Physicochemical changes in pyrogenic organic matter (biochar) after 15 months field-aging

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Abstract

Predicting the effects of pyrogenic organic matter (OM) addition (either natural or intentional as in the case of biochar amendment) on soil chemistry and crop yields has been hampered by a lack of understanding of how pyrogenic OM evolves in the environment over time. This work compared the physicochemical characteristics of newly-made and 15-month field-aged biochars and biochar-soil mixtures. After aging, biochars made by pyrolysis of wood and grass at 250, 400 and 650 ^{o}C exhibited 5-fold increases in cation exchange capacity (CEC), on average, appearance of anion exchange capacity (AEC) and significant decreases in pH, ash content and nanopore surface area. Cross polarization ^{13}C-NMR analyses indicated relative increases in O-containing functional groups including substituted aryl, carboxyl and carbonyl C, ~~likely via abiotic and microbial oxidation~~ and losses of O-alkyl groups~~, likely via leaching~~. Similar chemical trends were observed for soil-biochar mixtures suggesting the same biochar aging processes occurred in the soil environment. However, there was evidence for a ~~major~~ role of soil OM-microbe-biochar interaction during aging. Field-aging of soil with biochar resulted in large increases in C and N content (up to 124 and 143%, respectively) and exchange capacity (up to 43%) beyond that calculated by the weighted addition of the properties of biochar and soil aged separately. These beneficial interactive effects varied ~~greatly~~ with soil and biochar type. Scanning electronic microscopy (SEM) images of biochar particles~~, both~~ aged ~~alone and~~ with soil~~,~~ showed colonization by microbes and widespread ~~surficial deposits that were likely~~ OM coatings. Thus, sorption of both microbially-produced and soil OM are likely processes that enhanced biochar aging. ~~Among the important implications of these findings are that~~ Thus, biochar's full beneficial effects on soil properties ~~only occur~~likely increase over time and proper assignment of C sequestration credits to biochar users will require consideration of soil-biochar interactions.

1 Introduction

Pyrogenic organic matter (OM), or black carbon, represents a large, but poorly understood portion of global C cycling that may have played a role in climate changes, soil fertility, and now, pollutant fate and transport. Much recent work on understanding the cycling of pyrogenic OM has been carried out using biochar, biomass pyrolyzed in an oxygen-limited environment. Research on biochar has accelerated because of its possible uses for soil C sequestration, soil amelioration or as an environmental sorbent. The characteristics of biochar that make it well-suited to these purposes include its abundant and refractory OM (Glaser et al., 2001a; Glaser et al., 2001b; Glaser et al., 2002; Glaser et al., 2004), high pH, high cation exchange capacity (CEC, Mukherjee et al., 2011), high surface area (SA) and high organic compound sorption affinity (Chen et al., 2012; Hale et al., 2011; Kasozi et al., 2010; Smernik, 2009). However these characteristics have been shown to vary a great deal among parent biomass types and biochar production conditions (Mukherjee, 2011; Mukherjee et al., 2011).

More recently, it has become clear that the beneficial effects of biochar on soil vary temporally as well. For example, literature reviews have concluded that crop yields with added biochar are highly variable (Biederman and Harpole, 2013; Cornelissen et al., 2013; Jeffery et al., 2011; Mukherjee and Lal, 2014) and, in some cases, increases are only observed after several years or not at all (Gaskin et al., 2010; Jones et al., 2012; Kulmatiski and Beard, 2006). Laboratory chemical aging or mixing with soil was shown to decrease the capacity of biochar to sorb pyrene (Hale et al., 2011; Zhang et al., 2013), herbicides (eg., diuron and artrazine) (Martin et al., 2012) and allelopathic compounds(Cheng and Lehmann, 2009), but increased immobilization of heavy metals (Uchimiya et al., 2010). And biochar's impact on greenhouse gas (GHG) emissions also varied with time, sometimes increasing and sometimes decreasing with biochar weathering (Mukherjee and Lal, 2013; Spokas, 2013). Thus, in order to better understand the long-term effects of pyrogenic OM and to predict the shorter-term effects of biochar amendment on soil chemistry, study of how biochar surface and bulk chemistry and physical, chemical and biological interactions with soil evolve over time is urgently needed.

In general, biochars newly produced at higher temperatures have higher pH, ash content and surface area (SA) and lower volatile matter (VM) and acidic functional group content than those produced at lower temperatures (Mukherjee, 2011; Mukherjee et al., 2011). Increasing O/C and H/O ratios with production temperature indicate progressive depolymerization, loss of functional groups, and dehydrogenation reactions resulting in more condensed aromatic structures (Baldock and Smernik, 2002; Keiluweit et al., 2010; Knicker, 2007).

Biochar chemistry may also be altered by a variety of time-dependent processes that occur in the environment, termed here as 'aging', including abiotic and biotic redox reactions, solubilization and interactions with microbes, organic matter (OM), minerals and solutes in the soil environment. For example, oxidation reactions were suggested by strong correlations between rates of CO_2 production and O_2 consumption during incubations of a variety of biochars (Spokas and Reicosky, 2009). Also, analyses of laboratory-incubated or decades-old charcoal particles by titration and spectroscopic techniques have shown increasing amounts of O-containing functional groups with aging (Cheng et al., 2008; Cheng et al., 2006; Liang et al., 2006; Lin et al., 2012a; Lin et al., 2012b; Nguyen et al., 2009). These changes were accompanied by increases in biochar CEC, disappearance of surface positive charge and formation of surface negative charge (Cheng et al., 2008).

