

Short- and long-term impacts of plant-biochar-soil interactions on soil carbon cycling in a subtropical pasture

by

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Certification

I certify that the substance of this thesis has not already been submitted for any degree and is not currently being submitted for any other degree or qualification

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Abstract

While biochar has been widely promoted for its potential to stabilise soil organic carbon (SOC), many laboratory and glasshouse studies (mainly short-term) have demonstrated that biochar can increase (positive priming) and/or lower (negative priming) mineralisation of SOC. There is a clear knowledge gap on both biochar carbon (C) longevity, its priming effects on SOC, and recent root-derived C, under field conditions. This knowledge would allow the potential contribution of biochar to long-term soil C sequestration to be understood. Understanding plant-biochar-soil interactions (Chapter 1) over the long term is crucial to accurately predict the C storage potential of biochar-amended soil, additional to its intrinsic C content.

Most studies on biochar C longevity and its priming effect have been undertaken in plant-free laboratory incubations. To assess the impact of plants on biochar longevity and its priming effect (Chapter 2), a 388-d field study was carried out in the presence of annual ryegrass (C_3) grown on a rhodic Ferralsol with C_3/C_4 plant-derived SOC ($\delta^{13}C$: -20.2‰) in a subtropical climate. A ^{13}C -depleted hardwood biochar ($\delta^{13}C$: -35.7‰, produced at 450°C) was applied at 0 and 30 dry t ha⁻¹ and mixed into the top 100-mm soil profile (equivalent to 0 and 3% w/w, respectively). Root respiration and mineralisation of soil-C and biochar-C was differentiated and quantified in this field site. Periodic $^{13}CO_2$ pulse labelling was applied to enrich the $\delta^{13}C$ signature of root respiration during two separate winter campaigns ($\delta^{13}C$: 151.5-184.6‰) and one summer campaign ($\delta^{13}C$: 19.8-31.5‰). Combined soil plus root respiration was separated from leaf respiration using a novel in-field respiration collar. A two-pool isotope mixing model was applied to partition three C sources (i.e. root, biochar and soil). Three scenarios were used to assess the sensitivity associated with the C source partitioning in the planted systems: 1) extreme positive priming of the C_3 -dominant SOC derived from the current C_3 -ryegrass pasture; 2) equivalent magnitude of priming of recent C_3 and native C_4 vegetation-derived SOC; and 3) extreme positive priming of the native C_4 -dominant SOC. The biochar induced a significant negative priming of SOC in the presence of growing plants but no net priming was observed in the unplanted soil. I also demonstrated the importance of the experimental timeframe in capturing the transient nature of biochar-induced priming; from positive (day 0-62) to negative (day 62-388). The presence/absence of plants had no impact on biochar-C mineralisation in this Ferralsol during the measurement period. Based on a two-pool exponential model, the mean residence time (MRT) of biochar varied from 351-449 years in the intensive pasture system to 415-484 years in the unplanted controls.

Organo-mineral interactions serve as the principal mechanism for the stabilisation of soil organic matter (SOM) in mineral soils, which can be enhanced by the presence of biochar possibly via ligand

exchange (Singh and Cowie, 2014) or cation bridging mechanisms (Liang et al., 2010). In Chapter 3, I further assessed the contribution of organo-mineral interactions to the reported biochar-induced negative priming in a planted Ferralsol (shown in Chapter 2). Applying a detailed soil physical fractionation coupled with stable ^{13}C isotope partitioning, the magnitude and fate of recently fixed ^{13}C was quantified within various belowground C pools, including: soil plus root respiration, root biomass, soil aggregates and associated fractions. The aggregate-associated fractions consist of free- ($>1.6 \text{ g cm}^{-3}$) and occluded- ($>53 \mu\text{m}$) particulate organic matter (F- and O-POM), and mineral-protected SOM (M-SOM, $< 53 \mu\text{m}$). The biochar (*E.saligna*, 450°C) amendment increased total belowground ^{13}C recovery by 10% compared to the non-amended control. Retention of rhizodeposits (i.e. ^{13}C) was found in increasing concentrations in the order: O-POM of macroaggregates (macro-, $250\text{-}2000 \mu\text{m}$) $<$ O-POM of microaggregates (micro-, $< 250 \mu\text{m}$) $<$ micro-M-SOM $<$ macro-M-SOM. I provide evidence that rapid accumulation of rhizodeposits in organo-mineral (i.e. O-POM and M-SOM) fractions promoted biochar-induced negative priming over 12 months.

