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Biogeochemical Alteration of Particulate Pyrogenic Organic Carbon (pyC)

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The University of Southern Mississippi

BIOGEOCHEMICAL ALTERATION OF PARTICULATE PYROGENIC ORGANIC CARBON (*py*C)

by

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in Partial Fulfillment of the Requirements
for the Degree of Master of Science

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ABSTRACT

BIOGEOCHEMICAL ALTERATION OF PARTICULATE PYROGENIC ORGANIC CARBON (*py*C)

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The resistance of plant derived pyrogenic organic carbon (*py*C) to abiotic and biotic means of degradation has led to increased interest in the role of *py*C as a potential carbon sink as well as a strategy for sequestering atmospheric CO_2 to mitigate excess carbon emissions. Though much research pertaining to the fundamental properties of *py*C degradation have been performed in a controlled lab setting, there has been very little work regarding *py*C degradation in a field setting where factors such as precipitation and temperature fluctuate seasonally. This work focuses on various degradation characteristics of two different types of plant-derived *py*C, placed in both a burned and unburned setting, over a study period of 1-year. We observed that the potential for DOC from outside sources to adsorb itself to the surface of *pyC* increases in fall and spring due to increases in leaf litter and increases in temperature, respectively. We also observed that the overall recalcitrance of pine-derived *pyC*s decreases with time and the overall recalcitrance of CG-derived pyCs increases with time, as different portions of the *pyC*s are utilized by microbes depending on the microbial community present. Finally, we observed that *pyC*s degrade differently in burnt and unburnt sites. Namely, a more specialized microbial community found in burnt sites is able to utilize pine *py*Cs faster than the microbial community found in unburned sites. However, this specialization leads to the opposite trend occurring for CG *py*Cs, in which degradation occurs more quickly in unburnt sites than in burnt sites.

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CHAPTER I

INTRODUCTION

The Importance of pyrogenic carbon (*py*C)

The soil organic carbon (SOC) pool represents the largest reservoir of carbon in the biosphere-atmosphere system. Consequently, even small changes in the SOC pool can potentially have a significant impact on global C balance, and subsequently, global climate (IPCC, 2007). Recent evidence suggests that pyrogenic organic carbon (*pyC*) produced by burning biomass makes up a substantial part of the SOC pool, ranging from 2 to 45% of the total organic carbon in terrestrial systems (Lehmann et al., 2008, Skjemstad, Taylor, & Smernik, 1999; Skjemstad, Reicosky, Wilts, & McGowan, 2002). Estimated global rates of *pyC* production are small (40-259 million tons/yr) when compared to the global rate of total OC production. However, total amounts of *pyC* in soils are large because of its high innate resistance to both abiotic and biotic forms of degradation compared to non-pyrogenic carbon (Jaffe et al., 2013; Schmidt & Noack, 2000). The conversion of OC into *pyC* results in a shift of C to a more recalcitrant pool with a slower rate of microbial degradation and hence, longer turnover times. Compared to non-pyrolysed OC, with turnover times of a few years, turnover times for *pyC* are on the order of hundreds to thousands of years (Liang et al., 2008). The recognition of *pyC* being more resistant to abiotic and biotic degradation has led to increased interest in *pyC* as a potential C sink, as well as a strategy for sequestering atmospheric CO_2 to mitigate excess carbon emissions (Lehmann et al., 2006).

Current estimates for C sequestration potential by *pyC* are as high as $9.5x10^9$tons/yr and are comparable to estimated C emission from fossil fuel burning

(9.1×10^9tons/yr; Lehmann et al., 2006). However, this number will fluctuate as a consequence of varying *pyC* characteristics and environmental conditions. The composition of *pyC*s are known to be heterogeneous, with their innate recalcitrance being a function of their degree of thermal transformation as dictated by feedstock chemistry and pyrolysis conditions (Harvey, Herbert, Kuo, & Louchouarn, 2012; Zimmerman, 2010). For example, grass- derived *pyC*s show significantly faster turnover times than wood-derived *pyC*s produced under the same pyrolysis conditions, which reflects the differences in the chemical structure of the feedstock and the byproducts of pyrolysis. Recalcitrance of *py*C has also been shown to increase with pyrolization temperatures, due to the thermal transformation of *pyC* materials to form increasingly aromatized structures (Harvey et al., 2012). Variability in soil properties, temperature, nutrient availability and moisture conditions also impacts microbial community distribution and activity, and consequently the rate of *pyC* degradation (Torsvik & Øvreås, 2002: Waldrop & Fireston, 2006; Zogg et al., 1997). Recently, Mukherjee, Zimmerman, Hamdan, & Cooper (2014) observed that the degradation of *pyC* added to an agricultural soil was very different than when the same *pyC* was added to a forest soil. The difference in degradation in *pyC* was attributed to differences in soil composition and structure. Studies of microbial communities in ancient *pyC* enriched soils have also shown that these soils tended to have a greater microbial biomass and, in some cases, greater microbial diversity than surrounding non-enriched soils (O’Neil et al., 2009; Khodadad, Zimmerman, Green, Uthandi, & Foster, 2011). Khodadad et al. (2011) found that the growth of bacteria within the phyla *Actinobacter* and *Gemmatimonadetes* were stimulated in *pyC* amended soils compared to unamended soils. Studies across climatic gradients have also shown higher *pyC* degradation rates in areas of warmer temperatures and higher precipitation, due to increased rates of chemical weathering and microbial activity (Cheng, Lehmann, Thies, & Burton, 2008).

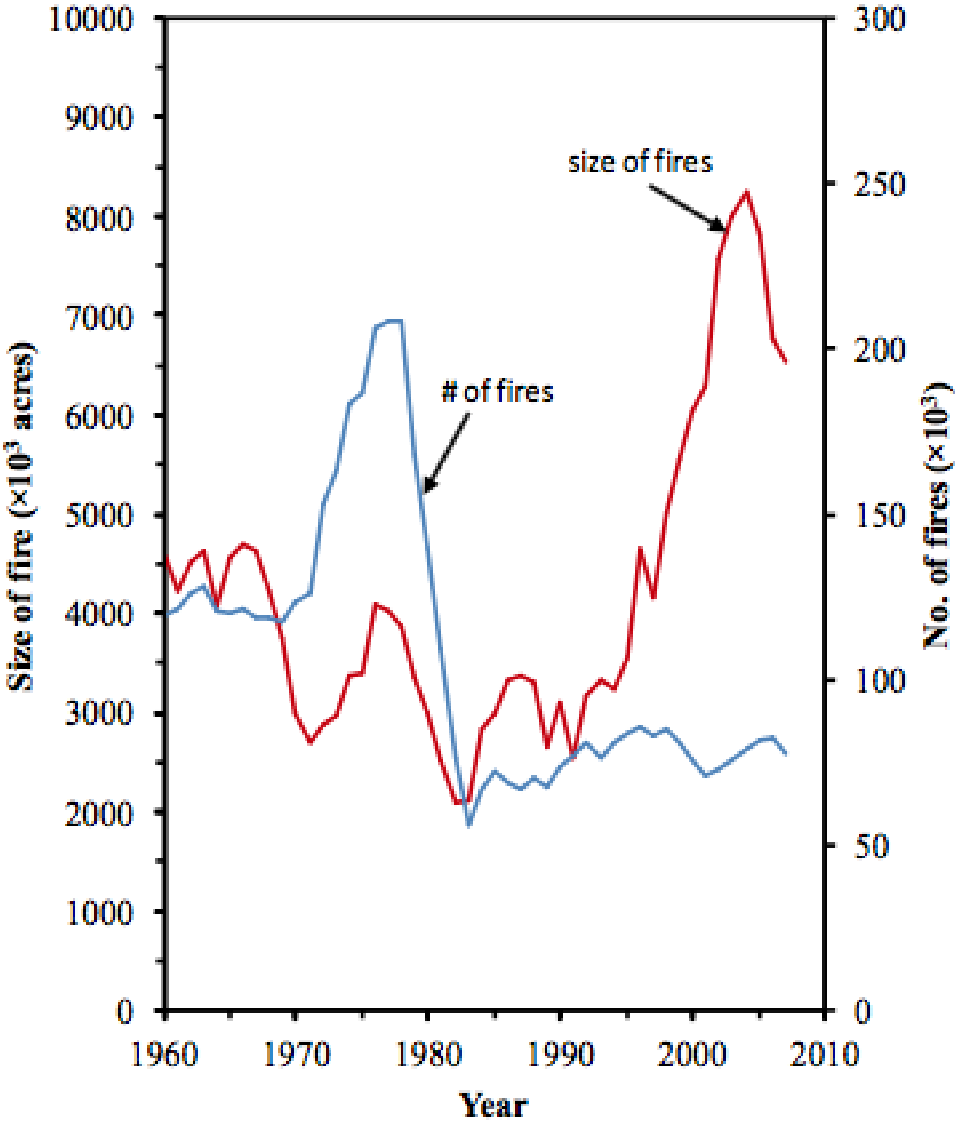

Figure 1. Wildfire size and occurrence in the United States since 1960.

In addition to increased *pyC* turnover times, increases in mean global temperatures could also have an effect on the amount of *pyC* generated by wildfires

(Flannigan, Stocks, & Wotton, 2000). Since 1980, wildfires have nearly tripled in size in the United States, while the number of wildfires has remained relatively stable (Figure 1). Approximately 3-5% of biomass is converted into *pyC* during a wildfire event, and as the total area of burnt land increases, we can also expect to see an increase in the amount of *pyC* generated, possibly increasing the potential amount of CO_2 sequestered (Skjemstad et al., 1999; Skjemstad et al., 2002; Forbes, Raison, & Skjemstad, 2006).

Wildfires, and therefore *pyC*s, are especially important in areas such as the Pascagoula River Basin where 52% of the total area ($24844km^2$) is covered by pine forest. Based on prescribed burning records obtained from the US Forest service, there are 80,000- 120,000 acres of government owned forested land that are burnt annually within the Pascagoula watershed, primarily near the end of winter when plants are dormant. Using a distribution factor of 136g *pyC*/m^2 (Alexis et al., 2007) and assuming *pyC* loss of 10.6% in dissolved form (Jaffe et al., 2013), an estimated ~46,000 to 69,000 tons of *pyC* is generated annually within the Pascagoula River Basin on government owned land. This number is very conservative and could be 2 to 3 times higher when prescribed burning on privately owned land is considered.

While *pyC* and the factors that influence its degradation has received increased attention within the past two decades, there are still significant knowledge gaps that exist in our understanding of *pyC* and its potential for CO_2 sequestration. Most studies concerning *pyC* have been conducted under laboratory conditions where specific components of *pyC* degradation are controlled in order to provide fundamental information on *pyC* behavior. However factors such as microbial influences and seasonal changes in environmental conditions (e.g. temperature and precipitation) cannot be taken into account within these experiments. Increasing wildfire size, and subsequent burning on previously unburnt areas, has the potential to form *pyC* from different feedstock materials, and involve complex interactions within microbial communities that are not adapted to utilizing *pyC* as a carbon source. The overarching goal of the present study was to address three questions concerning how increased fire size and seasonal variability in temperature and precipitation will impact *pyC* within the Pascagoula River Basin.

Specific questions are:

1. What effects do temporal fluctuations in temperature and precipitation have on field degradation of *pyC*?□We hypothesized that higher average temperatures in spring and summer will result in more rapid changes to the C content of buried *py*C.

2. Does *pyC* degrade differently in repeatedly burnt areas compared to an unburned setting? We hypothesized that, due to differences in microbial communities in burnt and unburnt sites, pyC in burnt areas will degrade faster than pyC in unburnt areas.