Though temporal variability in GHG emission, contaminant sorption and crop response to biochar amendment has been observed, their cause is poorly understood (Mukherjee and Lal, 2014). Very few studies, if any, have examined changes in soil/biochar mixtures in the field using a range of biochar types. Further, parallel studies of separate and mixed biochar and soil aging have not yet been conducted so that properties evolved from soil-biochar interactions could be identified. Thus, this study fills these gaps by examining the bulk and surface chemical characteristics of a range of fresh biochars and their aged counterparts. Both biochar-alone and soil/biochar mixtures were field-aged for 15 months under north Florida climatic conditions which, with high temperatures and abundant rainfall, can be characterized as extreme. While previous studies have shown biotic and abiotic oxidation to occur during biochar aging, we hypothesize that leaching/solubilization and microbial OM production and sorption of soil OM also occurs, resulting in a range of new organic functional groups that will vary with biochar and soil type. The overarching goal of this project was to understand how biochar amendments of different types affect soil chemistry and thus, fertility and C sequestration over longer timescales and to identify the types of biochar which may be better suited to specific purposes or soil types.

2. Materials and Methods

2.1 Materials

Biochar was produced from *Quercus lobata* (Laurel oak: Oak), *Pinus taeda* (Loblolly pine: Pine), and *Tripsacum floridanum* (Gamma grass: Grass) by combusting for 3 h at highest treatment temperature (HTT) of 250 ^{o}C in a closed oven with limited oxygen or 400 and 650 ^{o}C in a pyrolyzer continuously flushed with 99% pure gaseous N_2 (designated hereafter as Oak-250, Grass-650, etc.). The coarse size fraction (0.25 – 2 mm) of the biochars, separated by sieving and briefly rinsed with double distilled water to remove soluble salts, was used for all experiments and analyses. Detailed information on biochar preparation and chemical and physical characteristics of the freshly prepared biochars were presented elsewhere (Kasozi et al., 2010; Mukherjee et al., 2011; Zimmerman, 2010).

Duplicate samples of the nine biochars were ‘aged’ by placing in 2.5 quart plastic containers, screened above and below with 0.5 mm mesh metal screening as well as landscaping cloth above for shading (Blue Hawk™ Fabric), so that weathering by air and precipitation, but not sunlight and macro-fauna, could occur over 15 months (June 2009 – Sep. 2010) in Gainesville, Florida. During this period in the region, the average temperature was 20.6 ^{o}C and 92.1 cm of precipitation fell, all as rain, with 60% occurring during the two 4-month periods from May 1 to

September 1 of 2009 and 2010. Among the nine aged biochars, a subset of four biochars (oak and grass produced at 250 and 650 ^oC) were analyzed in greater detail for characteristics such as CEC and AEC and were incubated in combination with soil.

To study the effects of soil interaction with biochar over time, the four selected biochars were mixed with two north Florida soils, a fine sandy Entisol (PR: open agricultural field) and a forest Spodosol (BY: shaded oak-palmetto hammock forest). Characteristics of these soils are provided in Tables 1 and 2 and in Supplemental Table S1. Microbiological characteristics of these soils with and without biochar amendment were published previously in Khodadad et al. (2011). The soils were integrated from the 0 – 10 cm depth interval, sieved to 2 mm to remove plant roots and debris, and air dried for four days before fully homogenizing with the selected biochar. For each biochar type, 6 g biochar was mixed with 2.6 kg air-dried soil (0.23% biochar by weight), which resulted in soil that had organic C that was 4 – 6% and 13.1 – 18.8% pyrogenic and soil N that was 0.2 – 1.4% and 0.9 – 5.2% pyrogenic for BY and PR, respectively. These mixtures of biochar and soil (or soil alone as a control) were apportioned into duplicate 2.5 quart containers and screened as described above, but in this case, the containers were buried to a depth of 20 cm so that soil in the containers was level with the native soil. These samples were in the field during the same 15-month period and were kept free from debris by periodic removal of forest litter and weeds from the area around the emplacement.

2.2 Analytical Methods

The analytical methods used were standard methods adapted to the examination of the chemical and physical characteristic of biochar and are described in detail elsewhere (Mukherjee et al., 2011). Briefly, biochar VM content was quantified as weight loss after combustion in a ceramic crucible loosely covered with a ceramic cap at 850 – 900 ^oC for 6 min. Ash content was determined as weight loss after combustion at 750 ^oC for 6 h with no cover. The mesoporous (>1.5 nm pore diameter) SA was measured using N_2 sorptometry at 77 K using Brunauer, Emmet, and Teller (BET) theory (Brunauer, 1938) and microporous SA (<1.5 nm pore diameter) was measured using CO_2 sorptometry at 273 K and grand canonical Monte Carlo simulations of the non-local density functional theory (Jagiello and Thommes, 2004).