Nevertheless, the transient nature of biochar-induced priming highlights the need for longer-term studies to assess the persistence of negative priming as biochar ages in soil (Chapter 4). For this assessment, periodic $^{13}\text{CO}_2$ pulse-labelling was applied to ryegrass in the field, and belowground C allocation, SOC priming, and root-derived C stabilisation was monitored on a Ferralsol amended with biochar (hardwood, 550°C) over the period 8.2-9.5 years since amendment. I provide evidence that field-aged biochar (a) enhanced belowground C recovery by 20%, (b) promoted negative priming (lowered C loss through respiration by $160 \text{ g CO}_2\text{-C m}^{-2} \text{ y}^{-1}$) and (c) increased the retention of root-derived ^{13}C in the stable organo-mineral fractions ($<53 \mu\text{m}$) by 6% ($P < 0.05$). Through synchrotron-based spectroscopic analysis of bulk soil, field-aged biochar and microaggregates ($<250 \mu\text{m}$), I mechanistically demonstrate the role of biochar in accelerating formation of micro-agglomerates via organo-mineral interactions.

Despite the continued increase in the total soil C stock in the 9.5-year biochar-amended Ferralsol, a decrease in the rate of SOC build-up was detected in Chapter 4. This is interpreted as the biochar amended system approaching a new equilibrium of SOC. The reapplication of biochar has been suggested to restore the originally observed transient agronomic benefits, including improved soil quality by increasing phosphate availability and soil moisture (Quilliam et al., 2012). However, there is no literature on the effect of a repeated (2nd) application of biochar on the priming of native soil organic SOC in a clay-rich soil. This knowledge would allow the potential of recurring biochar application in long-term soil C sequestration to be established. In a previous field study established in 2006 as reported in Chapter 4, an *E. saligna* biochar (550°C) induced negative priming of native

and root-derived SOC after being incorporated in a Ferralsol over nearly a decade. The total soil C stock continued to increase beyond the amount of biochar-supplied C in this subtropical pasture. I aimed: 1) to further evaluate the extent of priming on this existing site, including the effects of the 2nd application of biochar in planted and unplanted Ferralsol; and 2) to assess the potential of resetting SOC stabilisation capacity through the use of a 2nd application of biochar. In April 2014, I superimposed a 488 d field trial on this long-term site. Three treatments were: 1) control (0 t biochar ha⁻¹), 2) fresh biochar applied to the unamended soil (10 t biochar ha⁻¹, “fresh biochar”), and 3) fresh biochar applied to the existing 9.5-year field-aged biochar-amended soil (10 t biochar ha⁻¹, “2nd application”). I showed that the 2nd application of biochar after nearly a decade doubled the increase of the total soil C stock found from the prior application. The negative priming of SOC by the 2nd application of biochar contributed to this increase in the total soil C stock. A lowered enzyme to microbial biomass ratio could increase microbial C use efficiency of both total and rhizodeposit-derived C in the biochar-amended soil, which may promote negative priming. The results provide fundamental field-derived evidence of the potential of repeated biochar application in resetting the SOC stabilisation capacity.