3. Do changes in feedstock associated with potential shifts in vegetation cover alter degradation characteristics of *pyC* within the same setting? We hypothesized that, based on differences in initial feedstock chemistry, cordgrass pyC will see faster rates of degradation than pine pyC in a field setting.

CHAPTER II

LITERATURE REVIEW

Pyrogenic carbon is often considered as continuum of materials, ranging from partially combusted plant remnants to charcoal, soot, and graphite (Preston & Schmidt, 2006). Components within this continuum are high in C content with significant aromatic structures present on the upper end (Masiello, 2004). Pyrogenic carbon is formed through the combustion of organic material in a low or oxygen-free environment, and can occur both naturally and by anthropogenic means. Pyrogenic carbon has been shown to exhibit a high degree of resistance to both biotic and abiotic forms of degradation. This resistance to degradation leads to *pyC* having a high relative stability within soil systems, where it has been show to represent the oldest fraction of total soil C (Pessenda et al., 2001). Soil turnover times for *pyC* have been found to range from hundreds to thousands of years. The ability of *pyC* to remain relatively un-altered over long time scales has led to increased interest in *pyC* as a potential carbon sink, as well as a method for sequestering atmospheric CO_2. When taking into account the total amount of *pyC* produced from any source, Lehmann, Gaunt, and Rondon, (2006) estimated that the maximum amount of potential sequestration of *pyC* is $9.5X10^9$tons/yr. There is, however, a two-way paradigm that has the potential to cause this estimate to fluctuate. The first paradigm involves the structure of the *pyC* itself, and is a factor of *pyC* feedstock material and pyrolysis conditions. The second paradigm involves the environment in which *pyC* resides, and is comprised of several factors including temperature, precipitation, soil structure, and the activity and diversity of the soil microbial community.

The key factor underpinning the structure paradigm is that, for a given environment, stability of *pyC* is known to increase with the degree of aromatic condensation/aromaticity (Harvey et al., 2012). There is now widespread consensus that aromaticity in *pyC* is a function of both feedstock chemistry and heat treatment conditions (Bruun et al., 2011). For example, woody feedstocks and increasing heat treatment temperatures (HTT) have been shown to produce *pyC*'s with increasing aromaticity. McBeath and Smernik (2009) used solid-state NMR to examine variations in the amount of aromaticity of 17 *pyC*s produced from different feedstocks and under varying HTT. The materials examined included wood-derived (e.g. Eucalyptus and chestnut), grass-derived (e.g. rice and Phalaris) and manure-derived *pyC*s produced at HTT of 250, 450 and 850 ^{o}C). Results from their study showed that aromatic C content in the *pyC*s produced at the lowest HTT (i.e. 250 ^{o}C) ranged from 33-41%, with most of the material consisting of carbohydrate (mostly cellulose) structures. In contrast, aromatic carbon content in *pyC*s produced at higher HTTs (i.e. 450 and 850^{o}C) was 83-84%. NMR spectra also suggested that the degree of aromatic condensation increased with lignin content, supporting similar observations made in previous studies (Freitas, Bonagamba, & Emmerich, 2001; Liou, Marayama, Kakimoto, & Imai, 1998).

Recently, Harvey et al. (2012) used 2 dimensional infrared spectroscopy (2D-IS) to examine the formation process of three different types of *pyC*'s along a HTT gradient. Materials studied included wood and grass-based *pyC*'s produced at HTT's ranging from 200-650^{o}C in increments of 50^{o}C. Results of 2D-IS suggested that defragmentation of the lignocellulose H-bonding network and subsequent changes to the lignocellulose fragments are primary drivers of *pyC* properties along the HTT gradient. Specifically, the dehydrogenation of methylene groups (R-CH_2-R □ R=C-R □ R=C=R) was linked to increasing amounts of aromatic condensation and *pyC* recalcitrance, and could possibly explain why plant biomass rich in carbohydrates (high in R-CH_2) tends to yield *pyC* with higher amounts of aromatic structures.

The effects of feedstock chemistry and pyrolysis temperature on *pyC* aromaticity, and therefore stability, were observed in a 5-year study by Singh et al. (2012). Pyrogenic carbons used in this study were created at two different temperatures (400 and 550C) from multiple feedstocks. Materials studied included wood, grass, and manure-derived *pyC*'s. Multiple samples of each type of *pyC* were incubated in darkness for a total of 5 years, with multiple sampling periods taking place within the study period. The cumulative amounts of *pyC* C lost as CO_2 over the 5-year period were then fitted to an exponential model in order to estimate the mean residence times of the *pyC* materials. Results of their study showed that between feedstocks, wood based *pyC*'s showed the least amount of C mineralization while manure based *pyC*'s showed the highest amounts of C mineralization. The amount of C mineralized was also found to be consistently lower for the higher HTT *pyC*'s than for the lower HTT *pyC*'s. These trends were reflected in the predicted mean residence times for the different *pyC*'s. For instance, when fitted to the exponential model, wood based *pyC*'s produced at 400C and 550C had mean residence times of 326 and 1271 years, respectively, while leaf based *pyC*'s had mean residence times of 270 and 572 years, respectively.

As previously mentioned, structural differences in *pyC* due to formation conditions are not the only factor when considering *pyC* stability and residence times. The presence and activity of microbes can play a major role in *pyC* turnover rates.

Khododad et al. (2011) used multiple techniques to study changes in soil microbial community as a result of pyrogenic carbon additions to various soil types. Materials studied include wood-derived and grass-derived *pyC*'s produced at two HTT's (250^{o}C and 650^{o}C). Mixtures of *py*C with two different soil types were also studied, one a forest soil with no prior burning history and the other a forest soil with a history of controlled burning dating back to the 1950's. After a 188-day incubation period, microbial community analyses were conducted through cultivation and various bacterial fingerprinting techniques (including ARISA, quantitative PCR, and DNA sequencing). Results of their study indicated that unburnt and burnt soil mixtures exhibited two different clusters of microbial populations, with a small amount of overlap (e.g., Gemmatinodates and Actinobacteria) between the two groups. Results of qPCR and direct plating indicated a decrease in microbial populations with the addition of low HTT *pyC*, but both burnt and unburnt soils showed an increase in population with the addition of higher HTT *pyC*. Overall, microbial populations and activity were also found to be higher in the burnt soils than in the unburnt soils, suggesting that the microbial communities found in the burned soils are better adapted to utilization of *pyC*.

Another key factor in *pyC* degradation is prevailing climatic conditions. The relationship between *pyC* degradation and environmental conditions, particularly temperature and precipitation, was studied by Cheng et al. (2008). Materials used in this study included wood-derived *pyC*'s collected from the remnants of historical charcoal blast furnaces. These furnaces were located at 11 different sites ranging from Quebec to Georgia, with mean annual temperatures (MAT) ranging from 3.9C to 15.7C. Samples were measured for OC using solid-state NMR methods, after which a double exponential model was used to determine OC mineralization rates. Results of their study showed a significant relationship between MAT and OC mineralization, where soils with higher MAT's displayed increasing amounts of OC mineralization when compared to soils with lower MAT's. These observations support previous work by Glaser and Amelung (2003), which found a similar relationship along a climate sequence in the Great Plains of the United States. There was, however, no significant relationship found between effective precipitation and the amount of OC mineralized.

CHAPTER III

MATERIALS, AND METHODS

Study Site

The study site was located at the Lake Thoreau Environmental Science Center, a 300 acre facility owned by The University of Southern Mississippi and located in the Lower Leaf River sub-basin of the Pascagoula River Basin. The study site is dominated by a pine forest ecosystem and was representative of the Pascagoula River Basin, which is comprised of 52% pine forest. Average seasonal temperatures for this area are 32.1, 25.5, 16.2, and 25.2 ^{o}C for the summer, fall, winter, and spring seasons, respectively. Average monthly precipitation values for the same seasons are 11.2, 10.5, 13.9, and 14.7 cm, respectively.

A total of six sampling locations, located along two transects, were selected for use in this study (Figure 2). Four of the sampling locations were in an area that has been consistently burnt on a two-year cycle, most recently in January of 2014. The remaining two sampling locations were in areas that have no records of being burnt. At each sampling location, samples of pine-derived (PI, *Pinus taeda*) and cordgrass-derived (CG, *Spartina alterniflora*) *pyC*s were placed in polypropylene litterbags (apparent opening = 210 µm) and buried at a depth of approximately 8 cm beneath the soil surface using a gridded outlay (Figure 3). A total of 11 PI and 11 CG *pyC*-containing litterbags were buried at each site. One litterbag of each pine and CG *pyC*s were removed on each sampling excursion, which occurred at 21, 42 63, 84, 105, 126, 147, 217, 294, 322, and 350 days after burial. Samples collected on each excursion were split 2:1, with 2/3 of each sample being dried (75^{o}C for 24 hr) and used for immediate analysis of pH, EC, relative recalcitrance, and total organic carbon content, while 1/3 of the sample was frozen and subsampled for use in ergosterol analysis.

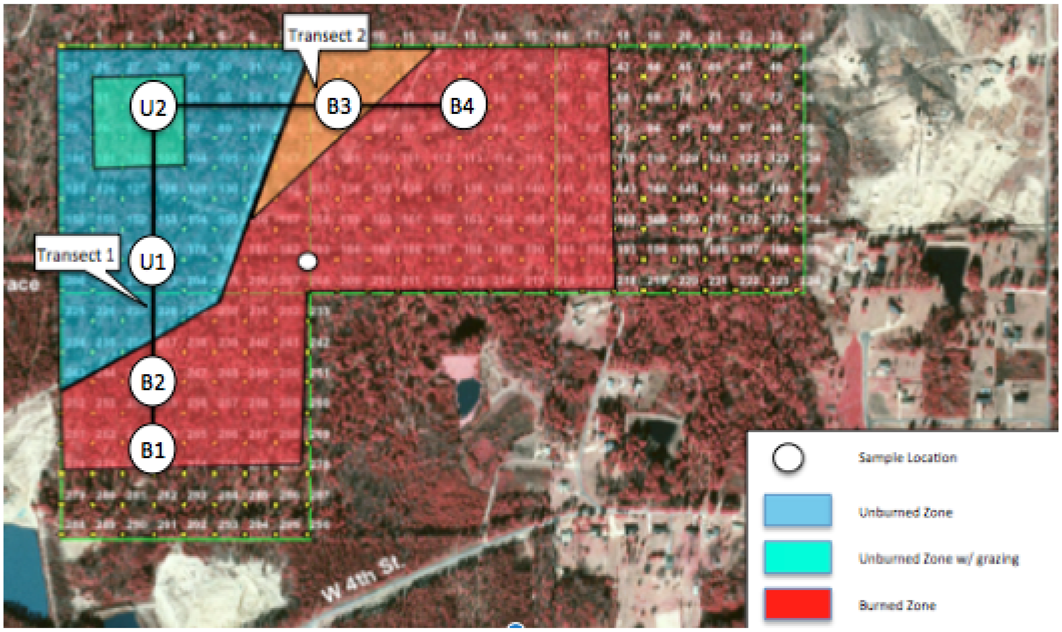

Figure 2. Map of study area in relation to burnt and unburnt zones.