Elemental C, H and N were analyzed using a Carlo Erba CHNS analyzer and the O concentration was calculated by weight difference after subtracting inorganic C, of which there was minor amounts, and assuming biochars consisted of only C, H, N, and O. Selected biochar and soil samples were also analyzed for total P, K, Ca, Mg, S, B, Zn, Mn, Fe, and Cu by inductively coupled plasma mass spectrometry (ICP-MS) following acid digestion (method AOAC 985.01). Biochar pH was measured using saturated paste approach in which 200 mg of biochar was mixed with 1.25 mL of water and pH was determined after two hour equilibration time. Soil pH was measured using the traditional method of mixing 10g of soil with 20 mL water (1:2 v/v) and pH was recorded after one hour equilibration time (Rao and Kennelley, 2002). Biochar and soil CEC and AEC were determined between a pH of 6 and 7 as detailed in Mukherjee et al. (2011) whereby all surface ions were replaced with K^+ and Cl^- ions and then fully exchanged with ions of $NaNO_3$. The CEC and AEC was calculated from the amount of K^+ and Cl^- released, respectively, after accounting for entrained ions. Each of the above analyzes were made twice on each sample.

Solid-state ^{13}C-NMR spectra were obtained with a widebore Varian Inova 500 MHz spectrometer operated at 125 MHz for carbon. Each sample was packed in a 4 mm O.D. zirconium rotor and sealed with KEL-F caps. Spectra were acquired using ramped-cross-polarization (CP) and magic angle spinning at 14 kHz. Spinning sidebands were eliminated using the total suppression of sidebands sequence. A 3 s pulse delay five times longer than the longest ^{1}H spin lattice relaxation time minimized saturation effects. From a series of variable contact time experiments, a 750 μs CP contact time was determined to yield the most representative spectra. Twenty thousand free induction decays were summed for each sample, zero-filled once, and processed with 50 Hz Lorentzian line broadening. Functional group distributions were determined by integrating over defined chemical shift regions following Hamdan et al. (2012) and Kögel-Knabner (2002): 0 – 45 ppm (alkyl C), 45 – 110 ppm (N-alkyl, methoxy and O-alkyl C, including carbohydrates), 110 – 140 ppm (aryl C and cyclic C), 140 – 160 ppm (substituted aryl, including primarily phenolic C and methoxy C in hydroquinones), 160 – 190 (carboxyl C in substituted/condensed acids and esters) and 190 – 245 ppm (carbonyl C in substituted/condensed amides, ketones and aldehydes).

The NMR spectra were acquired using CP rather than the more quantitative direct polarization (DP) technique because of the extremely long acquisition times required in the latter. We recognize that CP-NMR spectra will underestimate unprotonated carbon, and especially any carbon far from protons. Comparison of these CP spectra with quantitative DP spectra (> 95% C observed) obtained on the same fresh biochars (Podgorski et al., 2012) suggest that ~75% of the carbon in these samples were observed in the CP experiments. Thus, while the NMR results should only be considered semi-quantitative and are not useful in describing absolute functional group concentrations, these data are useful in comparing biochar types because calculations of the relative changes in each functional group normalize for variable carbon observability.

Scanning electron microscopy (SEM) images of the fresh and aged biochar particles were collected on an EVO MA10 (Zeiss®) equipped with a variable-pressure secondary electron detector which can dynamically image partially hydrated samples. The samples were dried at 60 °C for 48 h but were not coated or pre-treated in any other way. At least five particles were randomly selected and examined from each homogenized sample and attempts were made to present representative images.

2.3 Statistical Analyses

All of the data are presented as mean ± standard deviation of duplicate analyses of duplicate treatments except in the case of CEC, AEC and NMR analyses for which only one of the treatments was analyzed. Means, standard deviations and regression correlation coefficients were computed using Microsoft 2003 Excel software (MS, 2003). Type-1 paired t-tests were performed using Excel data analyses tool pack (MS, 2003) in order to test for significant differences between means of various parameters. Statistical differences in variables resulting from treatments were assessed using PROC-GLM in the SAS software (SAS, 2012) by three-way ANOVA with up to 2-way interactions for most variables and 2-way ANOVA without interaction for other variables, such as CEC, with only two replicate analyses.

3. Results

The chemical characteristic of the soils and freshly-produced biochars are listed in the two left-most columns of Tables 1 and 2 and in greater detail in Supplemental data tables S1-S4. These samples have been discussed in detail elsewhere (Mukherjee et al., 2011; Zimmerman, 2010). The following focusses on the properties of aged biochars and the differences between the properties of fresh and 15-month aged biochar.

3.1 Characteristics of biochars aged alone

As with freshly-produced biochars (Mukherjee et al., 2011; Zimmerman, 2010), the physicochemical characteristics of aged biochar varied most clearly with HTT. For example, both fresh and aged biochars exhibited significant increases in C, N and H content with increasing HTT (Table 3 and Supplemental Table S1). For both fresh and aged biochars, pH, ash content, and surface area (nanopore and micropore) increased with HTT and VM decreased with HTT. The prominent trend the parameters that were related to parent biomass type , such as the increase in pH and ash content from pine to oak to grass that were found in fresh biochars, were also found in aged biochars (Table 3, Fig. 1). This was also true for C content which increase and CEC that decreased from grass to pine to oak (Table 3).