Biochar-organo-mineral interactions control the long-term SOC stabilisation in the biochar-amended mineral soils. An *in situ* ¹³C₂-labelling study has shown significant stabilisation of recent (¹³C-labelled) pasture-derived rhizodeposits in a nearly decade-aged biochar-amended Ferralsol via enhanced organo-mineral interactions (Chapter 4). A repeated biochar application study on this aged biochar-amended Ferralsol was shown to promote negative priming of native and/or root-derived SOC (Chapter 5). In Chapter 6, I assessed the role of repeated biochar application on the stabilisation of recent root-derived C in the organo-mineral fractions in the aged biochar-amended Ferralsol. Repeated biochar application further stabilised recent rhizodeposits in the existing aged biochar-amended soil by facilitating the transformation of belowground ¹³C from root biomass and exudates to the aggregate-occluded particulates (8 month) and mineral (silt/clay)-protected (12 month) organic fractions. The increased stabilisation of root-derived C in the organo-mineral fractions may explain the reinvigorated soil C sequestration capacity following the 2nd application of biochar into a decade aged biochar amended soil (Chapter 5).

To conclude, using this replicated-design short- and long-term biochar field experiment, the turnover of both rhizodeposits and native SOC was lowered in biochar-amended soils, with biochar-induced negative priming counteracting the co-existing positive rhizosphere priming a decade after biochar incorporation. Further, I suggest that the formation and accumulation of organo-mineral complexes around catalytic biochar surfaces may counteract the dissolution of mineral-protected SOC by root

exudates. The results enable more accurate predictions of the C sequestration potential of woody biochars applied to Fe-dominated clay soils. Resetting the SOC stabilisation capacity is feasible via repeated biochar application.

Abbreviations

AS	Australian Synchrotron
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DAFF	Department of Agriculture, Fisheries and Forestry
NSW DPI	New South Wales Department of Primary Industries
ANOVA	analysis of variance
CEC	cation exchange capacity
CLPP	community level physiological profiles
DOM	dissolved organic matter
DOC	dissolved organic carbon
FOM	fresh organic matter
F-POM	free particulate organic matter
FTY	total fluorescence yield
LOM	labile organic matter
LSD	least significant difference
Mo	molybdenum
MOAs	mineral-organic associations
M-SOM	mineral-protected soil organic matter
MRT	mean residence time
MUF	methylumbelliferyl
OM	organic matter
O-POM	occluded particulate organic matter
POM	particulate organic matter
PDB	Pee Dee Belemnite
PE	polyethylene
PVC	polyvinyl chloride
PyC	pyrogenic carbon
PyOM	pyrogenic organic matter

SIR	substrate induced respiration
SOM	soil organic matter
SOC	soil organic carbon
SPT	sodium polytungstate
SSA	specific surface area
SXR	soft X-ray
UV	ultra-violet
XPS	X-ray photoelectron spectroscopy

°C	degrees Celsius
t	tonne/ metric ton
g	gram
L	litre
m	metre
s	second
ha	hectare (100 m × 100 m)
Pa	Pascals
mol	moles

Prefixes

G	giga (10^9)
M	mega (10^6)
k	kilo (10^3)
c	centi (10^{-2})
m	milli (10^{-3})
μ	micro (10^{-6})
n	nano (10^{-9})
p	pico (10^{-12})