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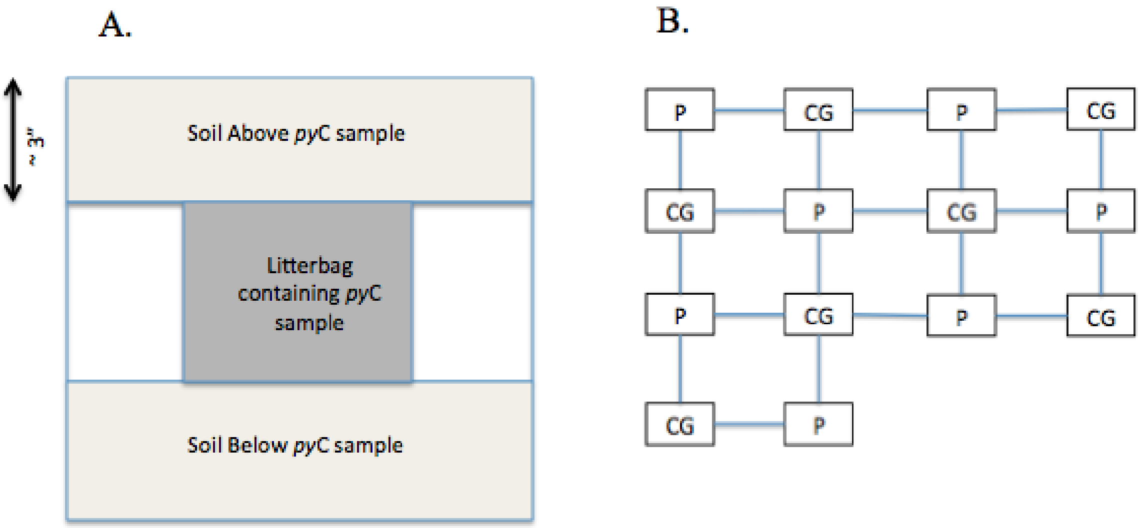

Figure 3. (A) Orientation of litterbags in relation to surrounding soils. (B) Example of grid system used for litterbag placement at each study site.

Preparation of *py*Cs

Pine-derived pyC was produced from pine needles collected from the study site, while cordgrass-derived pyC was produced from cordgrass straw collected from USM's Gulf Coast Research Laboratory. Prior to pyrolysis, needles and grass were dried at 60°C and cut to approximately 2 cm in length. Pyrolysis of plant material was performed in a muffle furnace (ramp rate = 25°C/min) under oxygen limited conditions at 450°C for a total of 1 hr to mimic average conditions for a typical vegetation fire in the southeastern United States (Alexis et al., 2007). Upon cooling and removal from the furnace, the *pyC* material was separated into a coarse fraction using 250 and 500 µm sieves. Approximately 1.5 g of the coarse *pyC* material was separated into 1.5 g portions and placed into pre-made 8X8 cm litterbags. The litterbags were comprised of needle-punched polypropylene material with apparent openings of 210µm (small enough to contain the coarse fraction of the *pyC*, let large enough to allow environmental interaction with the *pyC*).

Post-burial Analysis of *py*C

Pyrogenic carbon collected on each sampling excursion underwent analysis for total organic carbon content (TOC), base-extractable organic carbon (BEOC), pH, EC, relative recalcitrance (R_{50}), and fungal ergosterol.

Carbon content was determined via a Costech elemental combustion analyzer. 2 mg samples of pyC were weighed and place in pure tin capsules before undergoing combustion.

BEOC of *py*C samples was determined using UV-visible spectrophotometry. Samples were weighed to 150 mg and placed in 30ml of 0.5M NaOH solution before being heated for two hrs. at approximately 80°C. After heating, the *py*C:NaOH solution was analyzed at the 550, 365, and 250 nm wavelength using an Evolution 220 UV-VIS Spectrophotometer.

In order to test for pH and EC, 50 mg of each sample was weighed out and added to 5 ml of deionized water, creating a 1:100 *py*C:water solution. This solution was allowed to stand overnight before being measured for pH and EC by an Accumet pH/EC submersible probe.

Relative recalcitrance of the *pyC*s was analyzed by means of thermogravimetric analysis (TGA) and the use of the *R50* index as described by Harvey et al., (2012). Thermal analysis began at an oven temperature of 20°C, increasing at a ramp rate of 10°C per minute until 700°C, at which point no further weight loss was observed. The *R50* value of the *pyC* is a measure of the relative recalcitrance of the *pyC* material as a whole, and was calculated using the formula:

$$R_{50} = T_{50,pyC}/T_{50,graphite}$$

where $T_{50,pyC}$ and $T_{50,\mathrm{graphite}}$ are the temperature values corresponding to 50% oxidation of *pyC* material and graphite, respectively, and were obtained via use of TGA mentioned above.

The biomass of fungi associated with *py*C samples were estimated from ergosterol concentrations. Ergosterol was extracted from *py*C samples (~60 mg) by refluxing in alcoholic KOH (4% KOH in 95% methanol) for 30 minutes. The resultant extract was partitioned into n-pentane and evaporated to dryness under a stream of nitrogen gas. Ergosterol in dried samples was then redissolved by sonication in 0.5 ml of methanol and stored, tightly capped, in 1.5 ml screw cap HPLC vials at ~20°C until analyzed.

Separation and analysis of ergosterol was determined using High Pressure Liquid Chromatography (see Kuehn, Ohsowski, Francoueur, & Neely, 2011)

Soils and Soil Analysis

In addition to *py*Cs, soils were collected from directly beneath the litterbags on each sampling excursion. The collected soil samples were then air dried and ground to <2mm for use in textural, pH, EC, and OC analysis. Soil particle size was determined using the hydrometer method (Gee & Bauder, 1979). The pH of the soils was determined using the same procedure as for *pyC*s. OC of soils was analyzed using the same procedure as *py*C, albeit samples were weighed to approximately 20mg and was used as a standard.

Statistical Analysis

Multiple Two-way ANOVA, using various parameters such as location, feedstock, and site treatment were conducted across all sites using Graphpad Prism analytical software. The first tier of analysis involved the association of time and site location with changes in TOC and pH of both CG and Pine *py*C. This analysis was used to determine if data from certain sites could be proven statistically similar, and as such, could be grouped together in order to generate a more robust data set. The second tier of analysis involved the association of time and *py*C feedstock with changes in TOC, R50 and pH of *py*Cs in burnt and unburnt locations. The third tier of analysis involved the association of time and site treatment (burnt vs. unburnt) with changes in TOC, R50, and pH of both pine and CG *py*Cs.

CHAPTER IV

SITE CHARACTERIZATION

Site Elevation

Elevations of the study area varied from ~ 290 to 330 feet above sea level (Figure 4). Site B3 was located at the highest elevation (330ft), while site B2 was located at the lowest elevation (287ft.). The overall slope of the study area ran from NE to SW.

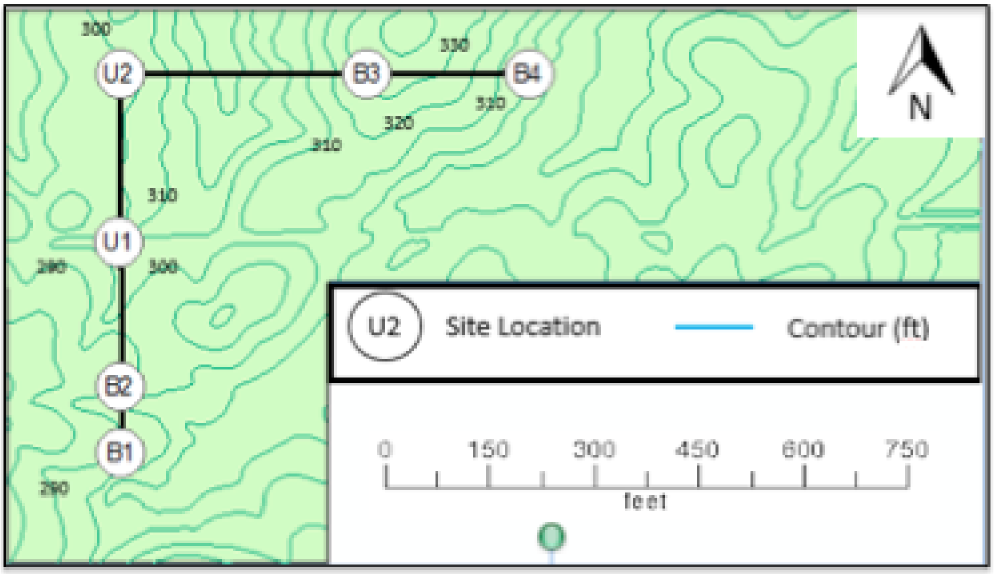

Figure 4. Elevation (feet above sea level) across study area.

Soil Type

With the exception of site B2, all sites were found to be composed of soils of the Freestone-McLaurin-Susquehanna Association (Figure 5A). The upper profile of these soils (where the *pyC* samples were located) consists mainly of moderately well drained sandy loam, and is typical of soils found in the coastal plains. Site B2 was found to be composed of soils from the Prentiss group. The upper profile of Prentiss group consists of moderately well drained fine sandy loam material, and is also typical of soils found in the coastal plains. Both soil types are derived from loamy alluvial parent material, and overall behave similarly.

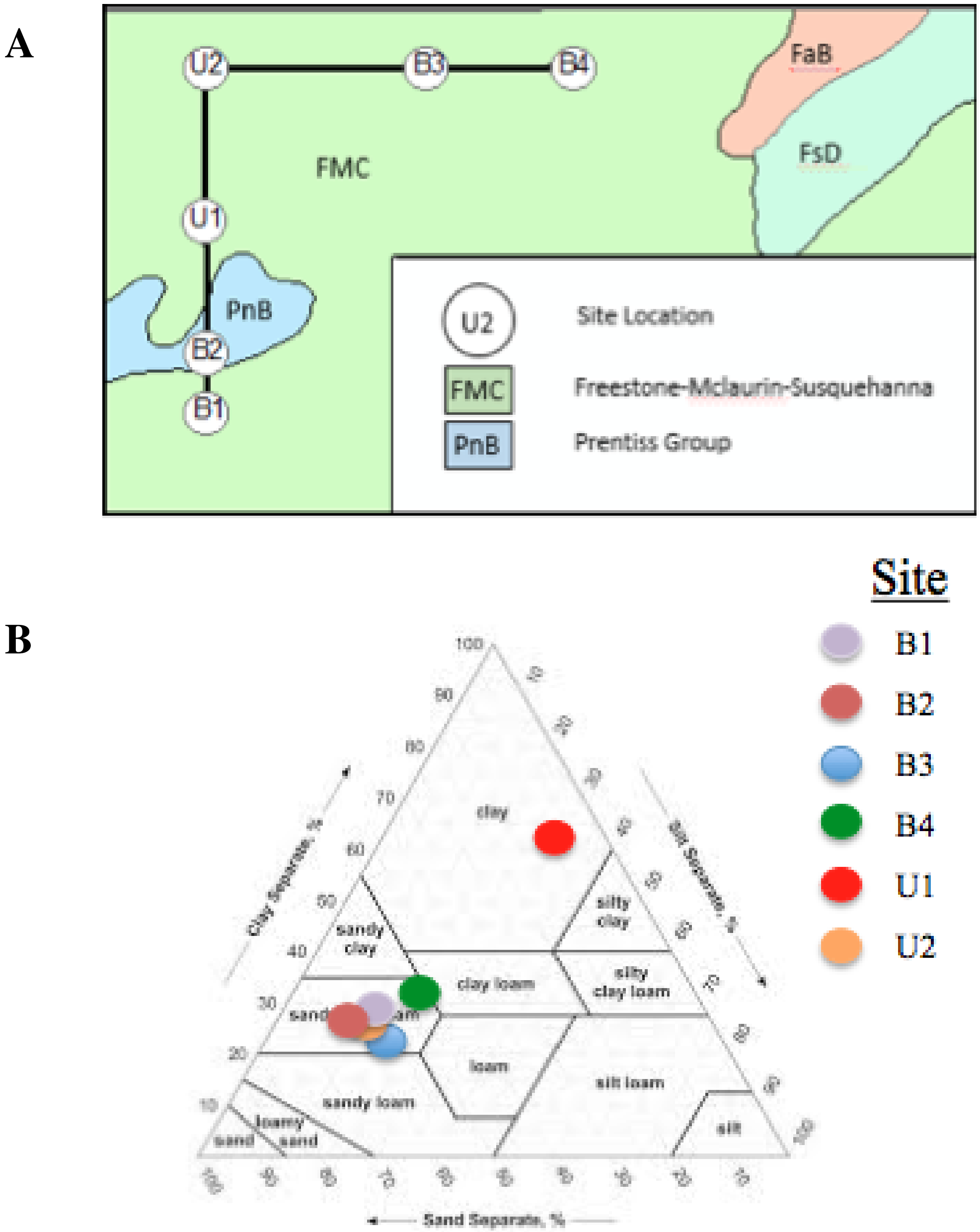

Figure 5. (A) Soil types across study area (soils data via WebSoilSurvey) (B) Soil textures for each site plotted on USDA textural triangle.

