



Positive and negative carbon mineralization priming effects among a variety of biochar-amended soils

Andrew R. Zimmerman^{a,*}, Bin Gao^b, Mi-Youn Ahn^c

^a Department of Geological Sciences, University of Florida, 241 Williamson Hall, PO Box 112120, Gainesville, FL 32611-2120, USA

^b Department of Agricultural and Biological Engineering, University of Florida, Gainesville, FL 32611, USA

^c Soil and Water Science Department, University of Florida, PO Box 110290, Gainesville, FL 32611, USA

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ABSTRACT

Pyrogenic carbon (biochar) amendment is increasingly discussed as a method to increase soil fertility while sequestering atmospheric carbon (C). However, both increased and decreased C mineralization has been observed following biochar additions to soils. In an effort to better understand the interaction of pyrogenic C and soil organic matter (OM), a range of Florida soils were incubated with a range of laboratory-produced biochars and CO₂ evolution was measured over more than one year. More C was released from biochar-amended than from non-amended soils and cumulative mineralized C generally increased with decreasing biomass combustion temperature and from hardwood to grass biochars, similar to the pattern of biochar lability previously determined from separate incubations of biochar alone.

The interactive effects of biochar addition to soil on CO₂ evolution (priming) were evaluated by comparing the additive CO₂ release expected from separate incubations of soil and biochar with that actually measured from corresponding biochar and soil mixtures. Priming direction (positive or negative for C mineralization stimulation or suppression, respectively) and magnitude varied with soil and biochar type, ranging from –52 to 89% at the end of 1 year. In general, C mineralization was greater than expected (positive priming) for soils combined with biochars produced at low temperatures (250 and 400 °C) and from grasses, particularly during the early incubation stage (first 90 d) and in soils of lower organic C content. In contrast, C mineralization was generally less than expected (negative priming) for soils combined with biochars produced at high temperatures (525 and 650 °C) and from hard woods, particularly during the later incubation stage (250–500 d). Measurements of the stable isotopic signature of respired CO₂ indicated that, for grass biochars at least, it was predominantly pyrogenic C mineralization that was stimulated during early incubation and soil C mineralization that was suppressed during later incubation stages. It is hypothesized that the presence of soil OM stimulated the co-mineralization of the more labile components of biochar over the short term. The data strongly suggests, however, that over the long term, biochar–soil interaction will enhance soil C storage via the processes of OM sorption to biochar and physical protection.

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1. Introduction

Pyrogenic organic matter, or black carbon (BC), the solid residuals of biomass combustion, has recently been recognized to represent a significant portion of sediment and soil organic carbon (SOC), ranging up to 40% but more typically about 10% of SOC (Masiello, 2004). Because of its highly condensed nature and resistance to chemical treatment, BC has generally been regarded as

biologically and chemically recalcitrant (e.g. Seiler and Crutzen, 1980; Kuhlbusch, 1998; Skjemstad et al., 2002). While BC is often found to be among the oldest pool of organic matter in soils, with ¹⁴C ages ranging to 10³ y (Schmidt et al., 2002), some studies have shown BC to be at least partially biotically and chemically reactive (Shneour, 1966; Baldock and Smernik, 2002; Hilscher et al., 2009; Kuznyakov et al., 2009; Zimmerman, 2010). The chemical or ‘combustion’ continuum of black carbon materials, in order of increasing charring temperature from slightly charred biomass to charcoal to soot (Masiello, 2004), likely corresponds to a lability continuum, with BC produced at lower temperatures (<500 °C) and from grasses degrading with shorter C half lives (10²–10⁴ y) than

* Corresponding author. Tel.: +1 352 392 0070; fax: +1 352 392 9294.

E-mail address: azimmer@ufl.edu (A.R. Zimmerman).

those produced at higher temperatures (>500 °C) and from hard woods (10^5 – 10^7 y) (Zimmerman, 2010).

The effect that BC may have on overall carbon cycling when added to a soil or sediment is controversial and not without important and immediate consequences. First, given the expected increase in fire frequency likely to occur with future climate change (IPCC, 2007), an understanding of the effects of BC addition on non-BC soil organic matter (OM) will be needed to model past and future changes in global carbon cycling or climate feedbacks. Second, stimulated by the observation that small plots of anthropogenic Amazon soil, called *terra preta*, are both extremely enriched in BC and highly fertile, there has been growing call to produce BC from organic wastes which can then be added to soils to enhance fertility and mitigate climate change by sequestering CO₂ from the atmosphere (e.g. Glaser et al., 2001; Lehmann et al., 2006). When BC is added to soils (then called biochar), it may be an ecosystem C source or sink, depending upon the nature of the interactions between BC, microbes, and non-BC OM.

'Priming effects', changes in the mineralization of native soil OM due to the addition of new substrates, have been observed in many types of laboratory and field studies and recently reviewed by Kuzyakov et al. (2000). Most commonly, it is 'positive priming' that is observed, i.e. the accelerated mineralization of a more refractory soil OM components when stimulated by the addition of a labile C source, but results are not always straightforward. For example, soil OM decomposition increased 3- to 5-fold or decreased by up to 30% in the presence of plant residues (Bell et al., 2003; Nottingham et al., 2009) or root exudates (Kuzyakov, 2002; Cheng, 2009). Cellulose additions resulted in a 100% increase in the mineralization of even 2500 y old OM from deep soils layers (Fontaine et al., 2007). Positive priming may be a direct effect of increased production of extracellular enzymes due to the added substrate which 'co-metabolize' soil OM, but indirect mechanisms are also possible, such as the stimulation of microbial activity through nitrogen or other nutrient additions or improvement in soils aeration, moisture or structure (Kuzyakov et al., 2000). On the other hand, 'negative priming', defined here (and by Kuzyakov et al., 2000) as any retardation in soil OM mineralization due to any treatment such as the addition of a new substrate, may occur due to the divergence of microbes or their enzymes to the more easily available substrate, or to the inhibition of microbial activity because of some change in the soil environment. Mineral adsorptive protection, often discussed as a soil OM preservation mechanism (e.g. Sollins et al., 1996), could be a form of negative priming if the added substrate contains a sorptive component.

Given its porous nature and high affinity for natural organic matter (Kasozi et al., 2010), it could be hypothesized that BC will sequester non-BC soil OM within its pore network, protecting it from degradation both by microbially-produced enzymes and abiotic oxidants. For example, charred biomass of different types had been shown to sorb a number hydrophobic agrochemicals, resulting in their decreased dissipation (Yang and Sheng, 2003; Cao et al., 2009; Spokas et al., 2009). More hydrophilic natural soil OM components have also been shown to sorb to BC, though to lesser extents, depending upon its charring temperature and the molecular size of the sorbate (Kasozi et al., 2010). Other signs of decreased microbial activity have been observed with addition of certain BC types, such as decreased N₂O production and CH₄ oxidation (Spokas and Reicosky, 2009) and production of the microbial inhibitor, ethylene (Spokas et al., 2010). Alternatively, BC may have a stimulatory effect on soil carbon mineralization. This positive priming could occur if BC acts as a mineralizable C source, and BC amendments may also provide nitrogen, phosphorous and micronutrients (Chan and Xu, 2009) or even a habitat favoring increased microbial heterotrophic activity (Thies and Rillig, 2009).

Positive priming effects on SOC degradation, as well as more labile amended substrates, have also been recorded in the presence of biochar. Wardle et al. (2008) observed greater mass loss from a litterbag containing a mixture of humus and charcoal than would be expected based on mass loss from separate litterbags of humus and charcoal. However, without the presence of soil minerals, the applicability of this finding has been questioned (Lehmann and Sohi, 2008). In another study, Steinbeiss et al. (2009) found an increase in respired CO₂ following addition of glucose-derived biochar to one of two soils tested, but no change to either when using a different biochar type. However, this change was only short term (several weeks) and the possibility of CO₂ degassing by the biochar alone was not examined. The presence of biochar in soils also enhanced the degradation of more labile C sources such as ryegrass residue (Hilscher et al., 2009) and switchgrass residue (Novak et al., 2010). On the other hand, the addition of glucose had a strong stimulatory effect on the oxidation of BC both in carbon-free sand (Hamer et al., 2004) and in soil (Kuzyakov et al., 2009).

In contrast to these findings, other studies have found no influence, or even a negative priming influence, of BC on OM degradation. For example, in three separate studies, after correcting for the CO₂ produced by biochar alone, there was no change in respired CO₂ in a biochar-amended versus biochar-absent loamy-sand (South Carolina, USA, Novak et al., 2010), a silty-loam (Minnesota, USA, Spokas et al., 2009) and a German loam and loess (Kuzyakov et al., 2009). Adding to the perplexity, Hilscher et al. (2009) recorded no increase in respired CO₂ when a Swiss loam was amended with pine wood-derived biochar but enhanced respiration with added grass-derived biochar. And in another study using sixteen chars and two soil types, about a third increased, a third decreased, and a third had no effect on SOC respiration (Spokas and Reicosky, 2009).

Further supporting the contention that BC amendments will enhance SOC preservation rather than encourage its degradation, *terra preta* soil are not only enriched in BC, but have also been found to contain greater non-BC amounts of natural OM compared to surrounding native tropical soils which are typically depleted in SOC (Glaser, 2007; Solomon et al., 2007). In addition, *terra preta* and other BC-containing soils such as those near historical charcoal blast furnace sites exhibit lower soil C respiration rates in incubations than adjacent soils with lower amounts of BC (Cheng et al., 2008b; Liang et al., 2008). Finally, another incubation study of *terra preta* soils found that, not only was 2–3 times less C mineralized in BC-rich versus adjacent BC-poor soils, but there was about 25% less C mineralized from an added labile carbon source (sugar cane leaves) in the former compared to the latter (Liang et al., 2010).

Clearly, a great deal of confusion exists as to the short and long-term effects that biochar amendment will have on soil C cycling and sequestration. Some of the discrepancy between studies that find a positive versus negative priming effect may lie in the materials used, including soil, biochar or priming substrate type, while others may have to do with the method by which the experiment was conducted, including water saturation, atmosphere, or timeframe over which the experiment was carried out. This study was intended to shed light on the interaction mechanisms between soil and biochar that may lead to variability in priming effects and natural variation in C mineralization by comparing C respiration rates among a variety of well-characterized biochars mixed with a range of soil types under constant laboratory incubation conditions. Modeling of the results and that expected from C mineralization by the soil and biochar alone was carried out to better illustrate the trends in soil–biochar priming that occurred during the experiment and to project them into the future.

2. Materials and methods

Five soil types were mixed and incubated with biochar produced from five biomass types under four combustion conditions. In addition, each of the soils and each of the biochar types were incubated separately to serve as controls. Carbon degradation was measured periodically as CO₂ evolution over the course of 505 days.

2.1. Materials

Biochar was produced from three woods: oak (Laurel oak: *Quercus laurifolia* Michx.), pine (Loblolly pine: *Pinus taeda* [L.] and bubinga (the tropical hardwood *Guibourtia demeusei* Harms), and two C4 grasses: Eastern gamma grass (*Tripsacum dactyloides* [L.]) and bagasse (sugar cane following industrial processing by Florida Crystal Corp.). After drying, biomass was cut into 1 × 1 × 5 cm pieces and either combusted in an oven under full atmosphere at 250 °C or pyrolyzed under N₂ at 400, 525 and 650 °C (hereafter designated Oak 250, Oak 400, Pine 650, etc.). The resulting biochars were then lightly crushed and sieved to obtain a uniform 0.25–2 mm particle size.

Details regarding the production and characterization of these biochars have been presented elsewhere (Kasozi et al., 2010; Zimmerman, 2010; Mukherjee et al., in press). Briefly, specific surface area was determined using both N₂ and CO₂ adsorption for nanopores and micropores, respectively. While both increased with combustion temperature, low temperature biochar (made at 250 and 400 °C) had mainly micropores ranging 129–522 m² g⁻¹, while high temperature biochar (made at 525 and 650 °C) possessed both nanopore and micropore-enclosed surfaces (396–627 m² g⁻¹). Volatile matter, the portion of biochar, by weight, that was lost after 6 min combustion at 900 °C (ASTM methods D-1762-84) ranged from 21 to 72% and decreased with biochar production temperature, reflecting progressive loss of its aliphatic and low molecular weight portion during pyrolysis. Correspondingly, elemental C increased and oxygen decreased with biochar combustion temperature.

The five experimental soils were all collected from within the Santa Fe River Watershed, North Florida, and were reported on as part of a separate study on labile soil C pools (Ahn et al., 2009). The soils were chosen to represent a range of chemistries and land use types and include two Alfisols, two Entisols and a Mollisol (Table 1). The soils, collected in Spring 2004 by hand auger and representing the integrated upper 30 cm of the soil column, were air-dried, sieved to <2 mm, and stored dried in the dark prior to chemical characterization and incubation starting Spring 2008 (Table 1). Methods used regarding their collection and characterization, including acid hydrolyzable C content, a measure of the soil OM lability, are detailed elsewhere (Ahn et al., 2009; Vasques et al., 2010).

2.2. Incubations experiment

Incubations of biochar-alone (200 mg quartz sand and 20 mg biochar), soil-alone (1 g), and soil–biochar mixtures (1 g and

100 mg, respectively), were carried out in triplicate for each treatment in sterilized 12 ml borosilicate vials with rubber septa. This soil–biochar mixture, or 10% biochar by weight, corresponds to 90 ton/ha application rate (10 cm tillage) which is in the upper end of application rates currently employed (Gaskin et al., 2010; Jha et al., 2010; Major et al., 2010). A microbial inoculate consisting of a forest soil extract (from within the same watershed) and an NPK nutrient solution similar to that of the soils [60 g of (NH₄)₂SO₄ + 6 g of KH₂PO₄ L⁻¹] was added to the biochar-alone incubations, whereas only distilled water was added to the soils, in each case to bring the soil or soil–biochar mixture to 50% water holding capacity (0.4–0.7 ml) was added to the soil-alone and soil + biochar incubations.