Table of contents

Acknowledgements	3
Publications	4
Abstract	5
Abbreviations	9
List of tables	14
List of figures	16
1. The role of plant-biochar-soil interactions in belowground carbon retention	
	18
1.1. Introduction	18
1.2. Change of global soil C stocks	19
1.3. Soil C sequestration potential	20
1.4. Proposed mechanisms for soil C sequestration	20
1.4.1. Physicochemical protection	20
1.4.2. Biochemical recalcitrance	22
1.5. Is there a soil C saturation level?	22
1.6. The contributions of biochar amendment to soil C sequestration	24
1.6.1. Direct addition of biochar-C and its longevity	24
1.6.2. Indirect effects from biochar amendment on soil C sequestration	25
1.6.2.1. Productivity	25
1.6.2.2. Biochar-induced priming of native soil organic matter	26
1.7. Mechanisms by which biochar amendments may change priming	29
1.7.1. Positive priming due to increased microbial activity	29
1.7.2. Positive priming due to co-metabolism of biochar-C and SOC	30
1.7.3. Negative priming due to depletion of labile C and organo-mineral interactions	30
1.7.4. Negative priming due to substrate switching	30
1.8. Theories on SOC stabilisation affected by plant-biochar-soil interactions	31
1.9. The role of long-term field experiments	33
1.10. Hypotheses	33
1.11. Aims	35
2. Plant-biochar interactions drive the negative priming of soil organic carbon in an annual ryegrass field system	
Abstract	38
2.1. Introduction	39
2.2. Material and methods	40
2.2.1. Biochar and site characteristics	40
2.2.2. Field set-up	42
2.2.3. ¹³ C pulse labelling	43
2.2.4. Quantification of biochar-C and soil-C mineralisation and root respiration	45
2.2.4.1. Total CO ₂ fluxes	45
2.2.4.2. Three-pool C source partitioning	46
2.2.4.3. Sensitivity of C source partitioning	47

2.2.5. Sampling and analyses	47
2.2.6. Mean residence time	48
2.2.7. Biometrical analysis	48
2.3. Results	49
2.3.1. Total CO ₂ fluxes	49
2.3.2. Three-pool C source partitioning	50
2.3.2.1. Sensitivity of C source partitioning	50
2.3.2.2. Biochar C mineralisation	50
2.3.2.3. Mineralisation of SOC and root respiration	52
2.3.3. Soil analyses and ryegrass yield	54
2.3.4. Mean residence time	56
2.3.5. Correlation and regression between biochar-C and soil-C mineralisation	56
2.4. Discussion	56
2.4.1. Biochar-induced priming of SOC in the planted and unplanted field systems	56
2.4.2. In-field biochar C mineralisation in the presence of plants	58
2.4.3. Mean residence time of biochar in the planted field system	59
2.5. Conclusions	60
3. Rapid accumulation of rhizodeposits in the organo-mineral fractions promoted biochar-induced net negative priming	
Abstract	64
3.1. Introduction	65
3.2. Material and methods	66
3.2.1. Soil, root and respiration sampling	66
3.2.2. Separation and analysis of aggregates and their associated organic fractions	66
3.2.3. Quantification of belowground ¹³ C allocation	67
3.2.4. Statistical analysis	67
3.3. Results and discussion	67
3.4. Conclusions	70
4. Biochar builds soil carbon over a decade by stabilising rhizodeposits in a managed subtropical pasture	
Abstract	74
4.1. Introduction	75
4.2. Material and methods	78
4.2.1. Field site and superimposed experimental setup	78
4.2.2. Periodic pulse labelling and calculations of SOC priming	79
4.2.3. Separation of aggregate sizes and associated organic fractions	80
4.2.4. Quantification of belowground C allocation	80
4.2.5. Substrate induced respiration	80
4.2.6. Catabolic enzyme activities	82
4.2.7. Soft X-ray (SXR) and X-ray photoelectron spectroscopy (XPS)	82
4.3. Results and discussion	83
4.3.1. Negative priming of native SOC after 9.5 years	83
4.3.2. Greater belowground C recovery and its retention in the organo-mineral fractions of the biochar-amended soil	86