Aging had a significant effect on some of biochar's characteristics but not on others. Overall, the the 15 month field aging only had a significant effect on biochar pH and nanopore SA, which decreased, and CEC, which increased (Table 3 and ANOVA analysis results in Supplemental Table S4). Other effects of aging were limited to groups of biochars made with specific production temperatures of biomass types. For example, only high temperature biochars and grass biochars displayed a significant increase in C content and only low temperature biochar displayed a significant increase in ash content with aging. Another large change was that, while fresh biochars were found to have little AEC (measured at pH 6 – 7), that of aged biochars was considerable, ranging 24.9 – 106.1 $\mathrm{cmol_c\ kg^{-1}}$ (Fig. 2a). The abundances of other elements such as S, B, Zn, Mn, Fe and Cu ranged between 0 – 0.6 $\mathrm{mg\ g^{-1}}$ and did not show any significant trends with HTT, biomass type or aging (Supplemental Table S2).

The distribution of organic functional groups in fresh and aged oak and grass biochars made at 250 and 650^{o} C, as determined by solid state ^{13}C-NMR, are shown in Figure 3 and full chemical shift spectra are provided in Supplemental Figures S1 and S2. Though only semi-quantitative, they show the greatest effect of aging on biochar bulk chemistry to be the loss of methoxy- and O-alkyl C groups, probably associated with carbohydrates. All the biochars showed gains in the relative abundance of other O-containing C groups with aging including substituted aryl, carboxyl and carbonyl-C. In contrast, relative changes in alkyl-C and aryl-C group abundances with aging were small and did not vary consistently with biochar HTT or biomass type.

The significant interrelationships found between the properties of aged biochar (Supplemental Table S5) were similar to those found for fresh biochars (Supplemental Table S6), which may reflect their common strong dependence upon HTT. For example, in both fresh and aged biochars, VM content was inversely related to both pH and CO_2-SA and directly related to O/C ratio. However, there were fewer strong relationships among the variables measured for all the biochars pooled (Supplemental Table S7) showing that aged biochar properties were distinct from those of fresh ones.

3.2 Characteristics of soil/biochar mixtures

The chemical characteristics of the control soil and the soil-biochar mixtures after 15 months of field-aging are listed in Tables 1 and 2 in the columns labeled ‘Aged Soil’ and ‘Aged Soil + Biochar (Meas.)’, respectively. While the C and N contents of the BY control soil were little changed after the aging period, those of the PR soil decreased by about half, likely due to the disturbance (drying/rewetting, homogenization, etc.). Soils mixed with biochar had, unsurprisingly, significantly greater C contents both initially and after aging. This was also true for N content in most cases.

Though the CEC of biochar aged separately was quite high, that of the aged soil/biochar mixtures was not much higher, and in fact, was sometimes lower, than the original soil (15.6 – 25.0 and 19.9 – 22.2 $\text{cmol}_c\ \text{kg}^{-1}$, for aged BY and PR soil respectively, Table 2). In contrast, the AEC of the aged soil/biochar mixtures was always the same or greater than that of the original soil, ranging 4.9 – 7.8 and 8.1 – 9.3 $\text{cmol}_c\ \text{kg}^{-1}$ for aged BY and PR soil, respectively. After aging, soil mixed with biochar, even those of higher pH, showed a decrease in pH, all falling within a narrow pH range (5.5 – 6.2, Table 2).

In SEM images surfaces of the fresh biochars were relatively clean showing cellulosic structure and no sign of microbial colonization (Fig. 4). In contrast, all aged biochars had abundant coccoid microbes 2 – 5 μm in diameter in about half of each randomly chosen view of the external surcfaces of each of the 10 biochar particles ~~sample~~examined. By comparison, biochar particles picked from aged soil-biochar mixtures had abundant microbial colonies on internal and external surfaces and coatings that may have been soil mineral phases, soil OM or microbial exudates. These were present in nearly all randomly chosen images of the charcoal particles. Filamentous and hyphal microorganisms were also observed less frequently on the outer surfaces of both biochar aged alone and when mixed with soil (Fig. 4c).

4. Discussion

4.1 Aging processes of biochar alone

The variations in physicochemical effects due to aging of the different biochar types observed in this study can explain some of the contradictory findings of previous biochar aging studies using laboratory incubations or examination of environmental charcoals. For example, little effect on biochar elemental composition was found after laboratory-simulated aging (Hale et al., 2011; Yao et al., 2010). In contrast, a significant decrease in C and increase in O was found after one year moist aging of ‘traditionally-made’ oak biochars at temperatures ranging from -22 to 70 ^{o}C (Cheng and Lehmann, 2009). Changes in bulk chemistry that occurred during aging were shown in the present study to vary with both parent material and production temperature (Supplemental Table S4). This is also true of changes in surface area (Table 3). Although this study and others (Hale et al., 2011; Liu et al., 2013) have found great variations between biochar types, the most common effect of aging on biochar surface structure is a decrease in nanopore SA (but little change in sub-nanopore SA~~), ~~). This may be due to pore blockage by dissolution and re-precipitation of inorganic minerals as only sparse ~~likely due to pore blockage by microbes, OM or inorganic precipitates as suggested by~~coverage by microbes or OM coatings were observed in the SEM images (Fig. 4).