Soil Size Analysis

With the exception of site U1, all soils were characterized as a sandy loam according to the USDA textural triangle (Figure 5B). The % clay associated with these soils varied from a minimum of 22% at site B2 to a maximum of 34% at site 63. Site U1 was characterized as a clay on the USDA textural triangle, containing a clay and silt content of 63 and 31%, respectively.

Soil pH

Soils immediately below the buried *py*C litterbags maintained a soil pH between 5.7 and 6.3 throughout the study period (Figure 6A). This was consistent with expectations, as soils in a pine forest have been shown to have a generally more acidic due to the nature of pine material (Falkengren-Grerup, 1987). The only noticeable variation in this stable trend was a slight drop in pH to 5.7 that occurred across all sites between days 217 and 300, after which the soil pH at all sites rose back into the normal range. There was no statistical difference in the pH of soils in burnt and unburnt sites, as well as no statistical difference in pH of soils located under pine and CG *py*C's.

Soil C%

Soil carbon in samples below the buried pyC litterbags ranged from 1 to 2.5% throughout the study period (Figure 6B). Soils in the unburnt sites generally had the lowest carbon content, while soils in the burnt sites generally had the highest carbon content. The higher carbon content in soils from burnt sites reflects the storage of pyC from previous burning events that would remain in the soil for long periods of time. A slight decrease in C% can be seen across all soils beginning at day around day 150, after which the C% in all soils increased for the remainder of the study period.

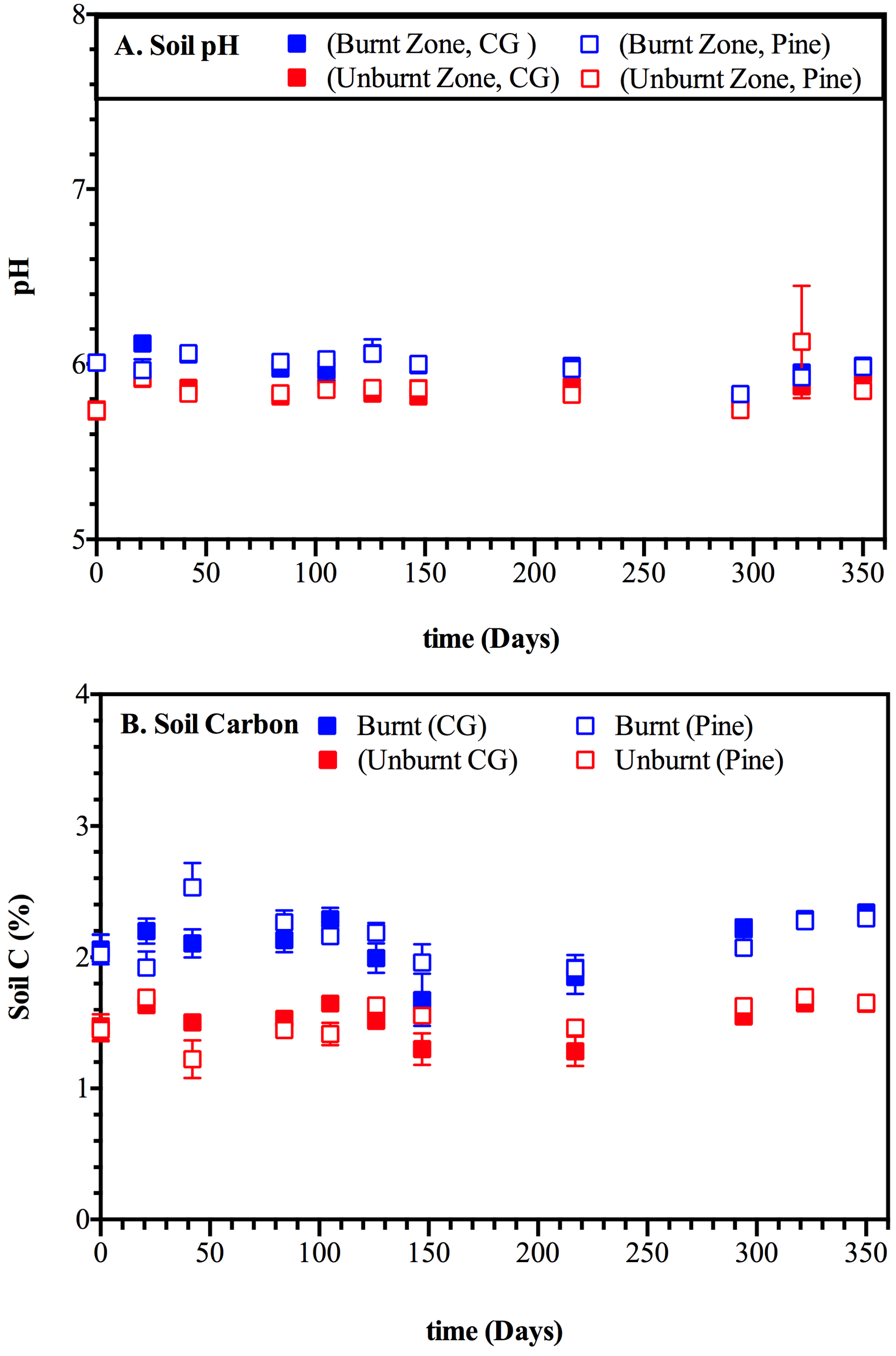

Figure 6. Changes in (A) pH of soils taken from underneath pine and CG pyCs in both burnt and unburnt sites. (B) Carbon content of soils taken from underneath pine and CG pyCs in both burnt and unburnt sites.

Overall Site Analysis

Overall, the six sites used in this study showed little variation to one another in terms of elevation, soil type, soil composition, pH, and C%. The only true exception to this was a higher clay content found in the soils at site U1. This similarity between sites should allow for relationships to be made regarding changes in pyC degradation without the grouping of particular sites based on attributes of the site itself.

CHAPTER V

LINKS BETWEEN TEMPORAL VARIATIONS IN TEMPERATURE AND PRECIPITATION, ORGANIC CARBON DYNAMICS AND PH IN BURIED PYROGENIC CARBON (*PYC*) MATERIALS.

Introduction

As seasons change, certain meteorological properties of the research area will be expected to change as well. For instance, in the Pascagoula River Basin the highest average temperatures are seen in the summer (90^{o}F) and spring (77^{o}F) months, while the highest average amounts of precipitation are seen in the winter (5.46”) and spring (5.77”) months. Previous studies have shown that *pyC* behaves differently in different temperature regimes (Cheng et al., 2008), and that temperature and precipitation effect the rates of both biotic and abiotic means of degradation (Liang et al., 2003; Aagaard & Helgeson, 1982). Based on what seasonal patterns in temperature and precipitation at the study site, we hypothesized that the higher average temperature and precipitation in summer and spring will result in more rapid changes to the C content of the buried *pyC*. In order to test this hypothesis, season to season changes in total organic content of the *pyC*, as well as changes in pH of the *pyC*, will be analyzed. Two-way ANOVA was used to compare changes in TOC and pH across site locations while keeping site treatments and *pyC* feedstock constant.

Seasonal Changes in TOC content of *py*Cs

Two-way ANOVA examining the effect of location and time on organic carbon content of the buried *pyC* showed that, irrespective of site treatment (burnt or unburnt) or *pyC* type, location had no significant effect on the quantity of organic carbon associated with the buried *pyC* (Table 1). The percent of variation in organic carbon attributable to location in the burnt treatments were 0.71 and 2.41% for PI and CG *pyC*s respectively. In unburnt sites the effect of location was even lower, accounting for 0.01% or lower variation in organic carbon content. This suggested that organic carbon associated with a given *pyC* type and within a given site treatment was; 1) similar at all sampling sites and, 2) could be considered as replicates (i.e., carbon content at U1 and U2 are replicates for unburnt treatment while B1, B2, B3 and B4 are replicates for burnt treatments). This allowed for the grouping of data sets based on *pyC* feedstock and site treatment. For instance, we were able to take organic carbon values for pine-derived *pyC* at all burned sites and combine them into a single dataset for use in further statistical analysis.

In contrast to location, time (and its interaction with location) had a significant effect on organic carbon content associated with the *pyC*s (Table 1). At burnt sites, time effects accounted for 83.4 and 90.3% of variation in organic carbon content in PI and CG *pyC*s, respectively. Very similar percent variations (80.5% for PI and 91.8% for CG *pyC*) were obtained for time effects on organic carbon content at unburnt sites, indicating that temporally-driven factors (e.g. precipitation and temperature) were by far the most significant driver of organic carbon dynamics over the course of the study. Consideration of the interaction between time and location also suggest that variations in temporally-driven factors at a given location also had some effect (2.3 to 2.7% in CG *pyC* and 13.1 to 16.6% in PI *pyC*s) on observed organic carbon dynamics. However, assessing such interactive effects would have required monitoring of temporal factors (in our case precipitation and temperature) at each individual site. Undertaking such activity would be excessive considering that a global site monitoring approach would explain up to 40 times more variability in organic carbon content than an individual site monitoring approach.

Table 1

Two way ANOVA analysis of the effects of time and location on changes in organic carbon content of cordgrass- (CG) and pine-derived (PI) pyC.