Tubes were incubated in the dark at 32 °C. Oxidation of biochar-C was determined every two weeks during the first three months and monthly thereafter by measuring CO₂ evolution into the vial headspace using an automated CO₂ coulometer (UIC Inc., Joliet, IL). Headspace CO₂ was carried with CO₂-free air into the coulometer during a 5 min flushing time, leaving the vials refilled with CO₂-free air for re-incubation. The analytical detection limit for CO₂ is 0.1 µg C and systems blanks, empty tubes, yielded CO₂ measurements of less than 2 µg for any given time period. Assuming 1:1 CO₂ production to oxygen consumption, O₂ was always in excess.

2.3. Stable carbon isotopic measurements

The carbon stable isotopic signature of CO₂ evolved during ‘early’ (between days 15 and 21) and ‘late’ (between days 542 and 548) stages of the some of the incubations was measured to estimate the fraction of respired CO₂ that was derived from biochar versus soil OM. While ‘early’ C isotopic measurements were made on parallel incubations, ‘late’ C isotopic measurements were made on the incubation tubes following all measurements of CO₂ evolution. Helium (UHP, 2 ml min⁻¹ flow) was used to flush incubation tube headspace gas into a ThermoFinnigan DeltaPlus XL isotope ratio mass spectrometer with a GasBench II universal online gas preparation device. For each sample, the average value of only the last seven of ten injections is reported here, for which the relative standard deviation was always less than 2%. Values are calibrated relative to a CO₂ reference gas and all isotopic ratios are expressed in standard delta notation as δ¹³CO₂ in units of per mil (‰) relative to the Vienna Pee Dee Belemnite (VPDB) standard.

Similar to Liang et al. (2008) and Cheng et al. (2008b), the fraction of evolved C from biochar (*f*_{BC}) and from soil OM (*f*_{SOC}) in the soil–biochar mixtures was calculated using a two-component isotopic mixing model such that:

$$\delta^{13}\text{CO}_{2\text{-measured}} = f_{\text{BC}} * \delta^{13}\text{CO}_{2\text{-BC}} + (1 - f_{\text{BC}}) * \delta^{13}\text{CO}_{2\text{-SOC}} \quad (1)$$

and

$$f_{\text{BC}} + f_{\text{SOC}} = 1 \quad (2)$$

where the carbon stable isotopic ratio of CO₂ derived from biochar (δ¹³CO_{2-BC}) is assumed to be that measured in biochar-only incubations and that attributable to soil OM (δ¹³CO_{2-SOC}) is assumed to be that measured in soil-only incubations (at the corresponding time period: ‘early’ or ‘late’).

The carbon stable isotopic signatures of the total carbon (TC) in soils and biochars were measured on duplicate ground solid samples loaded into tin capsules. After flash combustion in a Carlo Erba NA1500 elemental analyzer, δ¹³C-TC was measured in a ThermoFinnigan 252 mass spectrometer connected via a ThermoFinnigan ConFlo II interface.

Table 1
Location, type and chemical properties of experimental soils used.

Site code	Land use	Soil order	pH	Clay (wt.%)	Sand (wt.%)	C _{organic} (mg g ⁻¹)	Acid hydrolyzable C (mg g ⁻¹)
SF2074	Agriculture	Alfisol	4.7	11.1	84.6	7.1	3.0
SF33	Coniferous pine	Alfisol	3.9	3.1	95.2	13.7	2.1
SF1008	Upland forest	Entisol	5.3	7.8	88.1	17.5	3.7
SF2049	Upland forest	Entisol	5.1	2.2	94.0	20.4	3.8
SF922	Wetland	Mollisol	7.6	12.2	81.4	55.0	8.4

2.4. Statistic and modeling methods

For the sake of clarity, only the means of triplicate analyses of CO₂ evolution are plotted at each time point. For the biochars, relative standard deviation (standard deviation/mean) was always less than 10%, and for biochar + soil combined, always less than 50%, but usually less than 10%. Error in total C mineralization was calculated as the square root of the sum of squares of individual standard deviations whereas error in 'early' and 'late' C mineralization rates was calculated as the 90% confidence interval for the slope of the regression (i.e. product of standard error and critical value at alpha of 0.1). Analysis of variance (ANOVA) within the SAS package was used to determine significant differences between mineralization rates or priming effects among groupings of different sample treatments.

The course of CO₂ evolution from soil, biochar, and soil–biochar mixture incubations was fit to a double-exponential model using the equation:

$$M_t = M_1(1 - e^{-k_1t}) + M_2(1 - e^{-k_2t}) \quad (3)$$

where M_t = C mineralized (mg C g⁻¹); M_1 = amount of relatively labile mineralizable C, M_2 = amount of a relatively refractory mineralizable C, k_1 and k_2 = apparent first order mineralization rate constants for the labile and refractory pools (d⁻¹), respectively, and t = incubation time (d). The Levenberg–Marquardt algorithm was used to estimate the value of the model parameters to minimize the sum of the squared differences between model-calculated and measured values.

The sum of mineralized biochar-C_t and soil-C_t, each scaled to the initial amount of material used, was calculated to predict the amount of C mineralized in each corresponding soil–biochar mixture had no priming or other interaction occurred. This was compared with the measured CO₂ evolution to identify the time–course of priming effects.

3. Results

3.1. Carbon mineralization

Results and discussion of biochar-alone oxidation rate measurement and modeling have been published previously (Zimmerman, 2010). Briefly, biochar-C losses of 3–30 mg C g-

biochar⁻¹ y⁻¹ (1.4% on average) were recorded over a year. Abiotic biochar-C release rates were about 50–90% of those found to occur when amended with soil microbes. Biochars made at lower temperatures were found to degrade at faster rates than higher temperature chars, and biochar made from grasses generally degraded faster than those from hard woods. Tellingly, total degraded biochar C was most strongly directly related to the volatile content of the biochar.

Soil-only cumulative C mineralization rates (measured over 500 d, but normalized to 1 y) ranged 0.8–6.4 mgC g-soil⁻¹ y⁻¹ (Figs. 1 and S1). These C mineralization rates, when normalized to soil organic carbon (SOC) content were more narrowly ranging, 0.10–0.13 mgC mg-SOC⁻¹ y⁻¹ or 10–13% of the C present, suggesting SOC content to be the predominant factor in determining soil-only mineralization rates as has been found previously with a larger soil sample set from this region (Ahn et al., 2009). Although the decrease in C mineralization in soil, biochar and soil–biochar mixtures over time follows double-exponential function (as demonstrated in the following), it can be most simply represented as an 'early' and a 'late' average respiration rate during the first 90 d and the day 250–500 time period, respectively. This is justified given that the correlation coefficient (r^2) for the slope of the cumulative C mineralization versus time plots during these periods was never below 0.95 and usually better than 0.99. While a small pulse of CO₂ release occurred during the first few days, possible due to soil re-wetting as has been observed by others (e.g. Clein and Schimel, 1994; Fierer and Schimel, 2003), the pulse was small and did not affect the linearity of the early average respiration rate. The early soil C mineralization rates were 3–5 times greater than the late mineralization rate (0.8–7.7 mgC g-soil⁻¹ y⁻¹), except in the case of SF33 whose early and late mineralization rates were nearly the same.

Biochar amendments clearly had effects on soil CO₂ evolution which varied both with soil type and biochar type. Complete results from only two of the soils (SF33 and SF922) are presented here (Fig. 1) as these soils sufficiently illustrate the contrasting trends observed. However, the complete data is presented in the Supplementary data section. Cumulative C mineralized from biochar–soil mixtures generally corresponded to the same order of increasing mineralization observed in biochar-alone incubations (Figs. S1 and S2). That is, mineralization generally increased with decreasing combustion temperature and from woods to grasses. An exceptions to this generalizations is that biochar made at 400 °C

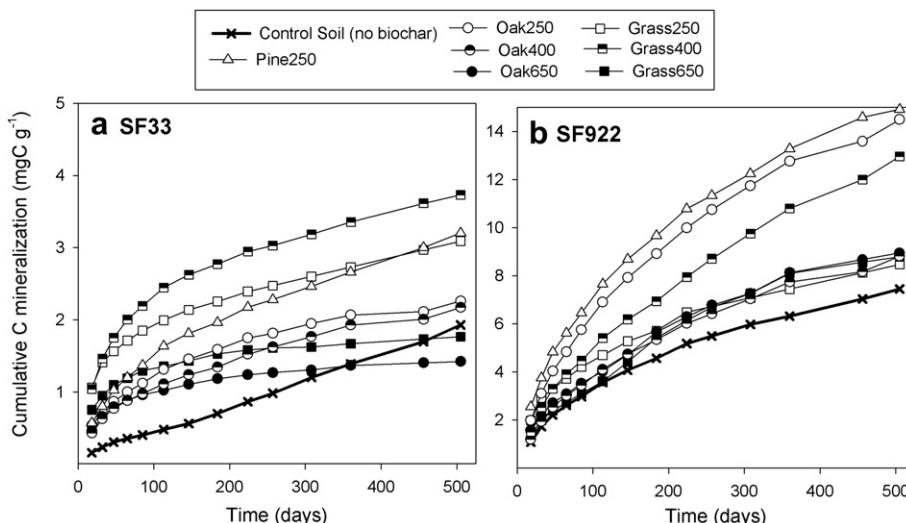


Fig. 1. Cumulative C released (as CO₂) from incubations of a) SF33 and b) SF922 soil alone and mixed with biochars of types shown in legend.

was sometimes more labile than that made at 250 °C, possibly due to the presence of oxygen during production of the latter.

During the early part of the incubations of biochar mixed with either SF33 (low SOC) or SF922 (high SOC), C mineralization rates were greater than that of soil alone (Fig. 1). However, during the later phase of the incubations, some mixture treatments continued to show C mineralization rates greater than that of the soil-alone incubations, while others returned to the same rate or decreased to a lower C mineralization rate than the soil-alone incubations. In SF33 soil, early C respirations rates were 0.7–4 times greater than that of late respiration rates for mixtures with low temperature biochar and 6–12 times greater than late respiration rates for mixtures with high temperature biochar. In SF922 soil–biochar mixtures, however, early C respirations rates were a more consistent 2.5–4.7 times greater than that of late respiration rates for all chars. Late respiration rates in SF33–biochar mixtures ranged from 0.2 to 2.9 times that of SF33 soil alone ($1.3 \text{ g}^{-1} \text{ y}^{-1}$), the slopes shown in Fig. 1) and SF922–biochar mixtures late respiration rates were 0.6–2.4 times that of SF922 soil alone ($2.8 \text{ g}^{-1} \text{ y}^{-1}$).

On a weight basis, biochar–soil mixtures with all but the most refractory biochar types released more CO_2 and had greater initial CO_2 evolution rates than their associated soils without biochar additions. This is most likely because biochar itself, especially freshly made biochar and lower temperature biochar is, itself, somewhat labile and so contributes to the degradative C loss of the mixture. It is important to note, however, that C normalized CO_2 evolution from the mixtures was always less than that of the soil alone (Fig. S2), so net C sequestration was always occurring. Whereas 10–13% of the C was lost from each soil during a year of incubation, soil–biochar mixtures lost an average of 3.4 times less C. Carbon-normalized average C oxidation ranged from 1.2 to 9.5 y^{-1} .

3.2. Indicators of biochar priming effect

To better understand the interactive effects of biochar addition to soil on CO_2 evolution, one can compare evolved CO_2 from soil and biochar separately (additive, no interaction) with that from corresponding biochar and soil mixtures (measured, interactive). In the figures that follow, positive priming is indicated when the measured evolved CO_2 (diagonal striped bars) is greater than the additive evolved CO_2 (wider unpatterned bars) and negative priming is indicated by the reverse. It should be understood, however, that no distinction is made here between priming caused by the influence of biochar on SOC oxidation and the influence of SOC on biochar oxidation (discussed further below).

Among all soil–biochar mixtures examined, priming effect on total C oxidation during the first year ($\text{mg C g}^{-1} \text{ y}^{-1}$) ranged from –52–89% (mean \pm stand deviation = $2 \pm 26\%$, Figs. 2 and S3). Clearly, overall priming direction and magnitude varied greatly with soil and biochar type. One apparent trend, however, is that, for a given biochar biomass type, priming effect on total C oxidation generally decreased with increasing combustion temperature. For 250, 400, 525 and 650 °C biochar, the average priming effect over one year was 16, 9, 5, and –12, respectively. In addition, negative priming was more prevalent in the two soils with the lowest SOC and least potentially mineralizable SOC (acid hydrolyzable C), SF33 and SF2074. Both of these soils happened to be Alfisols but had very different clay contents.