Decreases in pH during biochar aging of biochar has been observed previously (Cheng and Lehmann, 2009; Cheng et al., 2006) and has been explained as due to dissolution of basic species such as carbonates or oxy/hydroxides (Yao et al., 2010). However, in the current study, no significant decrease in base cation contents (other than potassium) after aging was detected and significant inverse correlations were found between pH and O/C of aged biochar (Supplemental Table S5). Thus, surface acidity is likely to have been generated through extensive oxidation, which is also supported by the ion exchange and NMR data. Increase in biochar acidity has been previously attributed to the abiotic chemisorption of oxygen and/or water from air onto biochar surfaces over time (Adams et al., 1988; Billinge et al., 1984; Cheng et al., 2006).

The increase in ion exchange sites with biochar aging indicated by the CEC and AEC data may be due either to progressive abiotic oxidation of surface functional groups (for CEC) or to ~~the sorbed OM produced by microbes colonizing biochar surfaces observed in SEM (Fig. 4)~~precipitation of minerals on the biochar surfaces as has been suggested by other work (Joseph et al., 2010). In fact, the CEC and AEC of these aged biochars are 10 times greater than that of most soils and more similar to that of soil humic acids (Harada and Inoko, 1975). However, aged biochars were previously reported to have no detectable AEC and to have CECs about 10 times less than that measured in the present study (Cheng and Lehmann, 2009; Cheng et al., 2008; Cheng et al., 2006). It may be that, in contrast to laboratory studies, the aging recorded here was more intense and microbial colonization encouraged to a greater extent by the humid tropical climate of the study site.

The role of oxidation in increasing surface acidity and CEC of the biochars is supported by the NMR data showing relative increases in oxygenated functional groups including substituted aromatic, carboxyl and carbonyl-C (Fig. 3). Amide functional groups may be among those detected as carbonyl-C group and could be responsible for the increased AEC of aged biochar. Increases in relative abundances of carboxyl-C and carbonyl-C during were previously detected on aged biochar surfaces using Fourier-transform-infrared (FT-IR) and XPS spectroscopy (Cheng and Lehmann, 2009; Cheng et al., 2008; Cheng et al., 2006; Lin et al., 2012b) and in bulk biochar using ^{13}C-NMR (Hilscher and Knicker, 2011; Yao et al., 2010). However, none of these studies detected increases in substituted aromatic (e.g. phenols, quinones and lactones), and one study even recorded major decreases in these functional groups during biochar aging (Nguyen et al., 2008). These groups are of particular significance in that they are intermediates in the formation of soil humic substances according to the polyphenol formation theory (Stevenson, 1994). In addition, these compounds serve as electron acceptors for microbial respiration (Scott et al., 1998).

In this study, the largest shift in biochar molecular chemistry indicated by NMR due to aging was the loss of methoxy- and O-alkyl C, probably associated with carbohydrates. One previous study that examined biochar aging in leached soil columns over 28 months recorded similar large losses in O-alkyl C (Hilscher and Knicker, 2011) whereas as all other studies either found no change or increases in these groups. Decreases in polysaccharides have been observed previously in soil OM aging experiments and have been attributed either to microbial decomposition (Baldock et al., 1992; Zech et al., 1992) or to solubilization and loss of carbohydrates during rewetting cycles (Preston et al., 1989; Schmidt et al., 1997). Loss of carbohydrates via solubilization seems the more likely explanation here since the O-alkyl C fraction is in much greater abundance in the low HTT biochar which also showed the greatest losses of organic C and N during laboratory successive leaching experiments conducted with same biochars (Mukherjee and Zimmerman, 2013). Biochar aging studies that did not record these losses were generally laboratory incubation studies in soil without water flow-through and, thus, may have shown greater effects of soil OM sorption versus leaching.

As a whole, the data suggest the creation or transformation of a wide range of functional groups on biochar surfaces~~, and likely interiors, ~~during aging~~ via oxidation, leaching and addition of microbially-produced OM~~. Evidence for the occurrence of a combination of aging processes is ~~also ~~present in the evolution of biochar's exchange capacities, both CEC and AEC. In fresh biochars, VM content was judged to be mainly responsible for its CEC (Mukherjee et al., 2011). However, biochar CEC increased after one year of aging though there was no significant change in VM content during this time-frame. Further, the CEC of aged low and high temperature biochars were similar though the VM content of the former was much greater. Thus, a different process, or more likely, a combination of processes, must be responsible for the enhanced exchange capacity of aged biochars. The similarity of aged biochar CEC to that of soil humic substances, the appearance of AEC~~, and the presence of microbes and OM in images of biochar surfaces,~~ suggest that biochar aging processes include: 1) addition and conversion of a wide variety of oxygen containing functional groups by abiotic and microbially-mediated oxidation, 2) leaching loss of more soluble biochar organic components, and, to a lesser extent, 3) sorption of microbially-derived OM onto biochar surfaces.