	Source of Variation		
	Time	Location	Interaction
<u>Burned Sites</u>	--------------------------- % of variation -------------------------		
CG	90.32 ****	2.409 ^{ns}	2.300 ****
PI	83.37 ****	0.706 ^{ns}	13.12 ****
<u>Unburned Sites</u>			
CG	91.78 ****	0.005 ^{ns}	2.665 ****
PI	80.50 ****	0.010 ^{ns}	16.56 ****

*,**,***,**** significant at the 0.05, 0.01, 0.001, and 0.0001 level, respectively

^{ns} not significant

Figure 7 shows daily precipitation and, temporal trends in maximum air temperature and organic carbon content associated with PI (Figure 7A) and CG (Figure 7B) *pyC*s in burnt and unburnt treatments. Over the study period, organic carbon content associated with *pyC*s varied between 80% to 120% of initial values (Figure 7), with trends in organic carbon content varying across seasons. Summer was characterized by a continued decrease in organic matter content (15-25% *pyC* loss) consistent with microbially-mediated degradation of pyC. Most of this carbon loss was focused towards the end of the summer and was associated with a 4-5 ^{o}F increase in air temperature (from 88 to 93 ^{o}F) between sampling days 42 and 84. Air temperatures during this period were

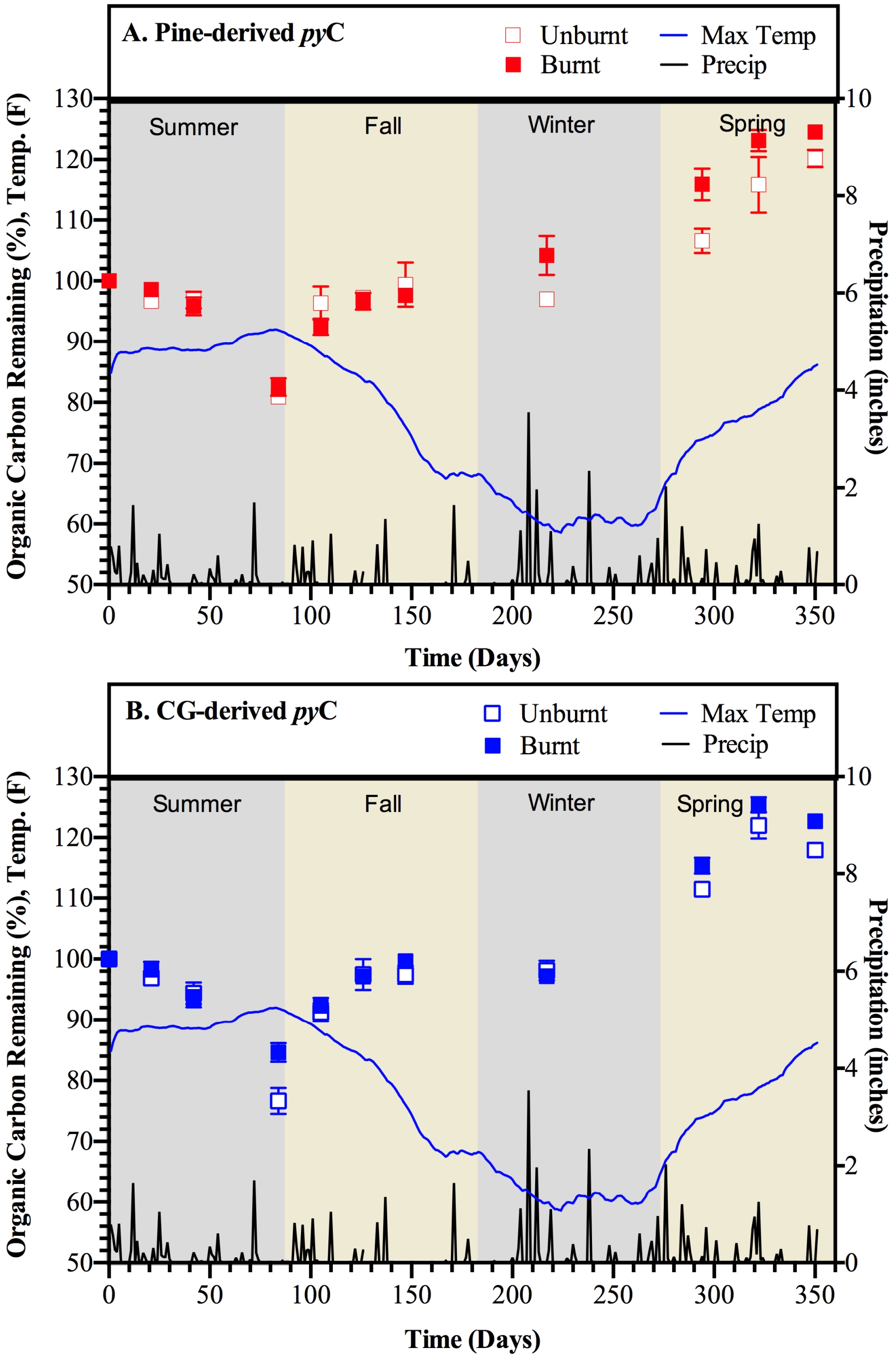

Figure 7. Changes in TOC in both pine-derived *py*C (A) and CG-derived *py*C (B) in both burnt and unburnt sites in relation to changes in temperature and precipitation

well within the optimal range for microbial activity and temperature increases in this range are known to be accompanied by increased microbial activity (Anderson &

Nilsson, 2001; Liang et al., 2003). Another contributing factor to the decrease in organic carbon associated with *pyC* during the summer, was the flushing of water soluble organic carbon from the buried *pyC* materials. A number of rainfall events, ranging in size from 0.1 to 2 inches, occurred during this period along with sharp declines in electrical conductivity of the *pyC,* providing evidence for the flushing of salts from the materials (Figure 8). Such flushing would most likely be accompanied by some flushing of soluble organic carbon. In fact, there is some anecdotal evidence to support microbial degradation and/or flushing as the primary processes accounting for the decrease in organic carbon content associated with CG and PI *pyC*. For example, microbial degradation will favor the removal of the most labile organic carbon fraction first. This

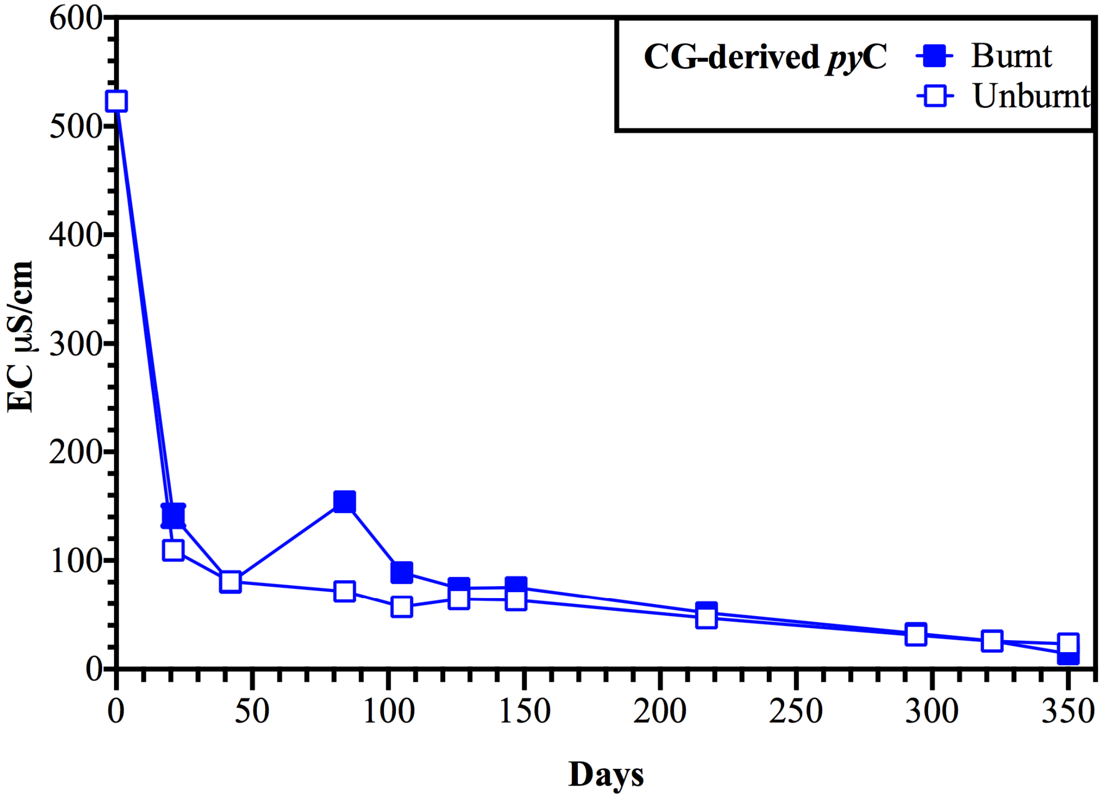

Figure 8. Changes in EC in CG-derived pyC in burnt and unburnt sites.

labile fraction is also the most water, acid or base soluble and consequently both microbial degradation and flushing of organic carbon would be expected to result in a decrease in this labile fraction.

In contrast to summer, organic carbon associated with *pyC* increased over the fall season. The increase followed a logarithmic pattern with time, reaching a plateau towards the latter part (day 147) of the fall season (Figure 7). The plateau corresponded to 100% of initial organic carbon content reflecting a re-accumulation of lost organic carbon associated with *pyC*. Re-accumulation of organic carbon during the fall was attributable to the accumulation and subsequent degradation of litter (from leaf and needle fall) resulting in the release of dissolved organic carbon (DOC) which gets adsorbed to the buried *pyC*. As fall progressed, the continued decrease in air temperature favored a decrease in microbial activity, and hence a decrease in DOC available for adsorption to the *pyC*. It is this slowing of available DOC for adsorption that results in the observed logarithmic trend in organic carbon with time throughout fall. The litter accumulation-degradation mechanism coupled to adsorption is consistent with both:1) studies which show that an increase in soil litter in the O horizon contribute large amounts to the DOC content of forest soils (Qualls & Haines, 1991; McDowell & Likens, 1988) and, 2) base-extracted organic carbon content BEOC which showed a similar temporal trend as in Figure 7 (Figure 9).

Even with increased precipitation during the winter, organic carbon associated with *pyC* remained relatively stable and comparable to the maximum observed for the fall season. This coincided with the lowest air temperatures (60-68 ^{o}F) during the study

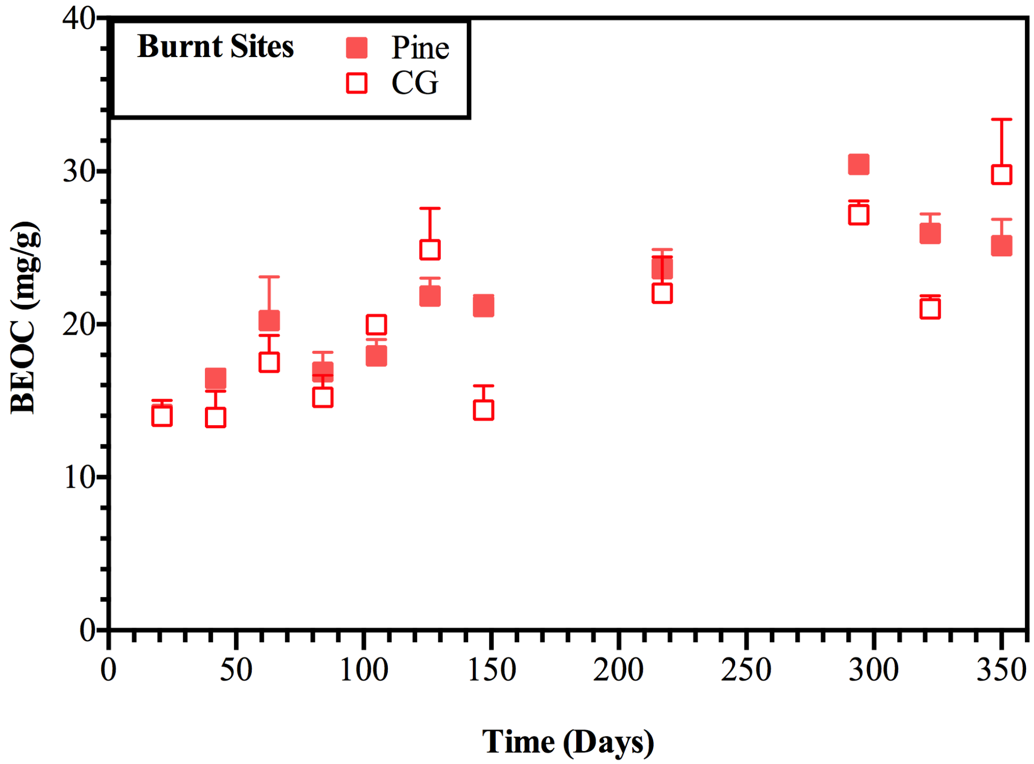

Figure 9. Changes in BEOC in pine and CG *py*Cs of burnt sites.

period and was attributable to further temperature-driven reduction in microbial activity. The transition from winter to spring was accompanied by a progressive increase in temperature and a concomitant increase in *pyC*-associated organic carbon content from 100 to 120% of initial values. Trends in base-extracted organic carbon were again supportive of an external source for the organic carbon responsible for the observed increase during spring. As temperature increases, biotic activity will increase, resulting in more DOC being available for adsorption to the *pyC* as in the fall. However, as opposed to the fall when DOC availability was driven mainly by microbial degradation of senescence of plant biomass, in spring, the growth of plant biomass (as plants shift from dormancy to active growth) could also be a significant contributor of DOC. For example, during active growth, roots are known release DOC as root exudates to enhance nutrient uptake (Ryan, Delhaize, & Jones, 2011). This non-microbial source of DOC would also be leached and could be plausibly adsorbed to *pyC,* thereby contributing to observed increase in *pyC*-associated organic carbon.