4.3.3. The role of biochar in the formation of stable organo-mineral complexes	90
4.4. Stabilising rhizodeposits: the impact on global soil C cycling	95
5. Resetting the soil carbon sequestration potential in a subtropical pasture through a second application of biochar a decade following its initial application	
Abstract	100
5.1. Introduction	101
5.2. Material and methods	103
5.2.1. Site and superimposed experimental design	103
5.2.2. Periodic ¹³ C pulse labelling to quantify SOC mineralisation and root respiration	104
5.2.3. Soil sampling and analysis	104
5.2.4. Catabolic enzyme activity and substrate induced respiration	105
5.2.5. Biometrical analysis	107
5.3. Results	107
5.3.1. Total CO ₂ fluxes	107
5.3.2. SOC priming and root respiration	110
5.3.3. Soil physical and biological properties	111
5.3.4. Catabolic enzyme activities and enzyme-microbial biomass ratio	113
5.3.5. Substrate induced respiration	114
5.4. Discussion	117
5.5. Conclusions	119
6. Repeated biochar application enhanced rhizodeposit stabilisation capacity of an aged biochar-amended Ferralsol under a subtropical pasture	
Abstract	122
6.1. Introduction	123
6.2. Material and methods	124
6.3. Results and discussion	124
6.4. Conclusion	126
7. Conclusions and future work	
7.1. Addressing the hypotheses with a synthesis of the impact of plant-biochar-soil interactions on organo-mineral stabilisation of rhizodeposits	129
7.2. Theories on the biochar-driven rhizodeposit stabilisation	133
7.3. Implications of plant-biochar-soil interactions for soil carbon sequestration	134
Reference	136
Appendix- Supplementary data and Preliminary investigation and design	145

List of tables

Table 1.1: Summary of studies of the impact biochar-soil interactions on soil organic carbon mineralisation

Table 1.2: Summary of experiments and analytical procedures in the thesis

Table 2.1: The chemical properties of the biochar used in the field experiment

Table 2.2: Cumulative biochar-C mineralised over 98 days and 388 days

Table 2.3: Cumulative root respiration over 388 days derived from the total CO₂ fluxes using the C mixing model

Table 2.4: Changes in soil chemical and biological properties over 12 months

Table 2.5: Estimated mean residence time of the labile C fraction of biochar and its proportion relative to the total biochar C

Table 3.1: $\delta^{13}\text{C}$ signatures of soil plus root respiration, root and soil components from the control and biochar-amended soils

Table 3.2: Proportion of belowground ^{13}C recovery relative to the total allocated ^{13}C enrichment (in percentage) within the control and biochar-amended soils

Table 3.3: Proportion of belowground ^{13}C recovery relative to the total allocated ^{13}C enrichment (in percentage) within the aggregate associated organic fractions

Table 4.1: The chemical properties of biochar and soil from the field experiment in 2014

Table 4.2: Belowground total C stocks measured at 4-month intervals in the unamended control and field-aged biochar-amended plots

Table 4.3: Metabolic quotient as microbial respiration (from total C and rhizodeposits, ^{13}C) over microbial biomass carbon (MBA) in control and biochar soils (9.5 years)

Table 4.4: Enzyme activities of β -glucosidase (Glc), xylosidase (Xyl), cellulase (Cel), N-acetylglucosaminidase (Nag) in planted unamended control and planted biochar-amended soil

Table 4.5: Proportion of belowground ^{13}C allocation relative to the total allocated ^{13}C enrichment (in percentage) within the control and field-aged biochar-amended soils

Table 4.6: Redox potentials, pH, mineral N, Fe²⁺ and Fe³⁺ and water and solid P content of the control and biochar-amended soils

Table 4.7 XPS of the C functional groups measured on finely crushed 250-2000 μm macroaggregates and <250 μm microaggregates from the control and biochar-amended plots

Table 4.8 XPS of the C functional groups measured on grain surface of the fresh and 9.5-year field-aged biochars

Table 4.9: Summary of XPS chemical elements characteristics found in the extracted fresh and 9.5-year aged biochars

Table 5.1: List of C substrates used for MicrorespTM system

Table 5.2: Contribution of root respiration to total CO₂ fluxes during each pulse labelling event derived from the total CO₂ fluxes using the C mixing model

Table 5.3: Changes in soil C stock of the biochar-amended soils over 12 months

Table 5.4: Microbial biomass carbon (MBC), metabolic quotient of both total and rhizodeposit-derived C in the fresh and 2nd applied biochar-amended soils

Table 5.5: Difference in enzyme activities between the unamended and biochar-amended soils (i.e. biochar - control) within the planted and unplanted systems