One reason it may be difficult to discern trends in total C oxidation and priming, however, is that these parameters integrate processes occurring both early and later in the incubation period, that is, during degradation of more labile and more refractory components of both biochar and soil OM. Thus, for each soil, an ‘early’ and a ‘late’ additive (soil-alone + biochar-alone)

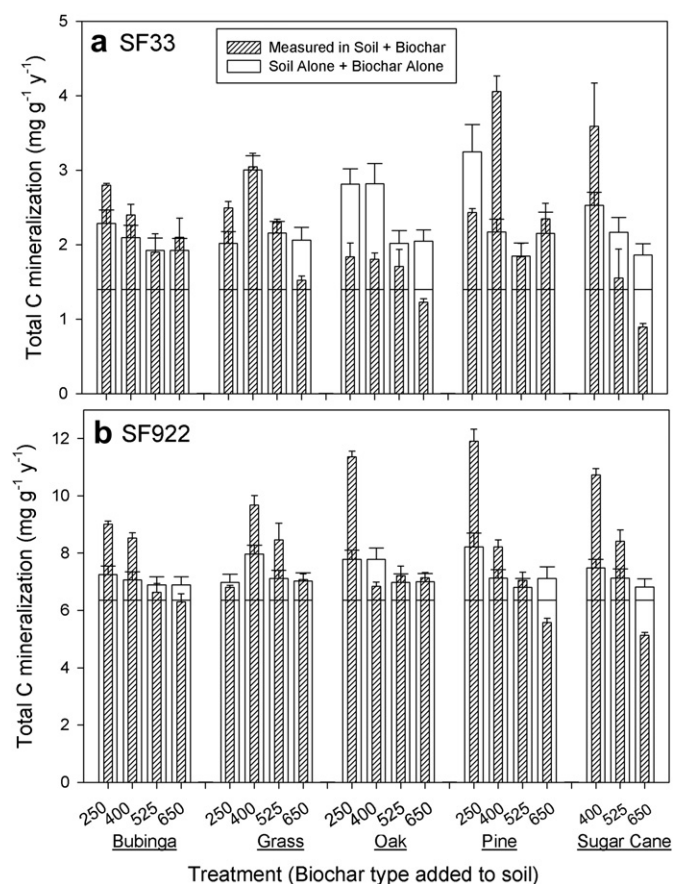


Fig. 2. Total C released (as CO_2) in 1 y incubations of biochar mixed with a) SF33 and b) SF922 soil (striped bars) compared with that calculated had C mineralization from soil (bottom portion of open bars) and biochar (top portion of open bars) been additive (i.e. no priming).

mineralization rate can be calculated and compared to that measured during each of these periods (Fig. 3 for SF3, Fig. 4 for SF922, Fig. S4 for SF2047, Fig. S5 for SF2059 and Fig. S6 for SF1008). Much as for total mineralization, in all soil, whether early or late in the incubation period, low temperature biochars tended to exhibit relatively more positive priming of its C mineralization rate compared to high temperature biochars. The two soils with the lowest SOC, SF33 and SF2074, exhibited significantly more negative priming of its mineralization rate during the late versus early portion of the incubation ($p < 0.05$, Figs. 3 and S4). For SF33, the average measured early mineralization rate was 2.3% greater than the predicted additive rate (–2.3% priming effect), on average, while that of the late mineralization rate was 31% less. For SF2074, mineralization rate priming effect was –8.3% early and –38% late, and for SF1008, +12% early and –15% late. In contrast, C mineralization rate priming effect for SF922 and SF2049 was not large and did not vary greatly with incubation stage; –1.1% early and –8.1% late for the former and –6.4% early and –6.9% late for the latter.

3.3. Carbon mineralization modeling

Although logic and previous work (Zimmerman, 2010) would dictate that both biochar and soil OM is composed of many, even infinite, OM fractions, each with their own degradation rate, the modeling carried out here demonstrates that the assumption of only two mineralizable OM fractions, one relatively labile and one refractory, is a reasonable approximation for data modeling

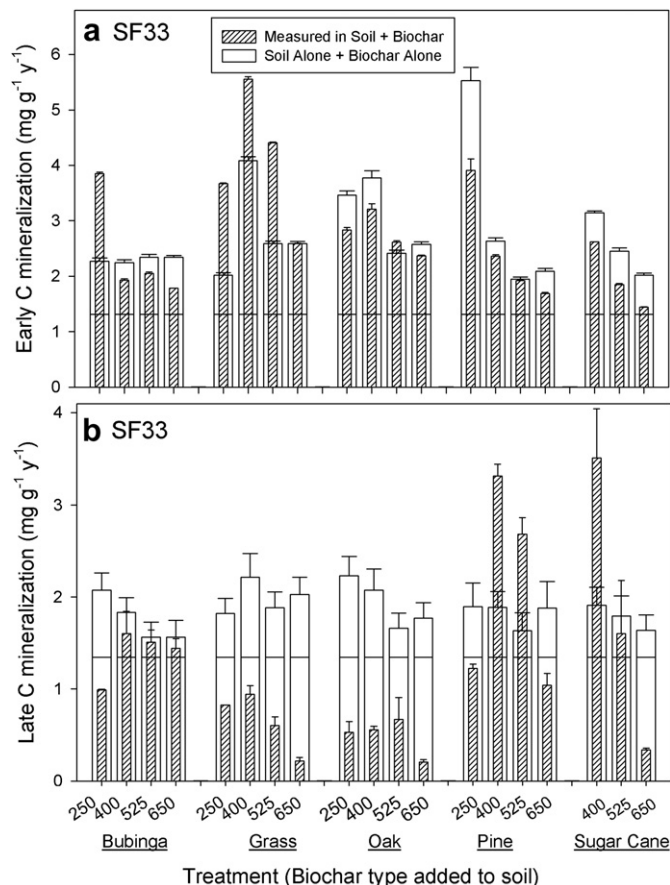


Fig. 3. C mineralization rate in incubations of SF33 soil mixed with biochars during a) the first 90 d ('early') and b) day 250–500 ('late') compared with that calculated had C mineralization rates from soil (bottom portion of open bars) and biochar (top portion of open bars) been additive (i.e. no priming).

purposes. The kinetics of CO₂ evolution from all incubations of soil, biochar, and soil-biochar mixtures were well-fit to the double-exponential model, with least-square correlation coefficients (r^2) of 0.97 or greater in all cases. All biochars and soils had much greater portions of potentially mineralizable OC assigned to the refractory versus labile pool (M_2 versus M_1 in Table S1), and this was usually the case for the biochar–soil mixtures as well. Half lives of C in the mineralizable labile pools in soil + biochar mixtures ranged widely from 2 to 462 d, as did those of C in the mineralizable refractory pools, 110 d to 76 y. This is to be expected given the broad range of soils and biochar types used in these experiments.

For each soil and biochar combination, a predicted CO₂ evolution time–course was calculated as the sum of CO₂ evolved from soil and biochar, separately, over a range of times, using the double-exponential model. These time–courses of additive C mineralization, which assume no soil–biochar interaction, were compared to those actually measured in the soil–biochar mixture incubations. Examples of these comparisons, shown for SF33 (Fig. 5a,b, and c) and SF922 (Fig. 5d,e, and f) with grass chars, express representative trends. Additional examples are provided in the Supplementary data section (Figs. S7, S8, S9, S10 and S11). For SF33 and other low OC soils (such as SF2074 and possibly SF1008), C mineralization priming is initially positive but becomes negative after some time. The crossover time decreases with biochar recalcitrance, from about 600 d to 375 d to 252 d for grass 250, 400 and 650 °C biochar, respectively, in SF33 soil (Fig. 5a,b, and c). On the other hand, for biochar mixtures with SF922 and other high OC soils (such as

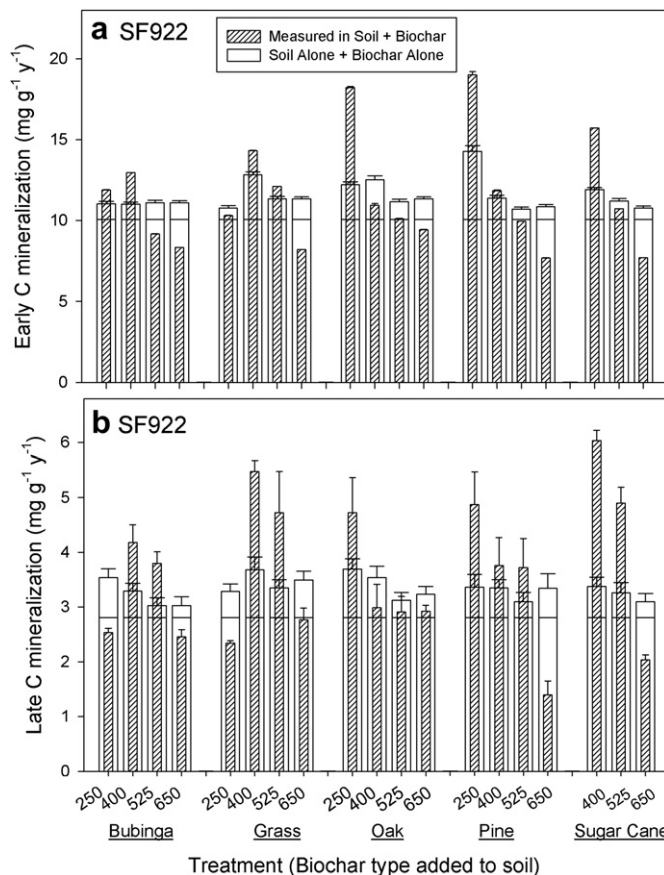


Fig. 4. C mineralization rate from incubations of SF922 soil mixed with biochars during a) the first 90 d ('early') and b) day 250–500 ('late') compared with that calculated had C mineralization rates from soil (bottom portion of open bars) and biochar (top portion of open bars) been additive (i.e. no priming).

SF2049), additive and measured C mineralization time–course plots were rather similar (Fig. 5d) or priming remained positive though the incubation period (Fig. 5e and f). It can also be seen that when the 'late' mineralization rate indicated positive or negative priming (Figs. 3b and 4b), the modeling results generally indicated persistence of these trends further into the future. Thus the 'late' mineralization rate may be good indicators of long-term priming effect.

3.4. Carbon stable isotopes/mixing model

After examining soil and biochar-C mineralization rates, a few representative soils (SF33 and SF922) and biochars (Oak and Grass) were chosen for the $\delta^{13}\text{C}_{\text{CO}_2}$ measurement. Both soils produced CO₂ with early and late $\delta^{13}\text{C}_{\text{CO}_2}$ values of about -27 and -24‰ , respectively, indicating a soil OM composition (at least the labile portion) derived primarily from C3 plants (Table 2). Enrichment in the $\delta^{13}\text{C}_{\text{CO}_2}$ of C respired over time from soils is commonly observed (Schweizer et al., 1999; Crow et al., 2006; Pendall and King, 2007) and is explained by the progressive utilization of increasingly greater proportions of refractory and ^{13}C -enriched soil C sources over time. For example, the heavy mineral-associated, and therefore, more processed portions of soil OM have been found to be isotopically enriched (Six et al., 2001; Fernandez et al., 2003; Crow et al., 2006).

The $\delta^{13}\text{C}_{\text{CO}_2\text{-BC}}$ of the pure oak and grass biochars reflected their C3 and C4 plant origins, respectively, and also became more

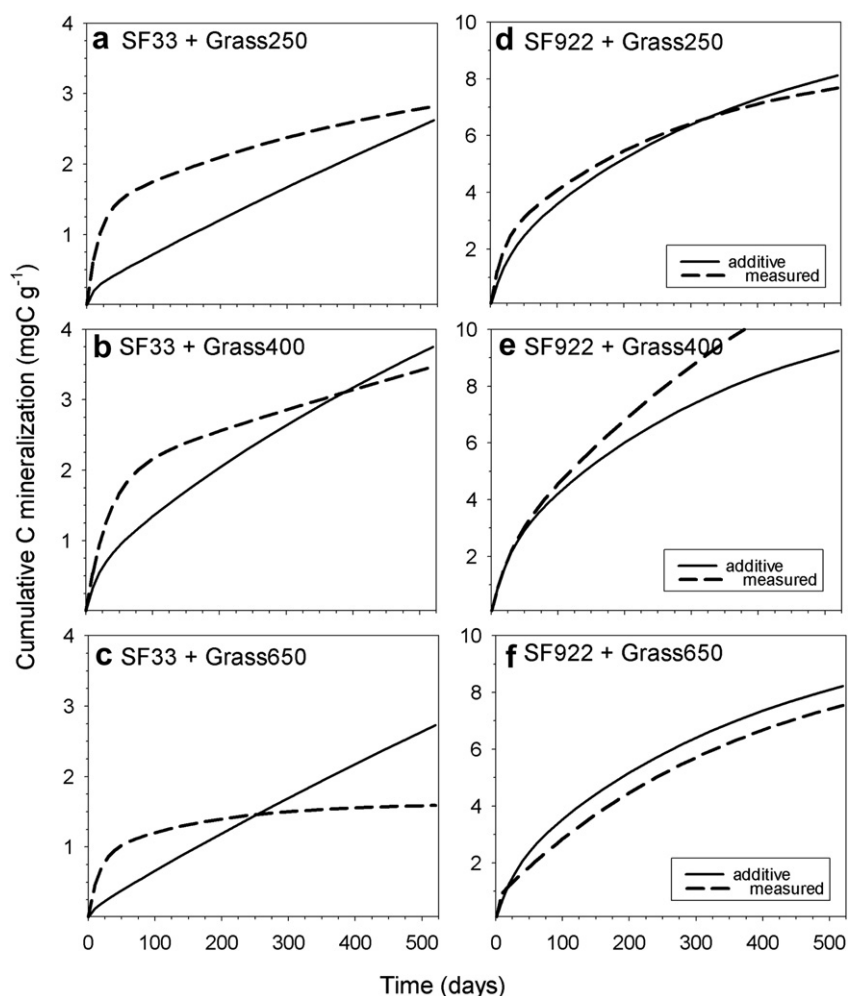


Fig. 5. Dual-exponential model of measured C mineralized (as CO_2) and that calculated had C mineralization from soil and biochar been additive (i.e. no priming) from mixtures of SF33 soil with a) Grass 250, b) Grass 400 and c) Grass 650 biochar and SF922 soil with d) Grass 250, e) Grass 400 and f) Grass 650 biochar.

enriched by 0.6–7.6‰, between the ‘early’ and ‘late’ incubations periods. The enrichment in biochar- ^{13}C that occurred with increasing combustion temperature supports the notion that biochar, itself, is composed of labile and refractory portions, the former

of which is more volatile and ^{13}C depleted and is progressively lost at higher combustion temperatures (Zimmerman, 2010).