Seasonal Changes in pH of *py*Cs

As with organic carbon, time accounted for the largest amount of variability in pH of buried *py*Cs (76-91%), indicating significant effects of temporally driven factors on soil acidity (Table 2). Location also had a significant effect on pH in buried *py*C's in burnt sites (2 and 3% of variability in PI and CG *py*C, respectively), indicating that certain characteristics of these sites may facilitate different processes in these *py*Cs. Further analysis of the burnt sites revealed that there were two distinct groups within the

Table 2

Two way ANOVA analysis of the effects of time and location on changes in pH of cordgrass and pine derived pyC.

	Source of Variation		
	Time	Location	Interaction
Burnt Sites	--------------------------- % of variation -------------------------		
CG	82.99 ****	1.976 **	12.82 ****
PI	75.94 ****	3.019 ****	16.99 ****
Unburnt Sites			
CG	90.73 ****	0.486 ^{ns}	4.859 ****
PI	83.12 ****	0.286 ^{ns}	12.83 ****

*,**,***,**** significant at the 0.05, 0.01, 0.001, and 0.0001 level, respectively

^{ns} not significant

overall burned group that were significantly similar (B1:B3, B2:B4), and therefore could be combined (Table 3). In the case of the unburnt sites, location had no significant effect on the pH of buried chars, regardless of feedstock. Interaction between time and location was significant in all sites (5-17%), though does not provide enough variability to warrant a site by site assessment of pH for each sampling period.

Table 3

Two way ANOVA analysis of the effects of location on pH in cordgrass and pine derived PyC across individual sites.

	Feedstock	
	Pine	CG
Comparison by site	% of total variation (location)	
B1:B2	0.978**	2.354*
B1:B3	0.088^{ns}	0.058^{ns}
B1:B4	2.025**	2.495***
B2:B3	3.426**	0.957**
B2:B4	0.127^{ns}	0.001^{ns}
B3:B4	5.375**	1.026**
U1:U2	0.286^{ns}	0.486^{ns}

*,**,***,**** significant at the 0.05, 0.01, 0.001, and 0.0001 level, respectively

^{ns} not significant

The general trend in pH was different for the pine and CG *py*Cs (Figure 10). Over the course of the study period, the pH of the pine *py*Cs remained relatively stable at around 6.2, with the exception of a rapid decrease to a pH near 5.8 occurring from mid-winter to the onset of spring (days 217 and 294, Figure 10A). The pH of the CG char decreased continuously from an initial value of 8 (pH of oceans in ~ 8.1) to a value of ~ 6.2 by the middle of winter (day 217, Figure 10B). At this point there was again a rapid decrease to a pH value of 5.8, followed by an increase in pH to a value of ~7 as temperatures warmed during the spring. The different general trend seen between the two *pyC* types is plausibly a function of the differences in feedstock chemistry. Pine-derived *pyC* would better reflect the acidic pH conditions typical of the soil environment in pine forests, and therefore remained relatively stable over the course of the study period.

Cordgrass, on the other hand, is native to coastal regions where pH is usually more basic. Basic material in an acidic environment will drop as it has to adjust itself to equilibrium conditions, thus the fall in pH seen in CG *py*Cs. The drop in pH at day 217 can be seen in both types of *py*C and is associated with the shift from winter to spring, suggesting that the change is likely linked to a shift in some environmental factor. The most likely trigger is the occurrence of several major precipitation events throughout winter into early spring (between days 210 and 240). This period represented the most intense period of precipitation and could have potentially brought in an influx of more acidic material from outside sources, resulting in the observed drop in pH of pine and CG *pyC*s between sampling day 217 and 294.

Concluding Remarks

The trends seen in *pyC* TOC during spring and winter agreed with our hypothesis that most changes in C content occurred in seasons with higher temperatures and amounts

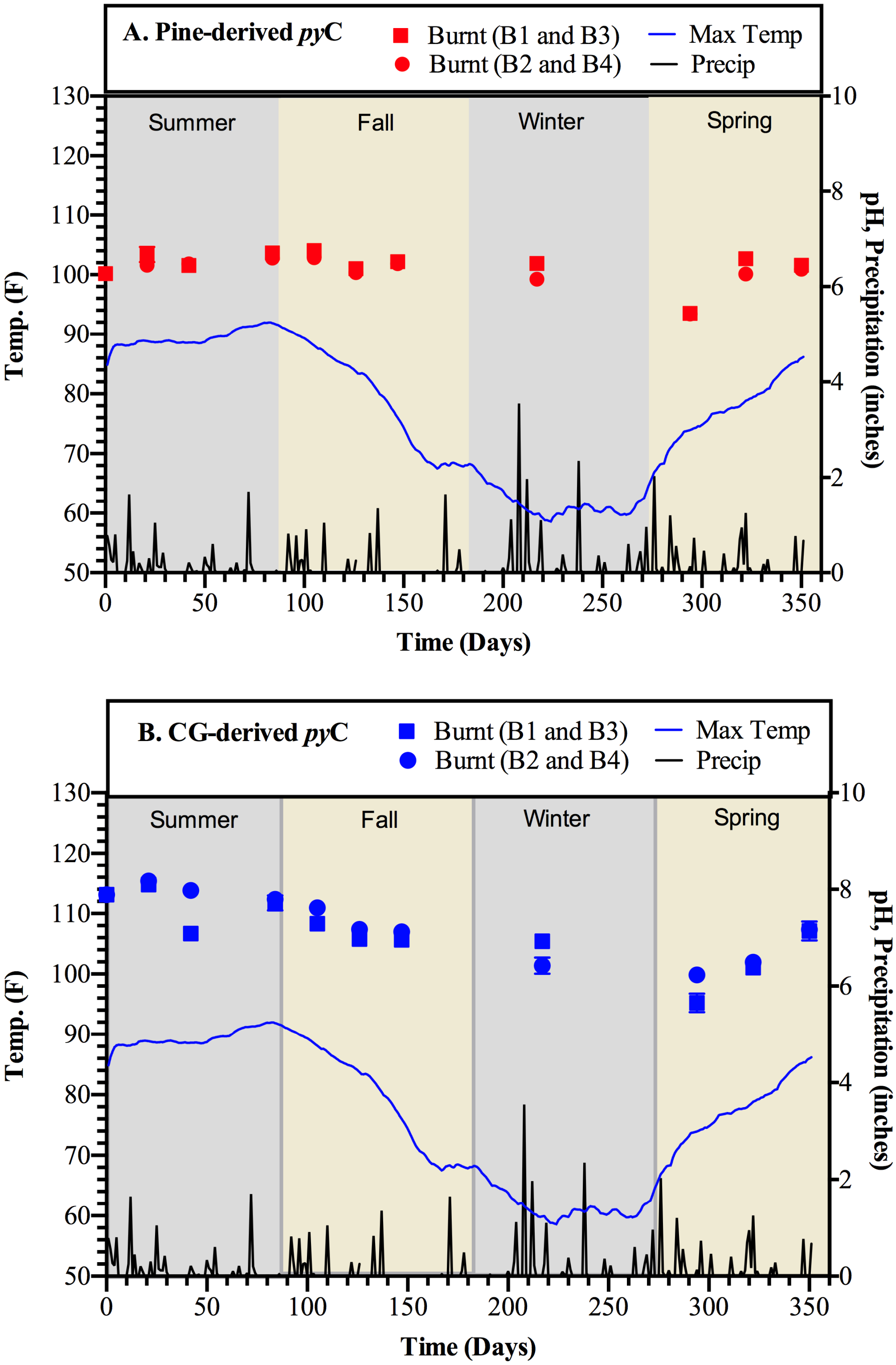

Figure 10. Changes in pH of both pine-derived pyC (A) and CG-derived pyC (B) of both burnt and unburnt sites in relation to changes in temperature and precipitation.

of precipitation, while changes would not occur as quickly in colder, drier seasons. We did not, however, expect to see rapid changes in TOC during the fall when temperatures were beginning to drop and there were low amounts of precipitation. This increase in TOC was attributed to the input of DOC from increased leaf and needle litter as trees shed their leaves (or needles) during the fall months. This interaction between DOC from outside sources and *py*C should be one focus in future work. Changes in pH were attributed to differences in *py*C feedstock, although the occurrence of heavy precipitation events was seen to correlate with a drop in pH, presumably due to the flushing of more acidic materials into the *py*C from outside sources. The role that this short term change in pH can have on C characteristics of buried pyC should also be noted in future studies, as pH has been shown to play a role in both rates of both biotic and abiotic forms of degradation (Pietri & Brookes, 2008; Palandri & Kharaka, 2004)

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CHAPTER VI

DIFFERENCES IN C DEGRADATION ASSOCIATED WITH *pyC* FEEDSTOCK

Introduction

With potential shifts in vegetation cover (such as clearing of forests to form grasslands), source materials for *pyC* will also change. It is likely that the microbial community will then have to adapt to seeing new forms of *pyC*, and *pyC* degradation will, at least initially, proceed at a slower rate. However, this decrease in microbial activity could be offset or even completely undermined by the shift to new feedstock material. It is already widely known that the recalcitrance of different forms of *pyC* is at least partially controlled by the structure of the feedstock material. Therefore shifts from areas such as forests (with an abundance of more recalcitrant, lignin rich material), to grassland regions (with an abundance of more labile, cellulose rich material) would also mean a shift from more recalcitrant *pyC* to an increasingly labile form of *pyC*. We hypothesized that, although microbes in a forest dominated region may not be adapted to seeing grass based *pyC* material, the grass based *pyC* material will see faster rates of degradation based on its initial feedstock chemistry. Changes in total organic content, R_{50} values, and pH of both pine and CG *py*Cs were used to test this hypothesis. Measurements of ergosterol as a function of fungal biomass were also used as supporting data. Two-way ANOVA was used to compare the differences in feedstock over time while holding location and site treatment (burnt vs. unburnt) constant.

Organic Carbon % in Pine and CG *py*Cs

Two-way ANOVA, examining the effects of time and feedstock on organic carbon content of the buried *pyC*, showed that, irrespective of site treatment (burnt or unburnt), feedstock had no significant effect on the quantity of organic carbon associated with the buried *pyC* (Table 4). The percent of variation in organic carbon attributable to feedstock in the burnt treatments was 0.030%. The percent of variation was even lower in unburnt sites, where feedstock only accounted for 0.018% of variability. In contrast, time had a significant effect on organic carbon content associated with *pyC*s, accounting for 86% of the variation at burnt sites and 85% of the variation at unburnt sites. This suggests that time is the most significant factor when considering changes in TOC of buried *py*Cs.

Table 4

Two way ANOVA analysis of the effects of time and feedstock (pine vs. CG) on changes in organic carbon content of cordgrass and pine derived pyC.