Table 5.6: The ratio between specific enzyme activities and total microbial biomass in the unamended control and biochar-amended soils within the planted system

Table 6.1: Proportion of belowground ¹³C recovery relative to the total allocated ¹³C enrichment (in percentage) within the freshly- and 2nd applied biochar-amended soils

Table 6.2: Proportion of belowground ¹³C recovery relative to the total allocated ¹³C enrichment (in percentage) within the aggregate-associated organic fractions

List of figures

Figure 1.1: Proposed mechanisms for priming of SOC induced by biochar amendment in the presence of plants

Figure 1.2: Conceptual diagram of the potential stepwise increase in total soil C resulting from repeated biochar dosing.

Figure 2.1: Diagram of the experimental layout of the circular microplots

Figure 2.2: Photographs of the soil respiration (a) and soil plus root respiration (b) collars before installation into the soil

Figure 2.3: Cumulative total CO₂-C fluxes for all treatments

Figure 2.4: Observed and estimated trends in CO₂ daily flux in the unplanted (a) or planted soil (b)

Figure 2.5: Top 80 mm soil temperatures and moistures

Figure 2.6: Biochar-C mineralisation rates derived from total CO₂ fluxes

Figure 2.7: Cumulative biochar-C mineralisation in the planted and unplanted systems

Figure 2.8: Difference between cumulative SOC mineralised in the biochar-amended and unamended soils for both planted and unplanted systems

Figure 2.9: Correlation between biochar-C mineralisation rates and soil-C mineralisation rates

Figure 3.1a-f: Belowground ¹³C recovery and retention (0-100 mm) in different C pools macroaggregates (a,c,e) and microaggregate (b,d,f) in the control and biochar-amended soils on day 15 at 4-, 8- and 12-month pulse labelling event

Figure 4.1 Proposed mechanisms for positive rhizosphere priming counteracted by biochar-induced negative priming and stabilisation of rhizodeposits (new C) in a ferralsol after 9.5 years

Figure 4.2: Priming on SOC as difference of cumulative SOC mineralisation between (a) planted and unplanted unamended controls (rhizosphere priming); (b) planted biochar-amended and planted unamended controls

Figure 4.3: Substrate induced respiration in the planted control (open) and 9.5-year field-aged biochar-amended (dark) soils

Figure 4.4: Belowground ¹³C recovery and retention (0-100 mm) in different C pools in control and biochar soils on day 15 at 8.9- (a), 9.2- (b) and 9.5- (c) year pulse labelling event

Figure 4.5: SXR spectra of bulk soils and biochars

Figure 4.6: Fe 2p XPS high resolution peak of fresh and field-aged biochars

Figure 4.7: 9.5-year field-aged biochar fragments embedded in microaggregates

Figure 5.1 a & b: Cumulative total CO₂-C fluxes for all treatments.

Figure 5.2 a & b: Priming as in difference between cumulative SOC mineralised in the biochar-amended and unamended soils for both planted and unplanted systems

Figure 5.3: Cumulative root respiration for all treatments

Figure 5.4 a & b: Difference in substrate induced respiration between the unamended controls (ie NPK plots no biochar) and 1) "fresh" (hardwood biochar applied to NPK plots 12 months prior to sampling) and 2) 2nd application (fresh hardwood biochar applied to plots previously dosed with biochar in 2006).

Figure 5.5: The stepwise increase in total soil C resulting from repeated biochar dosing

Figure 6.1a-f: Belowground ¹³C recovery and retention (0-100 mm) in different C pools macroaggregates (a,c,e) and microaggregate (b,d,f) in the freshly- and 2nd applied biochar-amended soils on day 15 at 4-, 8- and 12-month pulse labelling event

Figure 7.1: Synthesis of findings on belowground allocation and retention of rhizodeposits (¹³C enriched) in the unamended control (a) and biochar-amended soils (b-e) in a ferralsol over nearly a decade

Figure 7.2 Conceptual diagram of the formation of organo-mineral complexes on biochar surfaces over 9.5 years