Because $\delta^{13}\text{C}_{\text{CO}_2\text{-BC}}$ of oak biochars (–30 to –22‰) were in the same range as the $\delta^{13}\text{C}_{\text{CO}_2\text{-SOC}}$, the isotopic mixing model could not

Table 2

Stable carbon isotopic signature of soil and biochar total C and of CO_2 evolved from soil, biochar, and soil-biochar during early and later stages of incubation.

Incubation type	$\delta^{13}\text{C}\text{-TC}^a$ (‰)	$\delta^{13}\text{C}$ (early ^b) (‰)	f_{BC}^c (early)	$\delta^{13}\text{C}$ (late ^b) (‰)	f_{BC}^c (late)
SF33 soil	-26.5 ± 0.5	-27.0 ± 0.4	–	-24.4 ± 0.1	–
SF922 soil	-25.2 ± 0.4	-27.1 ± 0.1	–	-23.4 ± 0.3	–
Oak 250 biochar	-25.2 ± 0.1	-29.8 ± 0.3	–	-28.4 ± 1.0	–
Oak 400 biochar	-26.3 ± 0.1	-27.0 ± 0.4	–	-22.2 ± 1.2	–
Oak 650 biochar	-26.5 ± 0.1	-25.8 ± 0.1	–	-18.2 ± 0.1	–
Grass 250 biochar	-13.7 ± 0.3	-17.1 ± 1.5	–	-16.5 ± 0.7	–
Grass 400 biochar	-14.2 ± 0.0	-14.8 ± 0.9	–	-11.4 ± 0.4	–
Grass 650 biochar	-14.6 ± 0.1	-12.0 ± 1.2	–	-10.8 ± 0.4	–
SF33 + Grass 250	–	-17.4 ± 0.3	0.97 ± 0.03	-17.7 ± 1.8	0.84 ± 0.23
SF33 + Grass 400	–	-18.3 ± 0.8	0.72 ± 0.07	-18.7 ± 2.0	0.44 ± 0.16
SF33 + Grass 650	–	-20.2 ± 0.2	0.45 ± 0.01	-21.9 ± 0.1	0.18 ± 0.01
SF922 + Grass 250	–	-18.8 ± 0.6	0.83 ± 0.06	-20.1 ± 1.4	0.48 ± 0.21
SF922 + Grass 400	–	-22.6 ± 0.7	0.37 ± 0.06	-21.7 ± 0.4	0.14 ± 0.03
SF922 + Grass 650	–	-26.7 ± 0.1	0.03 ± 0.01	-22.1 ± 1.1	0.11 ± 0.09

^a Measured on duplicate ground solid samples (mean \pm standard deviation).

^b Measured on CO_2 evolved from incubations between days 15 and 21 (early) and days 543 and 548 (late). Mean \pm standard deviation of duplicate incubations.

^c Fraction of CO_2 calculated to be derived from biochar based upon two-component isotopic mixing model presented in text.

be used to differentiate respired C derived from soil versus from oak-derived biochar. Most CO₂ respired from oak biochar-soil mixtures were in the range of –27 to –23‰. However, the two-component model could be used with grass biochar–soil mixtures and illustrated clear trends. First, the fraction of respired C that could be attributed to biochar (f_{BC}), as opposed to soil OM, decreased with increasing combustion temperature of the chars with which the soil was mixed. Second, biochar was responsible for a greater portion of the respired CO₂ in early versus late incubation stages, by up to 100% more. Notably, the SF922 + Grass 650 incubation had very low f_{BC} during both early and late stages. Lastly, biochar was responsible for a greater portion of the respired CO₂ in when mixed with SF33 compared to SF922.

Using this data, the C mineralized by the biochar and soil components of grass biochar-soil mixtures (measured) was calculated and compared to that of soil-alone and biochar-alone incubations (Table 3). Measured mineralization rates greater than predicted indicate positive priming of a specific C source, whereas measurements of mineralized C lower than that predicted indicate negative priming. In general, low temperature (250 and 400 °C) biochar-C mineralization was positively primed during early incubation. Grass 650 biochar-C mineralization was reduced by its interaction with soil during both early and late incubation stages as was Grass 400 biochar-C during late stages. During the early incubation stage, the presence of Grass 250 biochar strongly reduced SOC mineralization in both soils, whereas other grass biochars had little effect on SOC respiration. During the late incubation stage, however, SOC mineralization was reduced (by 12–90%) due to its interaction with biochar in all cases except that of Grass 400 biochar in SF922 soil.

4. Discussion

There are a number of caveats that must be made when interpreting the data. First, the biochar inoculum from a forest soil may have had a different microbial population than the native population in each of the different soils. However, microbial activity in different soil types is often compared, though the microbes present may differ, and it is simply assumed that enough microbial diversity is present so that so that resources will be used to the fullest extent. Second, though each incubation was moistened to 50% water holding capacity, each soil and biochar had different textures such that water availability and resulting redox conditions could have varied among samples. With this said, however, consistent difference occurred across soil and biochar types which can offer insight into C dynamics in soil–biochar mixtures.

Table 3
Comparison of measured (includes priming) and predicted (neglects priming) C mineralization rates (in mgC g⁻¹ y⁻¹) attributable to biochar and soil organic carbon (OC) in incubations of biochar + soil.

Incubation type (soil + biochar)	Early incubation				Late incubation			
	Biochar C min.		Soil OC min.		Biochar C min.		Soil OC min.	
	Meas. ^a	Pred. ^b	Meas.	Pred.	Meas.	Pred.	Meas.	Pred.
SF33 + Grass 250	3.6	0.7	0.1	1.3	0.7	0.5	0.1	1.3
SF33 + Grass 400	4.0	2.8	1.6	1.3	0.4	0.9	0.5	1.3
SF33 + Grass 650	1.2	1.3	1.4	1.3	0.0	0.7	0.2	1.3
SF922 + Grass 250	8.6	0.7	1.8	10.1	1.1	0.5	1.2	2.8
SF922 + Grass 400	5.3	2.8	9.0	10.1	0.8	0.9	4.7	2.8
SF922 + Grass 650	0.2	1.3	8.0	10.1	0.3	0.7	2.5	2.8

^a Meas. = Measured total C mineralization rate $\times f_{BC}$ for biochar or $(1 - f_{BC})$ for soil (includes priming effect). 'Early' and 'late' refer to the first 90 d and the day 250–500 time periods, respectively.

^b Pred. = Predicted C mineralization from biochar-alone or soil-alone incubations (neglects priming effect).

The overall finding of this work is that black carbon (biochar) and soil OM interact when combined such that C mineralization (evolved CO₂) from each component is not additive. However, both the direction and magnitude of the priming effect observed varied with biochar type, soil type, and incubation stage. Generalized observations are enumerated here followed by proposed explanatory mechanisms.

- 1) Priming effect following the addition of biochar to soil tended to decrease from early to late biochar incubation, sometimes even swinging from positive to negative priming. This suggests that at least two types of interaction are at play, i.e. two different priming mechanisms. Based on the isotopic results, which indicate a loss of biochar-C during the early stages and a loss of SOC during the late stages of the incubation, we suggest that both SOC and biochar can act as priming agents: SOC may prime biochar decomposition initially while biochar may be responsible for retarding SOC decomposition in a later stage.
- 2) During early incubation, the magnitude of priming decreased, sometimes from positive to negative, from biochars made at lower to higher combustion temperatures and from those made from grass to pine to oak. This order mirrors that of decreasing biochar lability (Zimmerman, 2010), suggesting that it is only the more labile component of biochar whose microbial respiration is primed by the presence of soil OM. Biochar C mineralization has been found to be related to its volatile matter composition (Deenik et al., 2010; Zimmerman, 2010), thus identifying this component of biochar as microbially utilizable.
- 3) Biochar–soil priming was generally more pronounced, that is more positive (or less negative) early and more negative later, in SF33 and SF2074 soils (and perhaps SF1008) versus in SF922 and SF2049 soils. The most obvious factor distinguishing the priming behavior of these two soil groups is the lower SOC content (and mineralizable C content) of the former soils compared with the latter.

4.1. Biochar–soil early interaction mechanism – positive priming

The observation of early positive priming of biochar mineralization by soil seems consistent with the many previous studies that have seen rapid increases in mineralization after addition of a labile substrate to soil (e.g. Chotte et al., 1998; De Nobili et al., 2001; Hamer et al., 2004; Hamer and Marschner, 2005). The mechanism most often proposed involves growth of so-called 'r-strategist' microbes that are adapted to respond quickly to newly available C sources, remineralizing soil nutrients and co-metabolizing more refractory OM such as soil humic materials in the process (Kuzakov et al., 2000; Kuzakov, 2010). On the other hand, occasional findings of greater positive priming effect in response to additions of more complex substrates such as cellulose or straw (Wu et al., 1993; Shen and Bartha, 1997) implicate 'K-strategists', those that are continuously active soil OM feeders, which are more likely to co-metabolize OM substrates through their release of extracellular enzymes needed to breakdown complex biopolymers. And different soils have been found to be primed differently in response to repeated additions of different substrates (Hamer and Marschner, 2005). Fontaine et al. (2003) proposed that the priming effect is much more complicated, resulting from a balance between r- and K-strategists, depending upon the specific substrate and nutrient status of the system and the microbial community present.

Biochar mineralization has previously been found to be, at times, positively primed by the addition of a labile C source (Hamer

et al., 2004), soil humus (Wardle et al., 2008) and whole soil (Kuzyakov et al., 2009). Most of these findings are in line with r-strategist theory. That is, immediate increase in biochar decomposition was observed after the addition of glucose or other labile substrate which lasted a few days to weeks and has been attributed to co-metabolism (Hamer et al., 2004; Kuzyakov et al., 2009). And the addition of a more complex substrate, cellulose, did not induce a priming effect in soils mixed with a biochar (Nocentini et al., 2010).

The findings of this study are concordant with the r-strategist view in some respects and that of Fontaine et al. (2003) and others. As to be expected from an r-strategist response, positive priming was, in most cases, observed immediately when soils and biochar were combined. Greater priming effect was generally observed when soils were combined with the more labile biochars, i.e. those produced at lower temperatures and from grass. The biochars expected to increase microbial habitability more generally, i.e. the higher temperature and oak biochars due to their greater surface area and porosity and their greater tendency to increase pH (Zimmerman, 2010), did not produce as great a priming effect. On the other hand, positive priming often persisted over a few months and not the few days or weeks expected with r-strategist response. In some cases, soil C respiration rates were similar to biochar-C respiration rates, making it impossible to say which material was priming the other. And soils with lower OM biodegradability (SF33 and SF2049 with the lowest acid hydrolyzable C/SOC ratio, Table 1) often displayed the strongest early positive priming effect, a result not predicted by r-strategist theory. Lastly, it should be noted that all biochars leach both nitrate and phosphate into water, more so early and for the lower temperature chars. Thus increased nutrient availability is another possible explanation for the positive priming observed. And both microbial abundance and diversity have been shown to be altered differently in different types of chars and soil (Khodadad et al., 2011). All of these observations suggest a much more complicated biochar–soil OM–microbial interaction, not likely reducible to a single mechanism.

4.2. Biochar–soil later interaction mechanism – negative priming

We hypothesize that the negative priming observed, i.e. the repression of soil C mineralization through the interaction of soil and biochar, which appeared predominantly during the later incubation period, was caused by soil OM sorption to biochar, either within biochar pores or onto external biochar surfaces. The former can be termed encapsulation and would exclude biota and their extracellular enzymes from access to the OM within biochar pores. The latter has been called sorptive protection (Kaiser and Guggenberger, 2000). Both have been shown to strongly inhibit the degradation of OM sorbed onto silicate minerals (Zimmerman et al., 2004; Mikutta et al., 2007; Cheng and Reinhard, 2008) as well as carbonaceous sorbents (Yang et al., 2009).

While all BC has been shown to be highly sorbing of OM (e.g. Cornelissen et al., 2005; Sobek et al., 2009), the higher temperature biochars and the grass biochars used in these experiments have been shown to have relatively greater adsorption affinity for natural OM (Kasozi et al., 2010). This would explain their greater suppression of SOC mineralization. In addition, OM sorption onto biochar surfaces has been shown to be kinetically limited by slow diffusion into the sub-nanometer sized pores dominating biochar surfaces (Kasozi et al., 2010), thus suggesting an explanation for why the negative priming only became predominant later in the incubation period. For soils with lower OM contents (SF33 and SF2074), a greater portion of total mineralizable soil OM may have been sorbed onto the biochar surfaces and made unavailable, thus the negative priming effect was more strongly expressed for these

soils. Similarly, addition of labile OM to soils of low OC content have been shown to result in less additional mineralization than when added to soils rich in OM (Kimetu et al., 2009), apparently due to an upper limit to the amount of OM that can be physically protected.

At least in the case of grass biochar mixed with soil, suppression of SOC mineralization occurred with all the chars tested. However, repression of pyrogenic carbon mineralization also commonly occurred with higher temperature chars. A possible reason for this is that sorbed soil OM can cover surfaces and block biochar pore openings (Pignatello et al., 2006; Kasozi et al., 2010), thus blocking enzymes from accessing much of the biochar's surface and blocking desorption of biochar components into the solution phase, either of which would suppress oxidative degradation of biochar-C.

