic carbon (C) in soil is important in evaluating the role of specific practices for terrestrial C sequestration. However, knowledge about the effects of biochar addition on soil net C accumulation following various C input patterns remains limited. We incubated soil with aged and fresh biochar, and added ¹⁴C-labeled glucose at three frequencies over 120 days: single, repeated, and continuous additions. Regardless of glucose addition, the presence of aged or fresh biochar reduced the accumulative CO₂ emissions, by 23% on average, relative to the soil without biochar addition. Compared with the soil without biochar addition, glucose mineralization significantly increased by 1.4–2.0% in the soils with aged biochar, but decreased by 0.1–1.9% in the soils with fresh biochar. Relative to repeated and continuous glucose additions, the single addition may slightly overestimate priming effects, especially during the early incubation period. At the end of incubation, repeated and continuous additions showed a higher net C accumulation than the single addition. Regardless of the glucose addition frequency, net C accumulation was lower in soils with aged or fresh biochar than in the control. In summary, biochar amendment has an interactive effect with glucose addition frequency and thus affects the direction and magnitude of priming. Despite the direct contribution of biochar to C sequestration in soil, the low net C accumulation from glucose in the biochar-amended soil highlights the necessity of C balance accounting.

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1. Introduction

The amount of organic C stored in soils globally (2344 Pg C) is about three times that of the total C in either the atmosphere or terrestrial vegetation [1,2]. Small changes in the soil organic carbon (SOC) stock can significantly affect the global atmospheric CO₂ concentration and climate system [3]. It is essential to understand the factors regulating the accumulation and stability of soil C and thereby predict terrestrial feedbacks to future climate change [4,5]. Soil priming is a phenomenon defined as the altered decomposition of native SOC following the input of labile organic material (LOM) into the soil relative to a control soil without substrate addition [6]. Over the past decade, the priming effect (PE) of adding C (or N) on soil organic matter (SOM) mineralization has been well documented globally with divergent results being reported [7,8]. Therefore, quantification of PEs is of great importance to

predictions of SOC dynamics and C cycling, especially under climatic change conditions.

The mechanisms responsible for the PE have been comprehensively summarized [8,9]. As one of the most important factors, the type of C input is a critical factor in controlling the magnitude and direction of the PE [8]. Previous priming experiments conducted under controlled conditions have mostly focused on single LOM additions; whilst showing that priming can accelerate SOC decomposition, the effect is generally short in duration and small in magnitude [10–13]. However, the PE from repeated and continuous inputs of LOM is expected to be different from that of single addition. Compared with single additions, for example, research has shown that the repeated addition of substrate can induce a high positive PE over a 1–4 month incubation period [14], while the effects are less pronounced when substrates are added continuously [15]. A few studies have examined PEs induced by repeated [16] or continuous additions of LOMs [17–19]. However, only Qiao et al. [20] has compared PEs induced by single, repeated, and continuous inputs of glucose within the same experiment. In fact, previous pot and field experiments with plants have demonstrated

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that the rhizodeposit-induced PE can be a persistent phenomenon [21,22]. Consequently, our understanding of the specific effects of single, repeated, and continuous LOM inputs on the PE, and their effects on priming occurrence and magnitude, remains limited. This calls for studies of both continuous and repeated LOM inputs to evaluate the response of PEs.

Biochar, the carbonaceous residue of pyrolyzed organic materials under low oxygen conditions, has gained increasing attention in the last decade. The biochemical stability of biochar after application in soil is crucial in ensuring its long-term maintenance of soil fertility [23,24]. Biochar may have stimulative, suppressive, or negligible influences on native SOC mineralization [25,26]. In a recent review, we synthesized published studies and reported that biochar slightly retards the mineralization of SOM, with a mean of 3.8%, relative to soil without biochar addition [26]. Because of its high stability, the inert C content of biochar is the largest contributor to the mitigation of greenhouse gas emissions through soil C sequestration [27,28]. However, the overall effect of biochar on SOC dynamics depends not only on the stability of the biochar itself, but also on its effects on mineralization of SOM and plant residues. To date, conflicting results in the form of stimulated [29,30], inhibited [31,32] and non-significant [26] responses of added LOMs (e.g. plant residue and glucose) to biochar additions have been reported. Thus, the response of added LOMs in soil to the presence of biochar remains inconsistent.

While the divergent results of PE have been reported [9], components of the added primers remain in the soil and compensate for the SOC loss, thereby contributing positively to the net C balance in soil [10]. Under controlled conditions the supply of LOM may accelerate native SOC decomposition and, thus, induce a negative [10,21] and positive C balance [10,20]. Nevertheless, very few studies offer an insight into this issue, despite its importance for accurately estimating long-term soil C storage and nutrient mineralization [10,33]. Therefore, the specific objectives of this study were to (1) explore the PE responses to glucose addition frequencies including single, repeated and continuous additions; (2) examine the effects of aged or fresh biochar on the PE in agricultural soils; and (3) determine the net C balance in response to glucose addition patterns and associated interactions with biochar.