	Source of Variation		
Site Treatment	Time	Feedstock	Interaction
	% of variation		
Burnt	86.03 ****	0.030 ^{ns}	0.891 *
Unburnt	84.87 ****	0.018 ^{ns}	1.934 ^{ns}

*,**,***,**** significant at the 0.05, 0.01, 0.001, and 0.0001 level, respectively

^{ns} not significant

In the previous chapter, numerous links of total organic carbon of *pyC* to seasonal changes in precipitation and temperature were discussed thoroughly and can be seen in Figure 7. The same general trend of a decrease in TOC during the summer months, followed by increases in TOC during fall and spring months can be seen in both pine and CG-derived *py*Cs (Figure 11). If we consider the changes in TOC to be a factor that is mainly controlled by changes in precipitation and temperature regimes and associated inputs of DOC from an outside source, then it comes as no surprise that there is no statistical difference between TOC in *pyC* from different feedstocks. If possible, the measurement of TOC minus DOC from outside sources could give a better view of overall differences in recalcitrance of pine and CG *pyC*'s. This however, is beyond the scope of this paper and should be considered in future analysis.

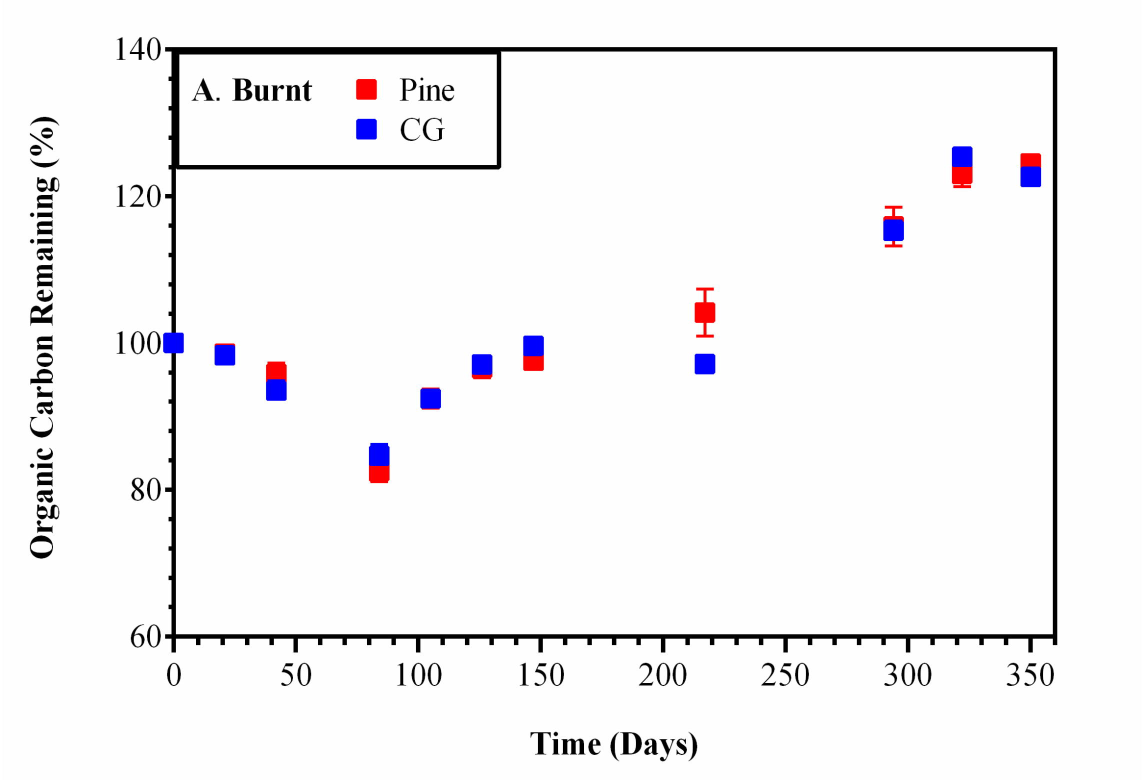

Figure 8. Changes in TOC% of both pine and CG *py*C's in burnt sites

Changes in R50 of Pine and CG *py*Cs

In contrast to organic carbon, differences in feedstock had a significant effect on the overall relative recalcitrance of the buried *pyC* (Table 5). The percent of variation attributable to feedstock in the burnt areas was 48.70%, by far accounting for the most significant percentage of total variation. This means that, at least in burnt sites, feedstock was the biggest cause of differences in relative recalcitrance of buried *py*C regardless of how long they had been subjected to environmental changes. In the unburnt sites, however, differences in feedstock did not have a significant effect on the overall recalcitrance of the buried *pyC*, accounting for only 18.78% of the variation in R_{50} values. Time and interaction had a significant effect on overall recalcitrance of *pyC* regardless of treatment, accounting for 9 and 30% of the variation, respectively, in the burnt zone and 27 and 40%, respectively, of the variation in unburnt zones. This means that, in the case of the unburnt sites, relative recalcitrance of buried pyC can differ depending on the whatever sampling date is taken into consideration.

Table 5

Two way ANOVA analysis of the effects of time and feedstock (pine vs. CG) on changes in R_{50} of cordgrass and pine derived pyC

	Source of Variation		
Site Treatment	Time	Feedstock	Interaction
	% of variation		
Burnt	8.805 ****	48.70 ****	30.01 ****
Unburnt	26.88 **	18.78 ^{ns}	39.28 ***

*,**,***,**** significant at the 0.05, 0.01, 0.001, and 0.0001 level, respectively

^{ns} not significant

R_{50} values are a measure of the relative recalcitrance of the *pyC* as a whole. Since DOC adsorbing to both forms of *pyC* is generally linked to the same outside source and therefore has similar properties, the changes in R_{50} must be attributed to changes to recalcitrance of the original *pyC* material. This logic means that the use of R_{50} should give a much clearer picture of overall recalcitrance than TOC. Figure 12 shows an initial difference in the recalcitrance of pine and cordgrass materials, with R_{50} values starting at 0.522 and 0.477, respectively. This initial difference can be attributed to structural differences between the pine and CG *pyC* (mainly the higher fraction of more recalcitrant lignin in pine char) that has been recognized in a number of previous studies (Harvey et al., 2012; McBeath & Smernik, 2009). As time progressed, the R_{50} values in the pine *pyC* decreased relatively slowly in a linear fashion, reaching an R_{50} of ~ 0.512 in burnt zone *pyC*s by the end of the study period. The R_{50} values of the CG *pyC*, on the other hand, increased as time progressed, also reaching a value of 0.51 by the end of the study period. The different trends exhibited by the two different types of *pyC* can be explained plausibly by considering differences in feedstock structure, availability of labile OC, and by adaptability requirements for the microbial community. The decrease in R_{50} of the pine *pyC* is attributable to the microbes in the burned zone being adapted to the utilizing

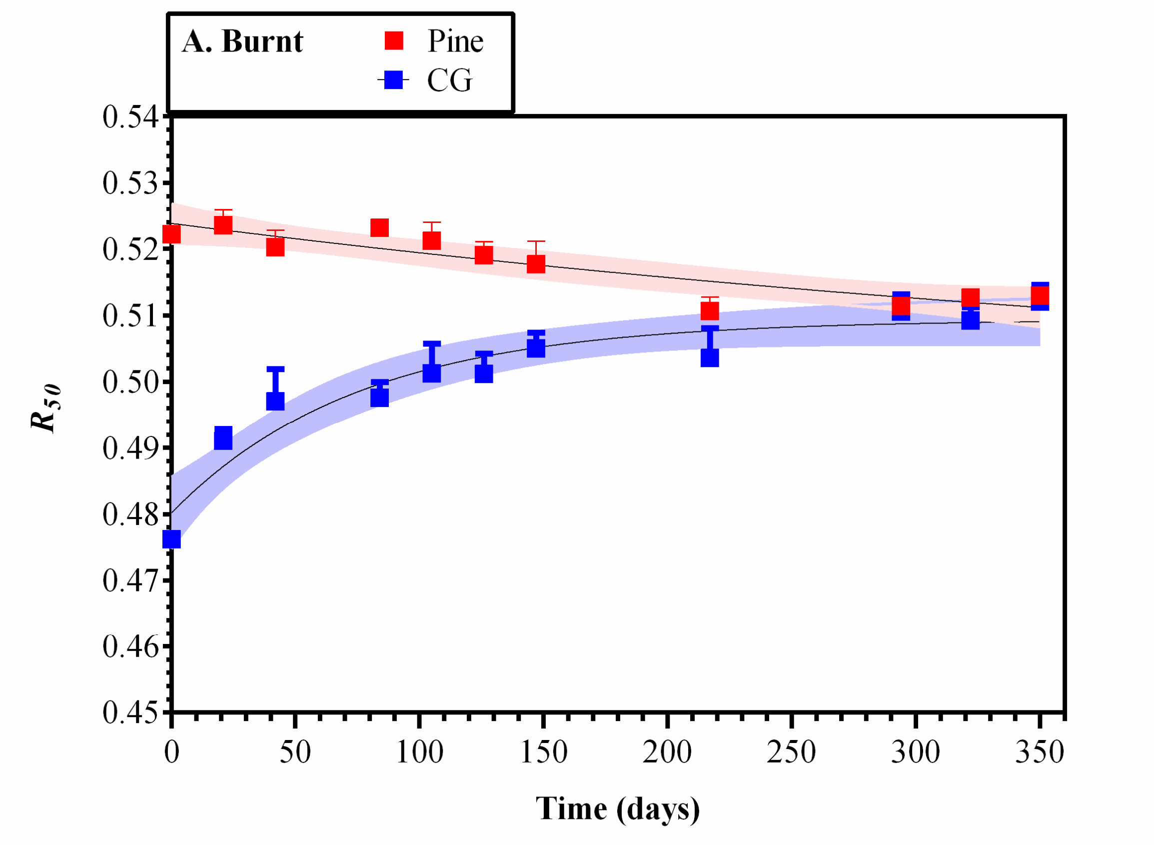

Figure 12. R_{50} values of pine and CG *py*Cs in burnt sites.

of pine *pyC* as a C source. These microbes are likely capable of utilizing the pine-derived pyC by some defragmenting mechanism, resulting in a decrease of the overall R_{50} values of the pine *py*C. The CG *py*Cs, on the other hand, are not native to the study area, meaning that the microbes are not used to using CG material and are therefore not adapted to utilizing it as a C source. They do not have as much access to the recalcitrant fraction of the CG *py*C, so must instead utilize the more labile fraction of the *py*C. As more of the labile fraction is mineralized and removed from the *py*C, the overall recalcitrance begins to increase, hence the increase seen in R_{50} of CG *py*Cs. At a point near the end of the study period (~day 280), the R_{50} values of both the pine and CG *pyC*'s converge at a value of ~0.51, following which the trends of each remain similar to one another. At this point the *py*Cs are becoming more similar to each other and therefore begin to behave in a similar fashion.

Not only were the trends in R_{50} values of pine and CG *pyC* different, but the general rate at which they changed varied as well. The most rapid increase in the R_{50} of CG *pyC* occurred over the period from 0 to 147 days, during which time the R_{50} increased by nearly 0.3 units from 0.477 to 0.505. During this same time period the R_{50} in the pine *pyC* only dropped by 0.004 units (from 0.522 to 0.518). After this point the rate of change in R_{50} remained similar for both types of *pyC*. The different rates at which R_{50} values of the pine and CG *pyC*s change is again most likely a factor of the initial *pyC* structure. Even though microbes in the burnt zone may be well adapted to the utilization of the pine *pyC*, it is still composed of a higher fraction of recalcitrant material that takes longer to metabolize. In the case of the CG *pyC*'s, the labile fraction is the major component that is being used by the microbes. Since this labile fraction is easier to metabolize than the recalcitrant pine material, the R_{50} values in the CG *pyC*s see a more rapid change.