Besides encapsulation and sorptive protection, additional mechanisms may explain at least some of the observed negative priming by biochar. First, sorption of microbial produced enzymes by biochar's 'external' surface or by desorbed biochar components may lead to their deactivation or activity suppression much as is observed for enzymes sorbed to many mineral surfaces or to natural OM (Zimmerman and Ahn, 2010). These changes in enzyme activity have been attributed to changes in pH or ionic strength that an enzyme experiences close to a mineral's surface, enzyme conformational changes upon sorption, or to steric hindrance (Quiquampoix et al., 1995; Quiquampoix and Burns, 2007). Second, biochar may encourage the formation of soil mineral aggregates which could, potentially, protect both pyrogenic C and SOC from degradation (Jastrow, 1996; Jastrow et al., 2007). Biochar has been found to be enriched in the organo-mineral fraction of soil (Brodowski et al., 2006; Liang et al., 2008) and it has been proposed that Ca^{+2} bridging stabilizes biochar through organo-mineral interaction (Czimczik and Masiello, 2007). Toxicity of the biochar to microorganisms is another possible negative priming mechanism. For example, biomass combustion products contain many toxic substances ranging from dioxins, furans, phenols and poly aromatic hydrocarbons to ethylene (Fierer et al., 2001; Fernandes and Brooks, 2003; Kim et al., 2003; Spokas et al., 2010), some of which have specifically been shown to reduce microbial activity (Porter, 1992; Jackel et al., 2004; Liu et al., 2009). However, if the greatest toxicity is found among volatile organic compounds (which is not necessarily the case), one would expect these effects to appear early and most strongly with low temperature biochars, which was the opposite of the experimental observations. On the other hand, both inhibitory and stimulatory effects have been found to be associated with volatile organic substances of many types, but these effects are far from well-understood (Insam and Seewald, 2010).

Lastly, it should be recognized that the change in priming effects from positive to negative may be related to the evolution of the physical properties of biochar that are known to occur over time. For example, the surface charge of biochar may switch from positive to negative as the surface oxidizes (Cheng et al., 2008a; Cheng and Lehmann, 2009) and functional group chemistry changes occur both with biotic and abiotic oxidation (Liang et al., 2006, 2008) and as soil OM sorbs to its surfaces. For example, only negative priming was observed when a labile OM source was added to a soil containing only highly weathered biochar (Liang et al., 2010). And Singh et al. (2010) observed that biochar added to soil had a positive effect on N_2O emission initially, but switched to an inhibitory effect after about a month. They attributed this to increased N sorption capacity of the chars after oxidative reactions. Other changes can be expected to occur with leaching or alteration in soil moisture regime, though tests for these effects on microbial processes and the possible influence of plant roots and macrobiota should be carried out under field conditions.

5. Conclusion

These experiments suggest possible mechanisms that may explain the seemingly contradictory results of previous works, showing biochar to have a positive priming effect in some cases and a negative one in others. That is, the biochar type, the soil type, and the period over which measurements are made, can strongly influence the direction and magnitude of priming effect recorded. While both positive and negative priming effects were observed in these incubations of biochar and soil, it is negative priming, that is, the enhanced storage of both biochar-C and SOC, which is expected to endure into the future. This is suggested by 1) the observation of negative priming during later incubation stages, 2) the modeling results showing that crossover to negative priming eventually occurs in most cases and increases in magnitude into the future, and 3) the mechanistic theory presented and supported by evidence which suggests that soil OM is progressively sorbed onto biochar surfaces and within pores where it is protected from degradation. The findings of a recent modeling study (Woolf et al., 2010) reported that biochar amendments to soil, when carried out sustainably, may annually sequester an amount of C equal to 12% the current anthropogenic CO₂ emissions. This estimate may have to be revised upward after taking into account the long-term effect of SOC mineralization repression that most biochar has on at least some soil OM.

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Appendix. Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.soilbio.2011.02.005.

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Supplementary Information:

Title: Positive and negative C mineralization priming effects among a variety of biochar-amended soils

For [Soil Biology & Biochemistry](#)

Andrew R. Zimmerman
Department of Geological Sciences, University of Florida
241 Williamson Hall
PO Box 112120
Gainesville, FL 32611-2120
Office: (352) 392-0070
azimmer@ufl.edu
Fax: (352) 392-9294

Bin Gao, Department of Agricultural and Biological Engineering, University of Florida, Gainesville, FL 32611

Mi-youn Ahn, Soil and Water Science Department, University of Florida, PO Box 110290, Gainesville, FL 32611

Table S1. Pool sizes and decay rates of soil, biochar, and soil-biochar mixtures using double-exponential decay model

Incubation		Decay Model Parameters ¹			
		k ₁ (d ⁻¹)	M ₁ (mgC/g)	k ₂ (d ⁻¹)	M ₂ (mgC/g)
Soil Type	Biochar Type				
-	Bubinga250	0.1100	1.4	0.0014	17.0
-	Bubinga400	0.0630	1.8	0.0021	8.9
-	Bubinga650	0.0530	1.8	0.0048	4.0
-	Grass250	0.0980	1.3	0.0018	9.2
-	Grass400	0.0510	4.6	0.0029	17.4
-	Grass525	0.0340	2.2	0.0003	6.1
-	Grass650	0.0090	1.0	0.0009	19.7
-	Oak250	0.0440	2.2	0.0028	17.9
-	Oak400	0.0540	3.4	0.0037	14.3
-	Oak525	0.0410	1.8	0.0033	6.2
-	Oak650	0.0310	2.4	0.0003	43.4
-	Pine250	2.4500	1.6	0.0099	17.0
-	Pine400	0.0200	1.9	0.0009	20.2
-	Pine400	0.0320	2.2	0.0006	18.3
-	Pine525	0.0600	1.1	0.0016	7.5
-	Pine650	1.8000	0.6	0.0028	10.5
-	SugCane250	0.5100	1.0	0.0052	8.5
-	SugCane400	0.0690	2.7	0.0040	11.0
-	SugCane525	0.1200	0.7	0.0040	9.1
-	SugCane650	0.0460	1.3	0.0027	4.8
SF2074	-	0.0052	2.2	0.0001	18.5
SF2074	Bubinga650	0.0710	0.3	0.0019	3.2
SF2074	Grass400	0.0460	1.2	0.0034	3.2
SF2074	Grass525	0.0330	1.5	0.0002	16.1
SF2074	Grass650	0.0420	1.8	0.0002	10.0
SF2074	Oak250	0.0180	1.7	0.0005	11.9
SF2074	Oak400	0.0230	1.3	0.0004	9.1
SF2074	Oak525	0.0210	1.5	0.0002	13.8
SF2074	Oak650	0.0480	0.9	0.0031	1.8
SF2074	Pine650	0.0690	2.0	0.0001	18.1
SF33	-	0.1500	0.1	0.0001	39.7
SF33	Bubinga250	0.0190	1.1	0.0001	25.1
SF33	Bubinga400	0.0340	0.3	0.0001	36.1
SF33	Bubinga525	0.0040	0.6	0.0007	7.0
SF33	Bubinga650	0.0015	0.2	0.0015	4.9
SF33	Grass250	0.0600	1.3	0.0021	2.2
SF33	Grass400	0.0300	1.9	0.0003	9.4
SF33	Grass525	0.0250	1.6	0.0001	20.6
SF33	Grass650	0.0670	0.8	0.0063	0.8
SF33	Oak250	0.0750	0.5	0.0047	1.7
SF33	Oak400	0.0280	1.2	0.0002	8.3
SF33	Oak525	0.0540	0.5	0.0027	2.0

SF33	Oak650	0.0290	0.9	0.0004	2.3
SF33	Pine250	0.0270	1.0	0.0009	5.1
SF33	Pine400	0.0035	0.0	0.0009	14.0
SF33	Pine525	0.0039	0.0	0.0001	88.4
SF33	Pine650	0.0023	0.0	0.0022	4.2
SF33	SugarCn400	0.0083	0.1	0.0008	13.2
SF33	SugarCn525	0.0860	0.3	0.0000	141.6
SF33	SugarCn650	0.0320	0.5	0.0002	5.0
SF1008	-	0.0190	0.5	0.0000	16.4
SF1008	Bubinga650	0.0120	0.7	0.0001	14.9
SF1008	Grass400	0.0450	1.1	0.0030	2.8
SF1008	Grass525	0.0420	1.2	0.0035	2.4
SF1008	Grass650	0.0370	1.3	0.0001	14.5
SF1008	Oak250	0.0370	1.2	0.0004	11.7
SF1008	Oak400	0.0230	1.3	0.0002	7.8
SF1008	Oak525	0.0230	1.2	0.0002	11.4
SF1008	Oak650	0.0280	1.1	0.0001	9.8
SF1008	Pine650	0.0390	0.3	0.0021	1.7
SF2049	-	0.0220	1.1	0.0001	16.6
SF2049	Bubinga250	0.0095	2.3	0.0002	14.2
SF2049	Bubinga400	0.0150	1.3	0.0002	16.2
SF2049	Bubinga525	0.0170	1.1	0.0001	18.4
SF2049	Bubinga650	0.0440	0.7	0.0031	1.8
SF2049	Grass250	0.0620	1.7	0.0032	3.4
SF2049	Grass400	0.0590	1.3	0.0013	9.7
SF2049	Grass525	0.0270	2.1	0.0005	8.8
SF2049	Grass650	0.0560	1.4	0.0033	2.0
SF2049	Oak250	0.0150	2.1	0.0002	29.8
SF2049	Oak400	0.0390	1.1	0.0027	3.0
SF2049	Oak525	0.0410	0.8	0.0035	2.5
SF2049	Oak650	0.0560	0.9	0.0044	2.1
SF2049	Pine250	0.0430	1.2	0.0009	9.7
SF2049	Pine400	0.0420	1.0	0.0009	4.7
SF2049	Pine525	0.0120	1.4	0.0001	13.4
SF2049	Pine650	0.0580	0.6	0.0030	2.6
SF2049	SugarCn400	0.0250	1.6	0.0003	8.3
SF2049	SugarCn525	0.0700	0.8	0.0018	3.9
SF2049	SugarCn650	0.0530	0.9	0.0001	15.1
SF922	-	0.0400	1.4	0.0028	7.8
SF922	Bubinga250	0.0530	2.2	0.0037	7.0
SF922	Bubinga400	0.0400	1.5	0.0025	12.0
SF922	Bubinga525	0.0520	1.3	0.0014	13.3
SF922	Bubinga650	0.1200	0.9	0.0034	7.7
SF922	Grass250	0.0640	2.1	0.0035	6.6
SF922	Grass400	0.0380	2.0	0.0016	17.7
SF922	Grass525	0.0510	1.6	0.0017	14.8
SF922	Grass650	0.3700	0.7	0.0033	9.2
SF922	Oak250	0.0410	2.0	0.0033	13.7
SF922	Oak400	0.0410	1.3	0.0030	8.5

SF922	Oak525	0.0790	0.9	0.0033	9.2
SF922	Oak650	0.1100	1.1	0.0032	8.8
SF922	Pine250	0.0440	2.8	0.0032	13.5
SF922	Pine400	0.0540	1.3	0.0030	10.7
SF922	Pine525	0.0470	1.4	0.0020	11.1
SF922	Pine650	0.1300	1.0	0.0047	5.6
SF922	SugarCn400	0.0410	2.1	0.0017	19.2
SF922	SugarCn525	0.0650	1.5	0.0013	17.9
SF922	SugarCn650	0.0640	1.3	0.0034	5.5

1. M_1 = amount of relatively labile mineralizable C, M_2 = amount of a relatively refractory mineralizable C, k_1 and k_2 = apparent first order mineralization rate constants for the labile and refractory pools (d^{-1}), respectively.

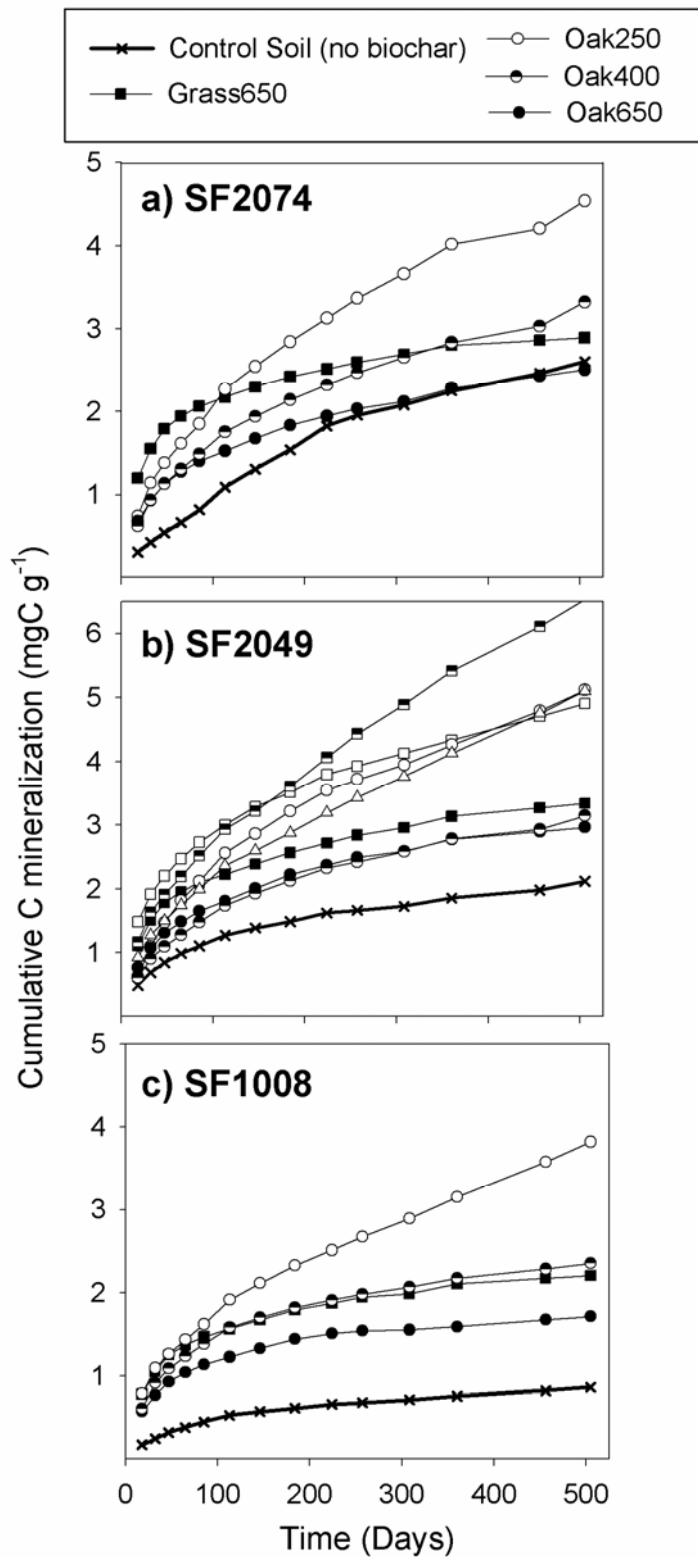


Figure S1. Cumulative C released (as CO₂) from incubations of a) SF2074, b) SF1008, and c) SF2049 soil and selected soil-biochar mixtures.