4.2 Aging processes in soil/biochar mixtures

While it seems obvious that soil amendment with a material such as biochar with high C content, surface area, ion exchange and pH (for high HTT biochar) would result in a soil enhanced in these properties, this was not always the case after a period of aging. This is certainly due to the changes in biochar properties during aging (discussed above), but also likely due to interactions between the soil and biochar during aging. To explore this, the chemical properties predicted by the weighted addition of biochar and soil aged were compared separately ('Aged Add' in Tables 1 and 2) to those measured in mixtures after aging ('Aged Soil + Biochar-Meas.'). For C and N content, this calculation shows that, except in the case of grass biochar with BY soil, the interaction was large and positive. That is, the interaction of soil and biochar during aging resulted in soil with about twice the C and N content of the additive combination of the aged soil and aged biochar. This has previously been termed 'negative priming' (the inhibition of OM mineralization due to the addition of a substrate) and has been previously observed in some laboratory incubations of biochar with soil (Jones et al., 2011a; Jones et al., 2011b; Santos et al., 2012; Zimmerman et al., 2011). A possible mechanism for negative priming is the enhanced sequestration of soil OM (both microbial and plant-derived) through biochar sorptive protection. This explanation is supported by the observation of greater positive interaction with the higher HTT and oak biochars which have greater surface area (Table 3) and sorb more OM (Kasozi et al., 2010) than low temperature and grass biochar. In addition, these finding are also in concordance with a previous study using the same biochars (but different soils) which showed that soil C mineralization was inhibited during long-term *in vitro* incubation of these high HTT and oak biochars mixed with soil (Zimmerman et al., 2011). ~~Another possibility is that biochar-derived OM was sorbed by soils minerals as this was shown to occur during laboratory soil/biochar column leaching experiments using same biochars (Mukherjee and Zimmerman, 2013).~~ The observation of abundant OM and microbes on biochar surfaces that had been incubated with soil (SEM in Fig. 4d) supports this mechanism. It should be noted, however, that the theory that biochar serves as a microbial habitat has been challenged recently. For example, only sparse microbial colonization was observed on the surfaces of biochar aged in the field over three years (Quilliam et al., 2013) or collected from centuries-old abandoned charcoal hearths (Criscuoli et al., 2014). However, neither of these study sites were as hot or moist as the subtropical environment in which our experiments were conducted.

Though biochar CEC and AEC increased significantly when aged alone, it was predicted to have only a small effect on the ion exchange capacities of the soil/biochar mixtures due to the small amount of biochar added ('Aged Add.' column in Table 2). However, except in the case of BY soil mixed with oak biochar, the interaction of biochars with soil during aging resulted in 26-43% increases in CEC and a 21-41% increases AEC beyond those predicted. The greater production of ion exchange capacity could be explained, as with C and N, by the additional storage of soil or biochar-derived OM. However, this would be expected to increase exchange capacity the most in those soils with the greatest C increase (BY/Oak mixtures). Thus, ion exchange capacity was increased through microbially mediated oxidation of biochar, which was stimulated to the greatest extent in the sandier agricultural soil with a lower native C content and by additions of grass biochars (Table 2) which release greater nutrients than oak biochars (Mukherjee and Zimmerman, 2013). No consistent interaction was calculated for pH suggesting a possible buffering effect by biochar-soil mixtures.

Clearly, predicting the longer term impacts of biochar addition on soil chemistry is not a simple case of proportional mixing of two substances with different starting compositions. Even after the chemical effects of aging of each separate material is accounted for, interactions between biochar and soil occurred, ~~possibly~~ likely including microbial colonization and OM production, soil and biochar OM remineralization, leaching and sorption during aging of biochar-soil mixtures. This wide range of processes, which would be expected to vary in extent with time and be both biochar and soil type-dependent, may explain why both increases and decreases in soil-C and biochar-C were observed in this study and others (Hilscher and Knicker, 2011; Lin et al., 2012b; Nguyen and Lehmann, 2009; Spokas, 2013; Yao et al., 2010).

5. Conclusions and Significance

The findings of this study have important implications for agricultural soils amended with biochar as well as natural systems subjected to repeated burning events. First, they show that aging of biochar and biochar-soil mixtures is involved in producing or enhancing many of the beneficial properties of biochar-amended soil including fertility and C sequestration-enhancement. For example, increases in exchange capacity over time may increase crop yields by decreasing losses of nutrients and organic matter, and increased abundance of certain functional groups, such as phenols and quinones, may encourage the humification process. The interaction between aged biochar and soil generally magnified the development of these beneficial properties and suggest the involvement of soil microbes and native soil OM in obtaining the full benefits of biochar for soil amelioration. Whether these aging processes continue along these same trends with greater passage of time is an important area for further research.