2. Materials and methods

2.1. Soil sampling and preparation

The soil samples were collected in November 2013 from the surface layer (0–20 cm) of a field that had been under intensive vegetable cultivation for approximately 10 years in Nanjing, Southeast China (31°01'N, 118°52'E). The soil is classified as *Irragric Anthrosols* [34] with a silty clay loam texture [35].

Two field treatments with three replications were conducted: without (hereafter, the control) and with biochar application at 40 Mg BC ha⁻¹ (equivalent to 16 g BC kg⁻¹ soil), which began in April 2011. Urea was used as an N fertilizer at 325 kg N ha⁻¹ in each crop for both treatments following local agronomic practices. The biochar was purchased from Sanli New Energy Company (Henan, China). Selected properties of studied soils and biochar are shown

in Table 1.

Air-dried wheat straw was cut into small segments and was heated in the reactor at 5–10 °C min⁻¹, which was then retained at the highest heating temperature (around 450 °C) for 4.5 h. Nitrogen gas was then added into the reactor during the cooling-off period to maintain an inert environment. The pyrolysis of wheat straw at 450 °C resulted in 30% biochar, >3% bio-oil, and <23% pyrolytic gas [36]. The biochar had a cation exchange capacity of 24.1 cmol kg⁻¹, surface area of 8.92 m² g⁻¹ and ash content of 20.8%. The biochar was ground to particle size ≤2 mm before mixing with the soil samples. The soil samples were stored field fresh in aerated polyethylene bags at 4 °C after sampling. Prior to the experiment, soil samples were sieved (≤2 mm) and homogenized, and fine roots and visible plant debris were carefully removed.

2.2. Experimental design

The experimental design included two factors: 1) biochar addition, and 2) frequency of glucose addition. The biochar treatments included: 1) soil without biochar: control, 2) aged biochar soil: soil with biochar applied in the field at 40 Mg BC ha⁻¹, and 3) fresh biochar soil: the control soil mixed with biochar in the lab at a rate of 10.6 g BC kg⁻¹ soil, to achieve the same amount of C as present in the aged biochar soil.

The glucose addition frequencies were: 1) control with distilled water, 2) single, 3) repeated, and 4) continuous additions. The treatments with glucose addition received a total of 87.6 µg C g⁻¹ soil over 120 days. Uniformly labeled ¹⁴C-glucose was added to the unlabeled D (+)-glucose before it was added to the soil. Controls received distilled water only at 10-day intervals throughout the 120-day incubation. Single addition treatments received all the glucose at the start of the experiment and all subsequent additions at 10-day intervals were distilled water only as in the control. Repeated addition treatments received 1/4 of the total glucose at 30-day intervals with the additions between being distilled water only at 10-day intervals. Continuous addition treatments received 1/12 of the total glucose every 10 days. Thus, the first glucose additions to each soil treatment varied in amount between treatments on a SOC-specific basis for soils with and without biochar addition (5.3 and 4.1 mg C g⁻¹ SOC, respectively). In total, the experiment included 12 treatments, each with three replicates.

2.3. Incubation and sampling

After mixing with biochar (only for the treatment with fresh biochar), air-dried soil samples (20 g of oven-dry weight) were moistened to 50% water holding capacity (WHC) and incubated inside 100-ml Schott jar with lids, in the dark, at 22 °C, for a pre-incubation period of one week, to stabilize the microbial activity, thus avoiding undesired microbial peaks. Three empty jars were used as controls to consider the background CO₂ in the headspace air. After pre-incubation, a glucose solution at the target concentration or distilled water in the control was added to achieve 60% WHC, which was maintained during incubation by adding additional distilled water periodically. Small vials with 3 ml of 1 M NaOH were placed in the jars to trap CO₂ throughout the incubation. The CO₂ measurements were performed at 10-day intervals. By the end of the incubation period, soil samples were destructively collected for the determination of microbial biomass and dissolved organic C (DOC).

2.4. Microbial biomass and DOC measurement

Microbial biomass was determined by chloroform fumigation extraction (modified after Vance et al. [37]). Briefly, after

Table 1
Properties of biochar and studied soils.

	Biochar	Soil	Aged biochar soil
Total C (%)	47.1	1.64	2.14
Total N (%)	0.77	0.26	0.27
C/N ratio	60.9	6.3	8.0
pH (H ₂ O)	10.40	7.09	6.52

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