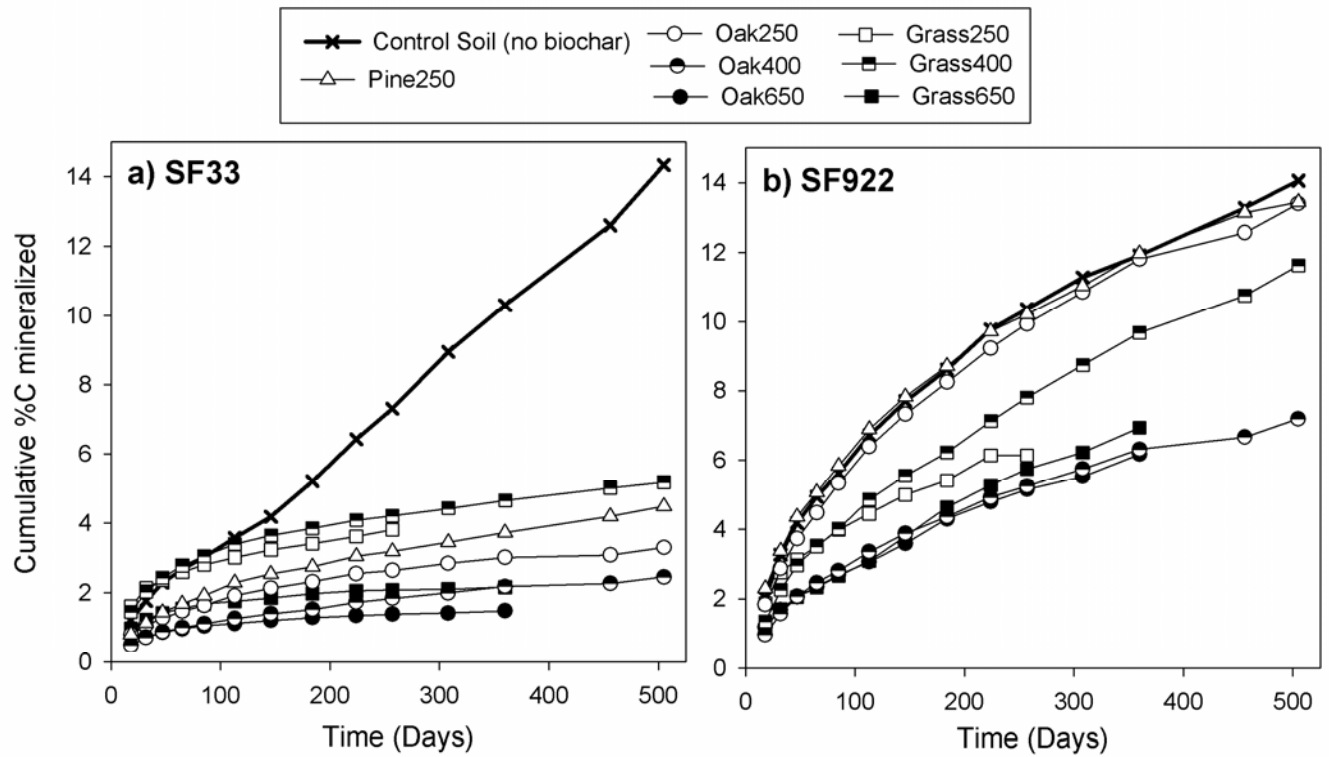


Figure S2. Cumulative C released (as CO_2) from incubations of a) SF33 and b) SF922 soil and selected soil-biochar mixtures normalized to total organic carbon content.

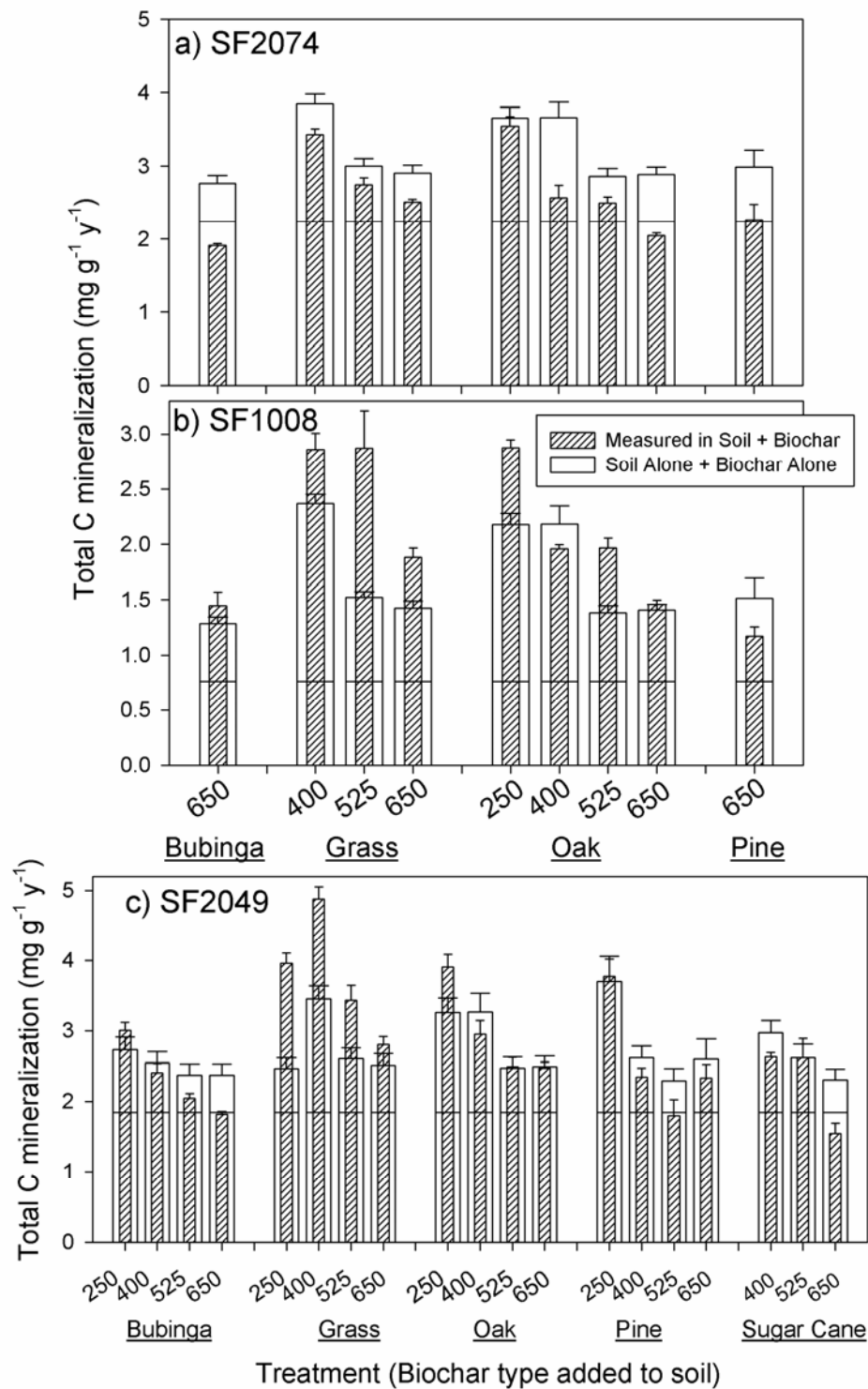


Figure S3. Cumulative C released (as CO₂) in 1 y incubations of biochar mixed with a) SF2074, b) SF1008, and c) SF2049 soil (striped bars) compared with that calculated had C mineralization from soil (bottom portion of open bars) and biochar (top portion of open bars) been additive (i.e., no priming).

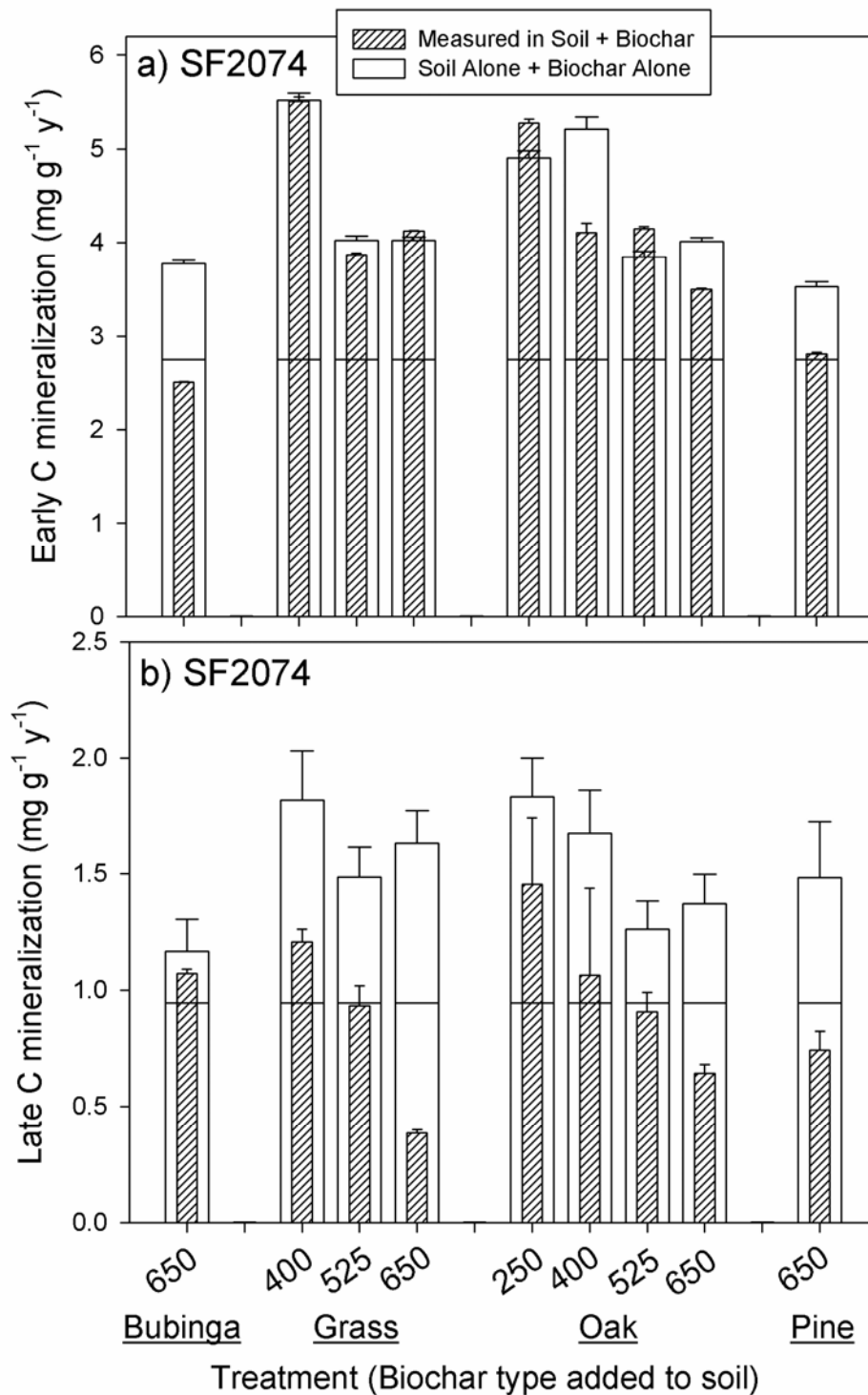


Figure S4. C mineralization rate from incubations of SF2074 soil mixed with biochars during a) the first 90 d ('early') and b) day 250-500 ('late') compared with that calculated had C mineralization rates from soil (bottom portion of open bars) and biochar (top portion of open bars) been additive (i.e., no priming).