Whether for modeling the effects of natural or anthropogenic biomass burning on atmosphere CO_2 concentrations or for assigning C credits to those practicing large-scale soil amendment projects, better assessment of the effects of pyrogenic C additions on soil C dynamics is needed. In documenting increases in soil C beyond those expected merely from the addition of biochar C, this study shows that simple C addition metrics, even when biochar C mineralization rates are factored in, may be inadequate as estimates of biochar's atmosphere C drawdown effect. Though positive priming (enhanced losses of soil OM due to biochar addition, or vice versa) has been observed in incubation studies (Steinbeiss et al., 2009; Wardle et al., 2008), this study, using a field approach, ~~confirms~~ suggests the importance of negative priming (reduced soil C losses due to biochar interaction) as has been observed in some long-term laboratory incubation studies (Jones et al., 2011a; Jones et al., 2011b; Santos et al., 2012; Zimmerman et al., 2011).

Finally, this study makes progress toward the goal of being able to produce and apply biochar types that are best suited for particular soil types and intended uses such as nutrient retention, C sequestration, or even contaminant immobilization. The effect of biochar on soil chemistry was not always what would be predicted given the character of fresh biochar. For example, after aging, low temperature biochars still had greater CEC than high temperature biochars, but the latter developed greater AEC. Thus low temperature biochars may be best suited to prevent nitrates or metal leaching while low temperature and grass biochars are better for improving phosphorus-deficient soils. These effects were greatest in low-C soils which may explain some observations of little fertility enhancement after biochar addition to already fertile OM-rich soils. While high temperature biochar C is known to be more resistant to mineralization and, thus, better suited to C sequestration objectives (Nguyen et al., 2010; Zimmerman, 2010), in the OM-poor soil examined here, low temperature biochar amendments resulted in similar C stocks over time due to its greater positive interaction with native microbes and soil-OM. Thus, a more complex picture is emerging of the effects of biochar amendment on soil chemistry. It may be that optimization will only be achieved by pre-testing soil-biochar combinations before carrying out large-scale biochar amendment projects. Studies incorporating microbial and molecular techniques are required to better identify the chemical changes and associated biogeochemical processes that occur during the aging of biochar in soil.

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Supplementary material

Supplementary data associated with this article can be found at XXXXX

Figure Captions

Figure 1. Relationship between pH and biochar production temperature for a range of fresh and aged biochars. Error bar shows the standard deviation of two replicate analyses.

Figure 2. Cation and anion exchange capacities (CEC and AEC, respectively) measured at pH 6-7 on a) fresh and ‘aged’ oak and grass biochars produced at 250 and 650 ^{o}C, and b) aged BY soil and BY soil/biochar mixtures

Figure 3. Relative functional group distributions determined by ^{13}C CP-MAS NMR for fresh and aged a) Oak-250, b) Oak-650, c) Grass-250, and d) Grass-650 biochars.

Figure 4. Representative scanning electron micrographs of fresh Oak-250 and Oak-650 biochar (a and b, respectively), and Oak-650 biochar field-aged alone (c) and with soil (d). Scale bars = 10 μm (a) and 5 μm (b-d). Coccoid microbes are indicated with arrows.

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Table 1. Average concentrations of C and N in fresh and 15-month field-aged soils, biochars and soil/biochar mixtures (Meas.) and those predicted by the arithmetic weighted combination of fresh and aged biochar and soil (Add.).

	Fresh Soil	Aged Soil	Fresh Biochar	Aged Biochar	Aged Soil + Biochar-Meas.	Aged Add.[1]	%Diff. (Aged Add/Meas.)[2]
				C (mg g^{-1})			
BY/Oak-250	27	27	626	594	49	28.3	73
BY/Oak-650	27	27	754	813	54	28.8	87
BY/Grass-250	27	27	494	575	26	28.3	-9
BY/Grass-650	27	27	557	704	31	28.6	9
PR/Oak-250	7.5	3.3	626	594	10	4.7	115
PR/Oak-650	7.5	3.3	754	813	11	5.2	113
PR/Grass-250	7.5	3.3	494	575	7.9	4.6	71
PR/Grass-650	7.5	3.3	557	704	11	4.9	124
				N (mg g^{-1})			
BY/Oak-250	1.8	2.2	1.9	2.5	3.1	2.2	41
BY/Oak-650	1.8	2.2	4.6	2.8	3.4	2.2	54
BY/Grass-250	1.8	2.2	12.0	9.8	2.0	2.2	-10
BY/Grass-650	1.8	2.2	5.7	15	2.1	2.2	-6
PR/Oak-250	0.5	0.2	1.9	2.5	0.4	0.2	95
PR/Oak-650	0.5	0.2	4.6	2.8	0.5	0.2	143
PR/Grass-250	0.5	0.2	12.0	9.8	0.4	0.2	80
PR/Grass-650	0.5	0.2	5.7	15	0.5	0.2	114

Notes/Abbreviations:

BY: agricultural Entisol, Gainesville, Florida, PR: forest Spodosol, Marion County, Florida, USA

1. Calculated as the weighted additive value of properties of aged biochar and aged soil
2. %Diff. (Aged Add/Meas.) = the difference between that predicted by the additive combination of the aged soil and aged biochar (Aged Add.) and that actually measured in the aged mixture [Aged Soil + Biochar-Meas.].