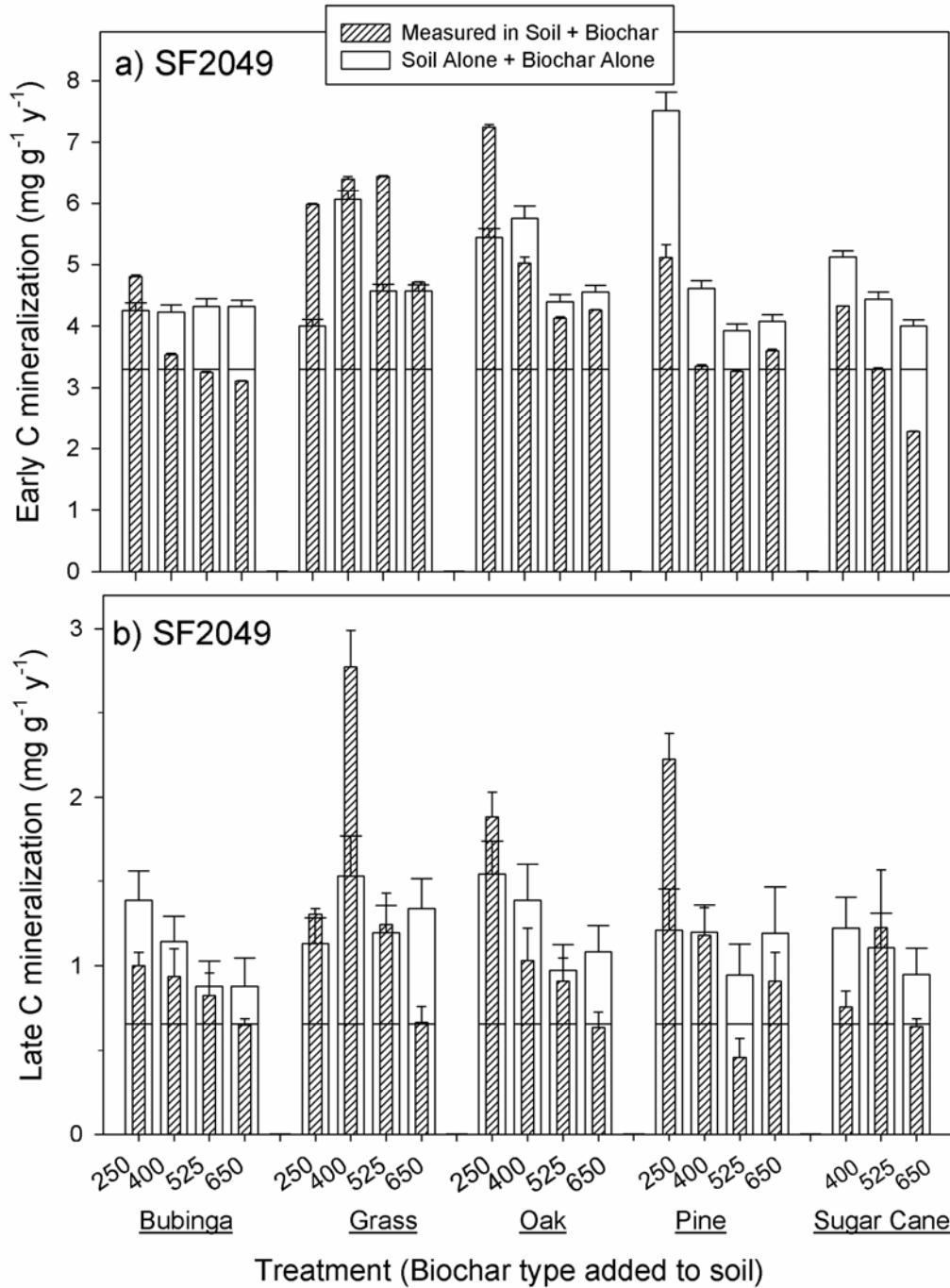


Figure S5. C mineralization rate from incubations of SF1008 soil mixed with biochars during a) the first 90 d ('early') and b) day 250-500 ('late') compared with that calculated had C mineralization rates from soil (bottom portion of open bars) and biochar (top portion of open bars) been additive (i.e., no priming).

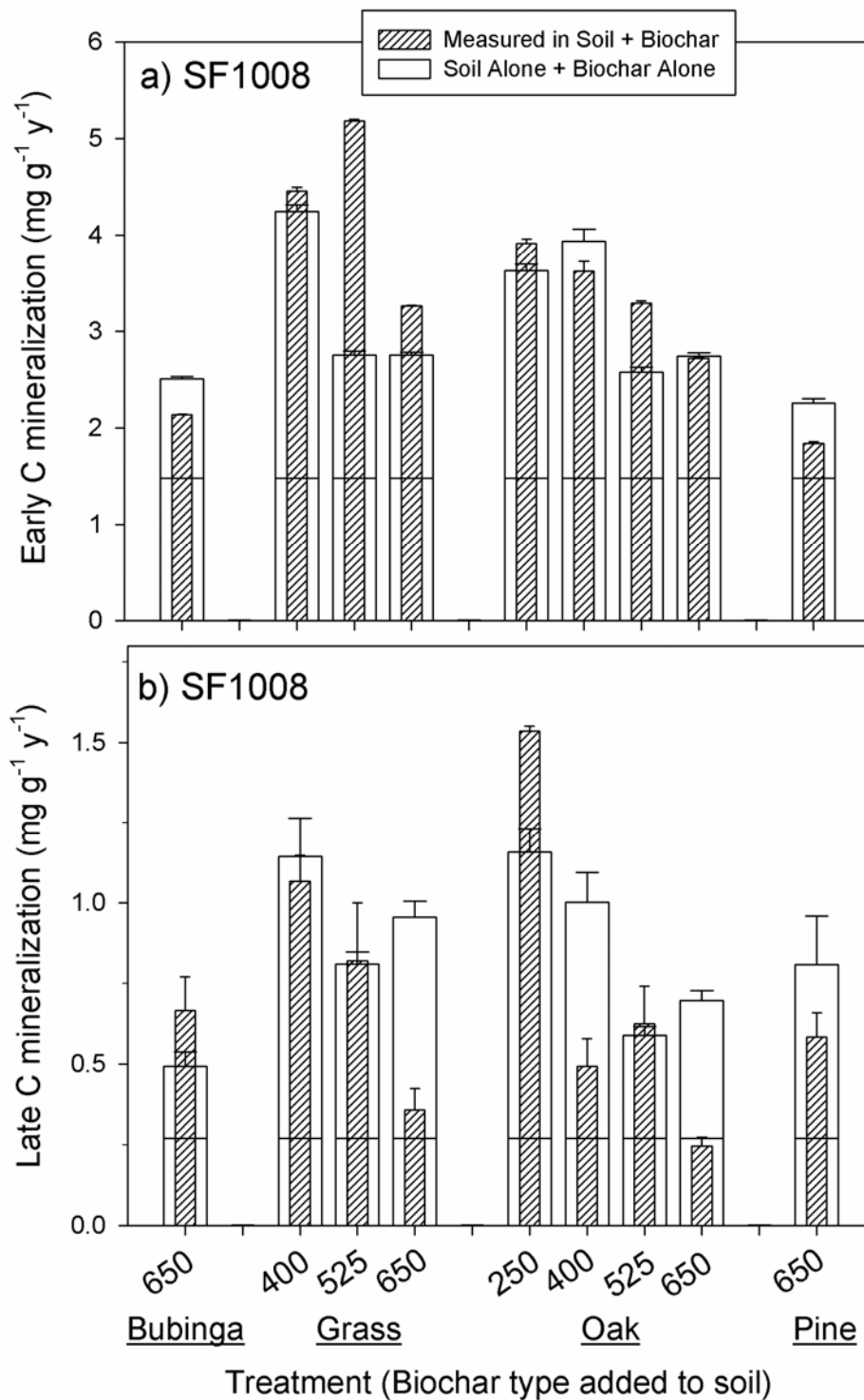


Figure S6. C mineralization rate from incubations of SF2049 soil mixed with biochars during a) the first 90 d ('early') and b) day 250-500 ('late') compared with that calculated had C mineralization rates from soil (bottom portion of open bars) and biochar (top portion of open bars) been additive (i.e., no priming).

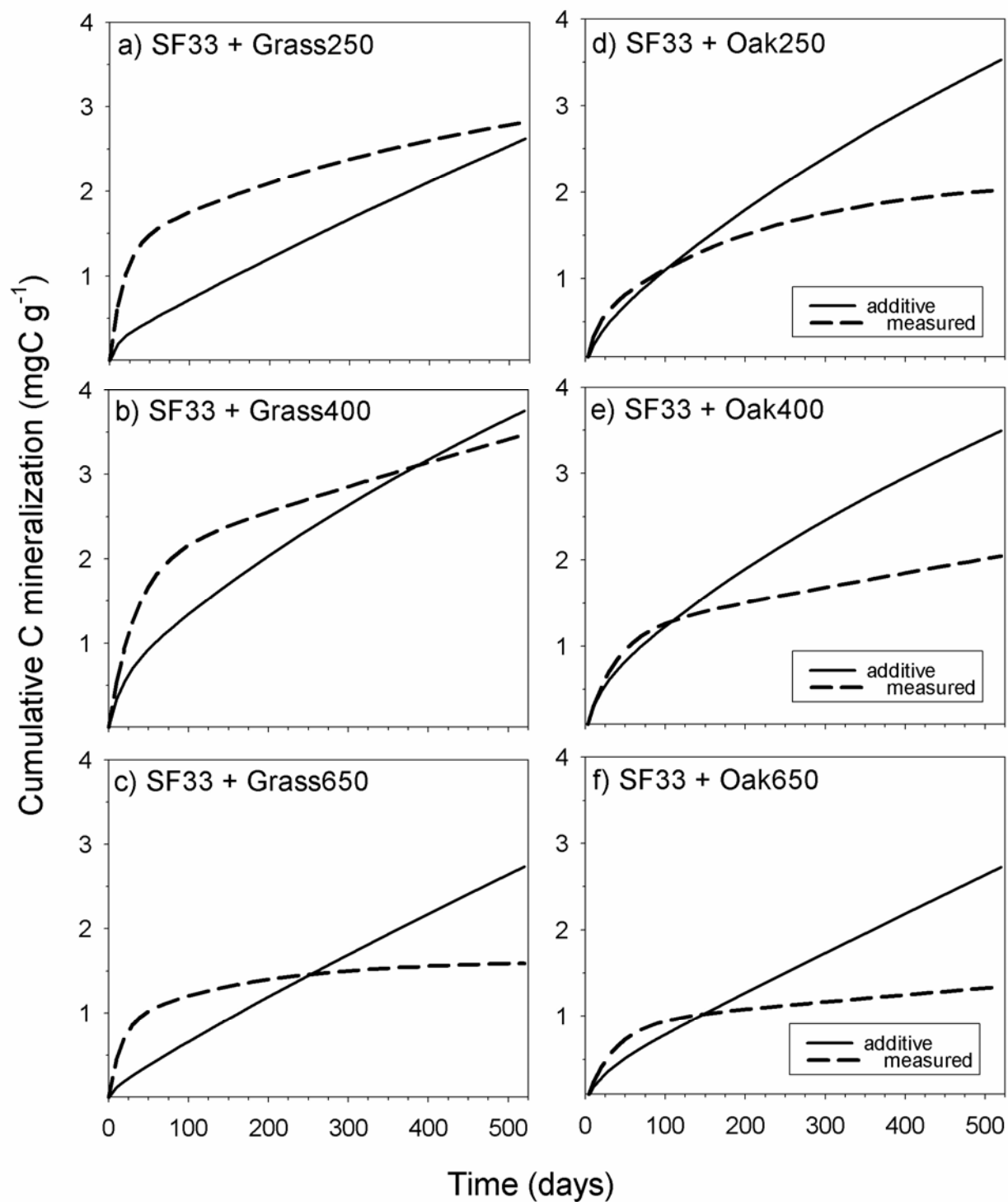


Figure S7. Dual-exponential model of measured C mineralized (as CO₂) and that calculated had C mineralization from soil and biochar been additive (i.e. no priming) from mixtures of SF33 soil with a) Grass 250, b) Grass 400, c) Grass 650, d) Oak 250, e) Oak 400 and f) Oak 650 biochar.

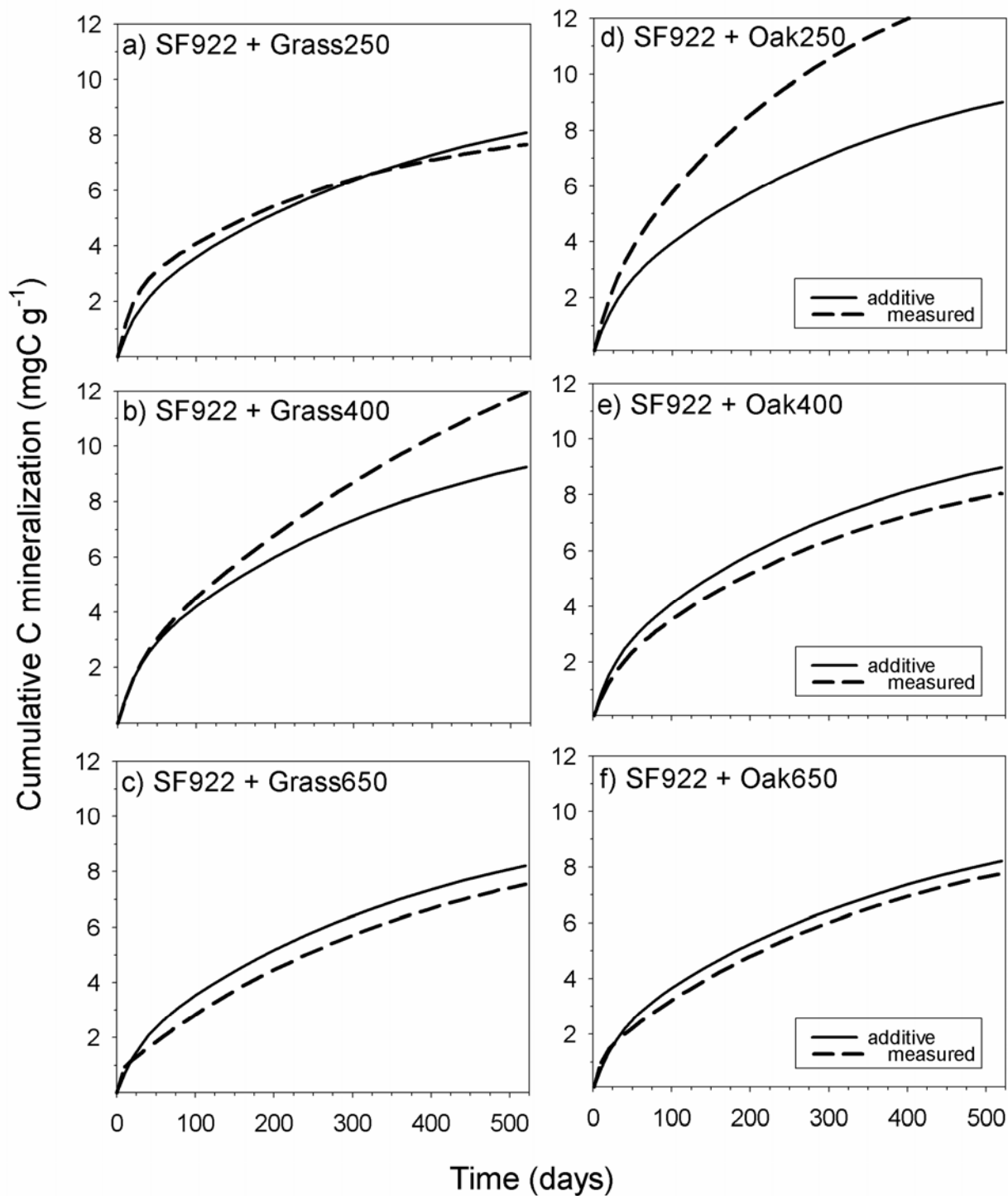


Figure S8. Dual-exponential model of measured C mineralized (as CO₂) and that calculated had C mineralization from soil and biochar been additive (i.e. no priming) from mixtures of SF922 soil with a) Grass 250, b) Grass 400, c) Grass 650, d) Oak 250, e) Oak 400 and f) Oak 650 biochar.