Table 2. Average cation and anion exchange capacity and pH of fresh and field-aged soils, biochars and soil/biochar mixtures (Meas.) and those predicted by the arithmetic weighted combination of each biochar and soil (Add.).

	Fresh Soil	Aged Soil	Fresh Biochar	Aged Biochar	Aged Soil + Biochar-Meas.	Aged Add.	%Diff. (Aged Add/ Aged Meas.)
				CEC at pH 6 – 7 ($\text{cmol}_c\ \text{kg}^{-1}$)			
BY/Oak-250	21	17.7	39.9	136	15.6	18.0	-13
BY/Oak-650	21	17.7	10.2	111	12.4	17.9	-31
BY/Grass-250	21	17.7	69.2	206	23.1	18.1	28
BY/Grass-650	21	17.7	40.8	238	25.0	18.2	38
PR/Oak-250	15	15.3	39.9	136	20.5	15.6	32
PR/Oak-650	15	15.3	10.2	111	22.2	15.5	43
PR/Grass-250	15	15.3	69.2	206	21.5	15.6	38
PR/Grass-650	15	15.3	40.8	238	19.9	15.8	26
				AEC at pH 6 – 7 ($\text{cmol}_c\ \text{kg}^{-1}$)			
BY/Oak-250	4.7	5.7	4.9	70	4.9	5.9	-16
BY/Oak-650	4.7	5.7	4.5	13	4.7	5.8	-18
BY/Grass-250	4.7	5.7	1.8	34	7.1	5.9	23
BY/Grass-650	4.7	5.7	1.4	15	7.8	5.9	36
PR/Oak-250	6.5	6.5	4.9	70	8.7	6.6	32
PR/Oak-650	6.5	6.5	4.5	13	9.3	6.6	43
PR/Grass-250	6.5	6.5	1.8	34	9.2	6.6	40
PR/Grass-650	6.5	6.5	1.4	15	8.1	6.7	24
				pH [1]			
BY/Oak-250	6.7	5.8	3.5	4.0	5.8	5.7	1
BY/Oak-650	6.7	5.8	9.1	6.7	5.5	5.8	-5
BY/Grass-250	6.7	5.8	4.5	4.2	5.8	5.8	1
BY/Grass-650	6.7	5.8	10.0	7.0	6.1	5.8	5
PR/Oak-250	6.5	6.3	3.5	4.0	6.0	6.1	-2
PR/Oak-650	6.5	6.3	9.1	6.7	6.2	6.3	-2
PR/Grass-250	6.5	6.3	4.5	4.2	6.2	6.2	0
PR/Grass-650	6.5	6.3	10.0	7.0	6.1	6.3	-3

Notes/Abbreviations:

Abbreviations used are those listed for Table 1.

1 Additive values were calculated after converting pH to H^+ concentrations, which were then converted back to pH units.

Table 3. Mean values of various parameters for fresh and aged biochars as a whole (n = 18) and grouped. The difference between fresh and aged biochar is significant, computed by Type-1, paired T-test, at $p < 0.1$ when underlined and only $p < 0.05$ level when also with an asterisk.

Biochar	pH		VM (%)		Ash (%)		N_2-SA ($m^2 g^{-1}$)		CO_2-SA ($m^2 g^{-1}$)		C (mg g^{-1})		O/C		CEC ($cmol_c$ kg^{-1})	
Group	Fresh	Aged	Fresh	Aged	Fresh	Aged	Fresh	Aged	Fresh	Aged	Fresh	Aged	Fresh	Aged	Fresh	Aged[a]
Overall	$\underline{6.3}$	$\underline{5.3}$	49.6	50.8	5.1	3.9	$\underline{63.2}$	$\underline{9.4}$	351	343	617	667	0.5	0.5	$\underline{26.2*}$	$\underline{173*}$
250 °C	3.7	4.0	63.2	65.0	$\underline{2.8*}$	$\underline{3.6*}$	1.6	1.9	277	232	581	576	0.8	0.7	$\underline{43.6*}$	$\underline{171*}$
400 °C	6.5	5.5	54.0	55.5	5.4	3.8	$\underline{3.7}$	$\underline{1.4}$	252	247	653	660	0.5	0.5	13.8	-
650 °C	$\underline{8.6*}$	$\underline{6.4*}$	31.5	31.9	6.9	4.4	$\underline{184*}$	$\underline{25*}$	523	550	$\underline{621}$	$\underline{766}$	0.3	0.5	$\underline{18.4*}$	$\underline{174*}$
Oak	6.4	5.4	51.4	48.6	2.6	2.4	76	12	351	349	686	706	0.5	0.4	$\underline{18.4*}$	$\underline{123*}$
Pine	5.0	4.7	48.3	50.5	0.6	0.8	86	1	$\underline{423}$	$\underline{349}$	645	658	0.4	0.7	22.7	-
Grass	7.4	5.7	49.0	53.3	12.0	8.6	28	15	278	331	$\underline{525*}$	$\underline{638*}$	0.6^*	0.5^*	$\underline{37.4}$	$\underline{222}$

Abbreviations: SA = surface area, VM = volatile matter, CEC = cation exchange capacity measured at pH 6-7.

a: n = 4