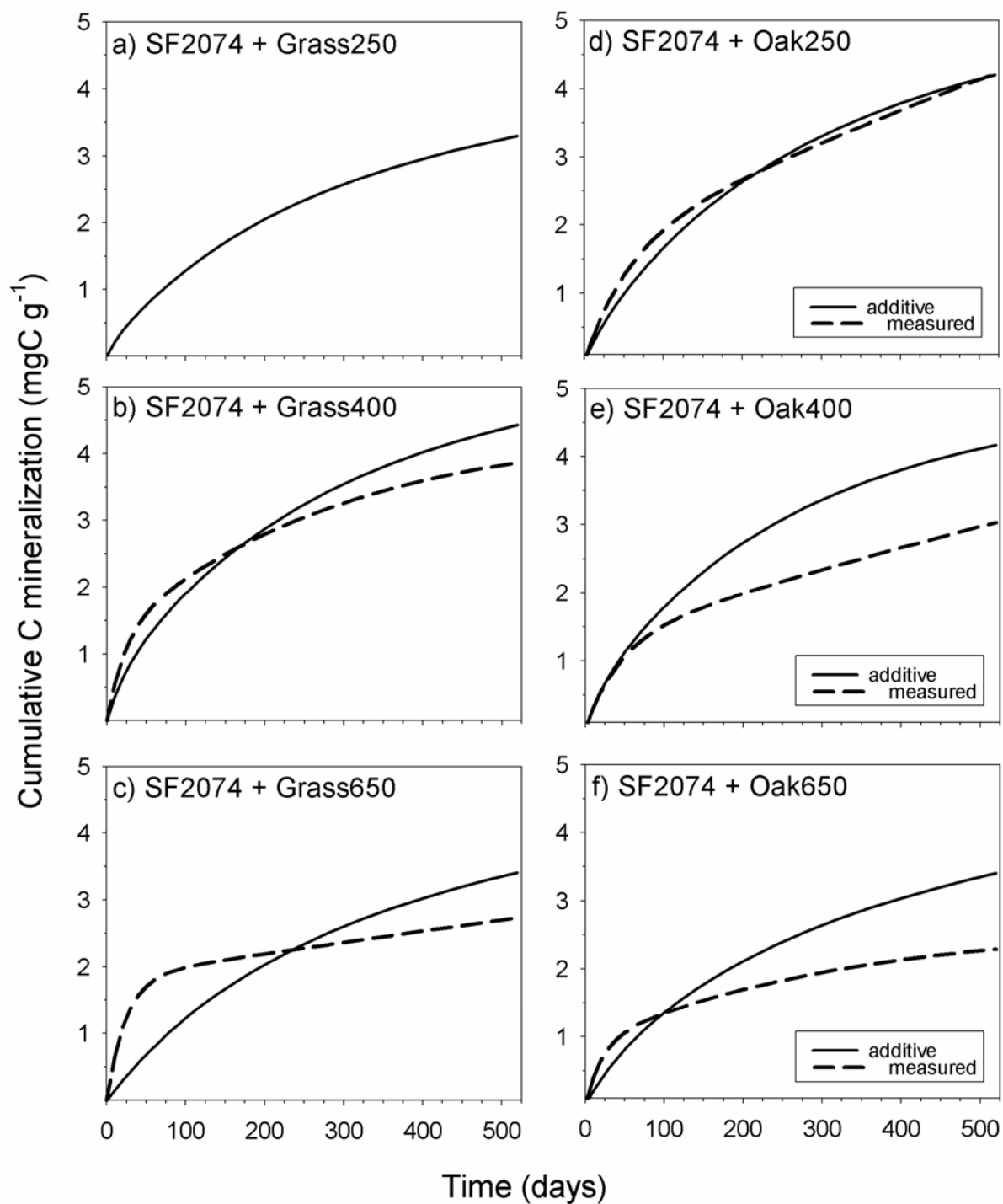


Figure S9. Dual-exponential model of measured C mineralized (as CO₂) and that calculated had C mineralization from soil and biochar been additive (i.e. no priming) from mixtures of SF2074 soil with a) Grass 250, b) Grass 400, c) Grass 650, d) Oak 250, e) Oak 400 and f) Oak 650 biochar. Grass250 + SF2074 incubation was not carried out.

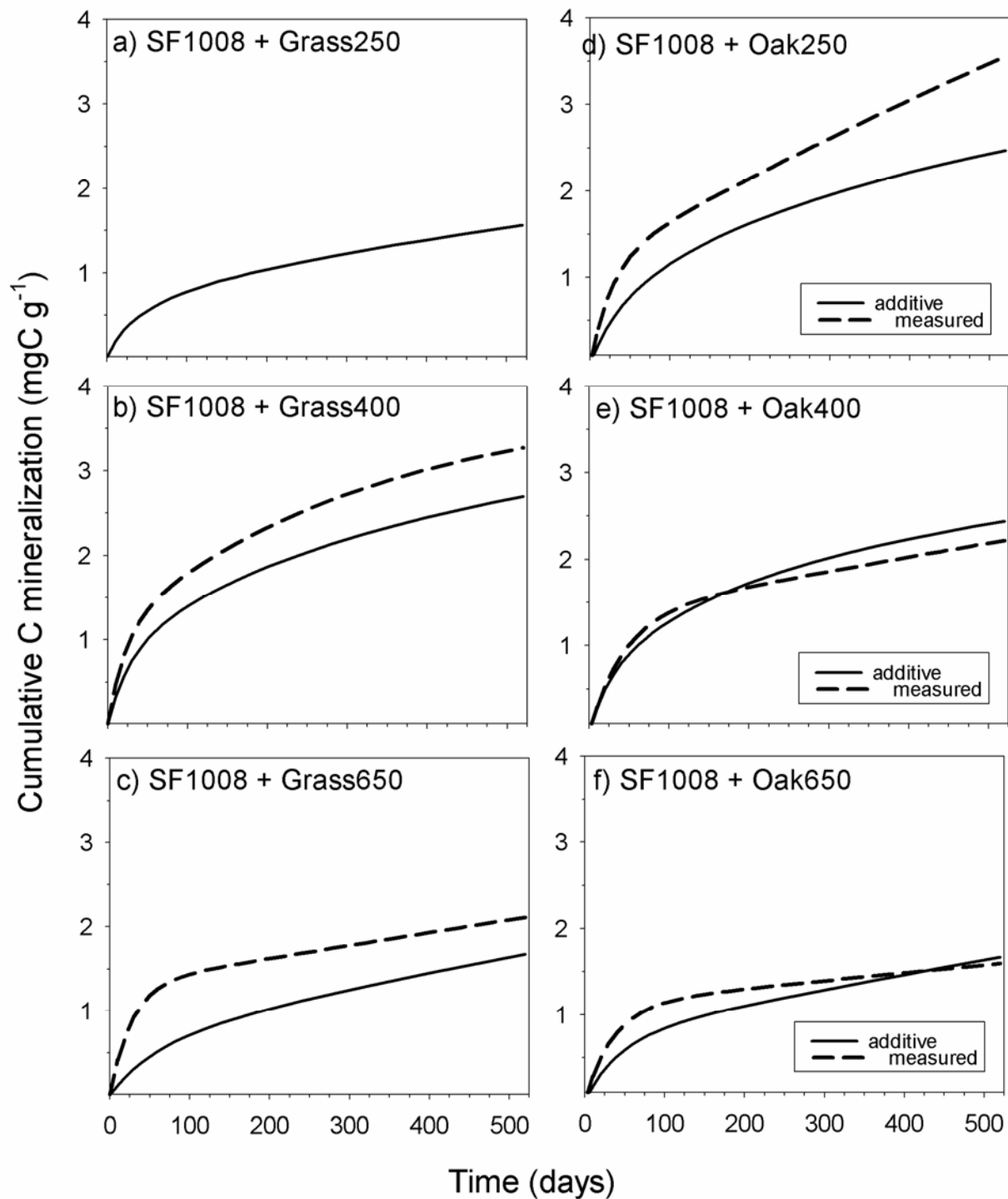


Figure S10. Dual-exponential model of measured C mineralized (as CO₂) and that calculated had C mineralization from soil and biochar been additive (i.e. no priming) from mixtures of SF1008 soil with a) Grass 250, b) Grass 400, c) Grass 650, d) Oak 250, e) Oak 400 and f) Oak 650 biochar. Grass250 + SF1008 incubation was not carried out.

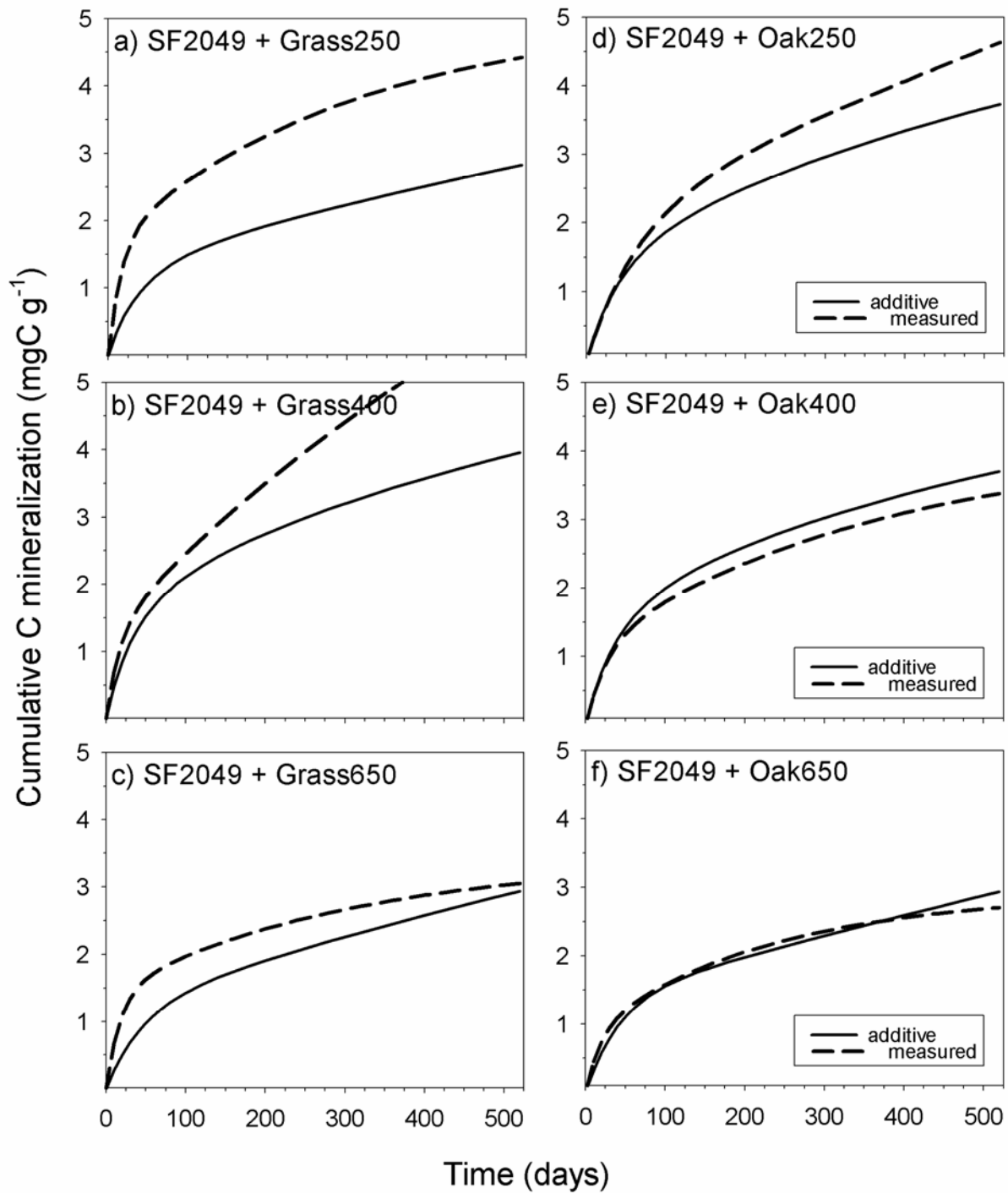


Figure S11. Dual-exponential model of measured C mineralized (as CO₂) and that calculated had C mineralization from soil and biochar been additive (i.e. no priming) from mixtures of SF2049 soil with a) Grass 250, b) Grass 400, c) Grass 650, d) Oak 250, e) Oak 400 and f) Oak 650 biochar.