Fungal Biomass (ergosterol)

Fungi have been shown to be a primary utilizer of *pyC* in forest soils (Steinbeiss, Gleixner, and Antonietti, 2009), and as such, can be considered a reliable marker for general microbial biomass within *pyC* material. Ergosterol levels within the *py*C were measured at 5 points (42, 84, 105, 147, and 322 days) over the course of the study period and can be seen in Figure 13. In general, ergosterol levels increased with time, and in burned sites, were higher in pine *py*Cs than in CG *py*Cs. At day 42 in the burned zone, ergosterol levels in the pine *py*C were at 5ug/g of char. By day 432, this value had increased to 13ug/g of char. Ergosterol levels in the CG char on the same dates were 4 and 8ug/g of *py*C, respectively. This data supports the argument that microbes in the burnt environment are more adapted to utilization of the native pine material than the

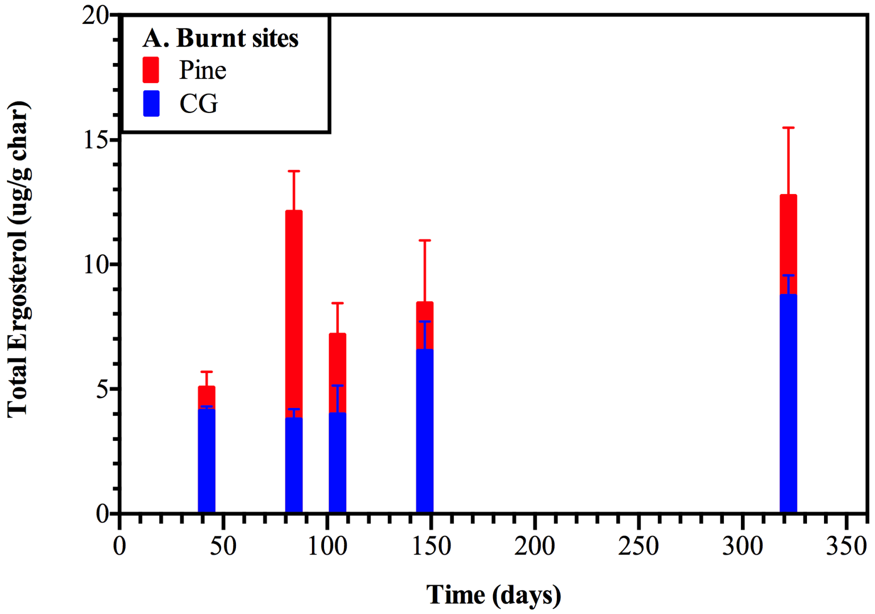

Figure 13. Total Ergosterol of pine and CG *py*Cs in burnt sites

introduced CG material in that there are presumably higher amounts of fungal biomass that are utilizing the pine *pyC*.

Changes in pH of Pine and CG *py*Cs

Irrespective of site treatment (burnt or unburnt), feedstock had a significant effect on pH of the buried *pyC* (Table 6). The percent of variation attributable to differences in feedstock was 26 and 46% for group B1:B3 and group B2:B4, respectively. Feedstock differences accounted for only 7% of the variation between pH in the unburned group. This shows that changes in pH of buried *py*Cs can, at least partially, be linked to structural differences in *py*C feedstocks. Time also had a significant effect on the pH within all treatments, accounting for 39 and 48% for groups B1:B3 and B2:B4, respectively, and for 64% of the variation in the unburned group. This shows that although, feedstock does play a role in the changes of pH of buried pyCs, changes in site conditions over time still account for most of the variation.

Table 6

Two way ANOVA analysis of the effects of time and feedstock (pine vs. CG) on changes in pH of cordgrass and pine derived pyC

	Source of Variation		
Site Treatment	Time	Feedstock	Interaction
	--------------------------- % of variation --------------------------		
Burnt: (B1,B3)	48.45 ****	25.84 ****	13.98 ****
Burnt: (B2,B4)	38.64 ****	46.25 ****	11.34 ****
Unburnt	63.64 ****	6.793 ***	20.00 ****

*,**,***,**** significant at the 0.05, 0.01, 0.001, and 0.0001 level, respectively

ns not significant

As discussed in Chapter V and exhibited by the 2-way ANOVA shown in Table 6, the general trend in pH differs significantly for pine and CG pyC's (Figure 14). The pH of the pine pyC's in both burnt groups remained relatively stable in the 6.2-6.8 range for the majority of the study period. Conversely, the pH of the CG pyC's in both burnt groups dropped continuously over the course of the study period, starting in the 8-8.2 range before dropping to the 6.6-7.0 range by day 217. At this point, the pH in both pine and CG pyC's drops significantly (as was alluded to in Chapter V, where the drop was proposed to be a result of a number of heavy precipitation events) before rising again to a range of 6.2-6.2 and 7-7.2 for pine and CG pyC's, respectively. The difference in trends seen between the pH of pine and CG pyC's was discussed briefly in Chapter V, and is most likely a factor of the different origin of the respective *pyC* feedstocks. The pine *pyC*s, being native to the study area, began with an initial pH that was already similar to

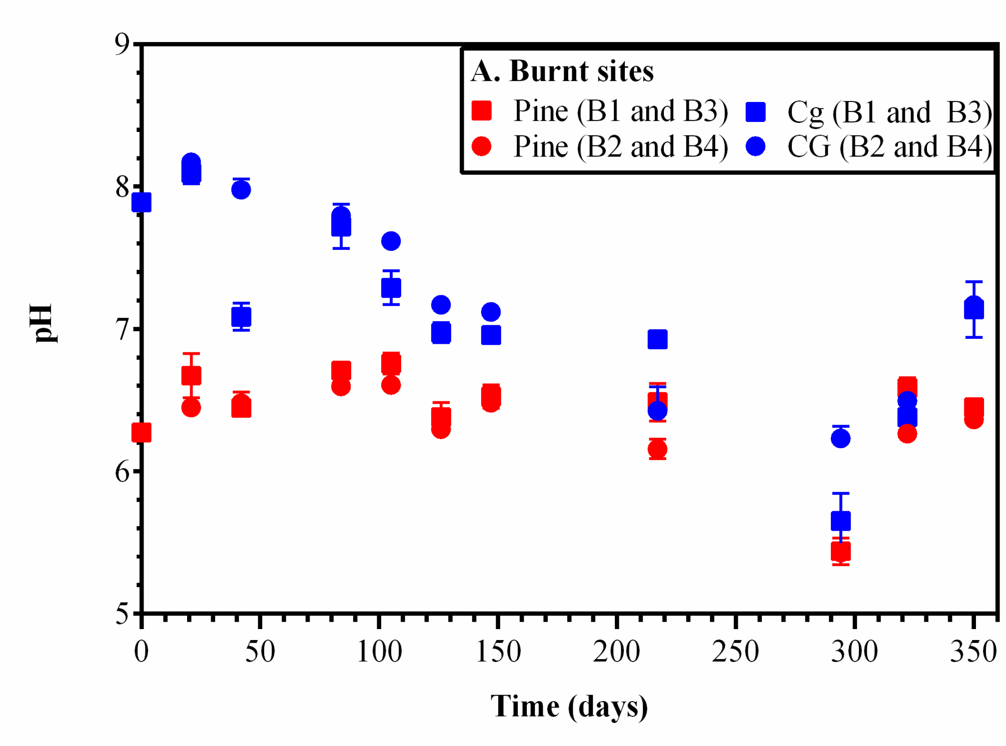

Figure 14. pH of pine and CG *py*Cs in burnt sites.

the soils found in a pine forest, and therefore did not have to adjust itself to reflect the surrounding environment. The CG *pyC*'s, on the other hand, are native to a more basic, coastal environment, and begin with an initially higher pH. The drop in pH that is seen in the CG *pyC* over the course of the study period reflects the adjustment of the *pyC* to the more acidic pine soils.

Concluding Remarks

The hypothesis that CG *pyC*s would see faster rates of degradation due to its initial feedstock chemistry was supported by the more rapid increase of R_{50} values seen in the CG *pyC*'s. The reason for the increase in R_{50} was that the microbial community was restricted to utilizing the more labile fraction of the CG *pyC* as a source of C. As this labile fraction was removed from the *pyC*, the overall recalcitrance of the CG *pyC* increased. The opposite trend was seen in the pine *pyC*s, where the R_{50} values decreased over time. In this case the microbes in the burnt zone were adapted to the utilization of the more recalcitrant pine material. As this material was utilized, the overall recalcitrance of the pine *pyC* was lowered. The more aromatic structures found in the pine *pyC* took longer to metabolize than the more labile CG material, and as a result, the changes in pine R_{50} occurred more slowly. Ergosterol data supported the idea that microbes in the burnt zone were more adapted to the utilization of pine *PyC*, as higher levels of ergosterol were found in the pine *pyC* throughout the study period. As well as further research into differences in R_{50} trends between *pyC* from different feedstocks, future studies should look into the measurement of TOC of the *pyC* after removal of DOC from outside sources, as the presumed differences in TOC between different feedstocks was masked by the large input of DOC that was seen across the study area. A more in depth analysis of the microbial community would also be beneficial to the understanding of *pyC* degradation between *pyC* of different feedstocks.

CHAPTER VII

DIFFERENCES IN CARBON DYNAMICS OF *py*Cs LOCATED IN BURNT AND UNBURNT SITES

Introduction

As wildfires continue to increase in size, they will presumably begin to encroach into new areas that have not previously been effected by burning events. In this case, the microbial community in these new areas will most likely not have encountered *pyC* material before, and will therefore not be adapted for the utilization of *pyC* as a C source. As a result, rates of *pyC* degradation can be expected to occur at a slower rate in these newly burned zones than would have occurred in areas that are subjected to routine burning events. This mode of thinking led us to hypothesize that *pyC*s in the burned zones of the study area will degrade more rapidly than *pyC*s located in the unburned zones, due to a lack of adaptation to the presence of *pyC* seen by the microbial community in the latter. Changes in total organic content, *R50* values, and pH were used to test this hypothesis. Measurements of ergosterol as a function of fungal biomass were also again used as supporting data. Two-way ANOVA was used to compare differences in burned and unburned zones while keeping location and feedstock constant.

Organic Carbon % of *py*Cs Located in Burnt and Unburnt Sites

Two-way ANOVA, examining the effects of time and site treatment (burnt vs. unburnt) on organic carbon content of the buried *pyC*, showed that, irrespective of feedstock, site treatment had no significant effect on the quantity of organic carbon associated with the buried *pyC* (Table 7). The percent of variation in organic carbon attributable to site treatment in the CG and pine *pyC*s was 0.570 and 0.649%, respectively. In contrast, time had a significant effect on organic carbon content associated with the *pyC*s, accounting for 79% of the variation in CG *pyC*s and 66% of the variation in pine *pyC*s. This shows that changes in relevant parameters at each site over time were the main drivers in changes of organic carbon content of buried *py*Cs.

Table 7

Two way ANOVA analysis of the effects of time and site treatment (burnt vs. unburnt) on changes in organic carbon content of cordgrass and pine derived pyC

	Source of Variation		
Feedstock	Time	Site Treatment	Interaction
	--------------------------- % of variation -------------------------		
CG	79.09 ****	0.570 ^{ns}	0.918 ***
PI	65.97 ****	0.649 ^{ns}	2.190 *

*,**,***,**** significant at the 0.05, 0.01, 0.001, and 0.0001 level, respectively

^{ns} not significant

The same general trend of a decrease in TOC in the summer months, followed by increases in TOC during the fall and spring months can be seen in *pyC*s from both the burned and unburned sites (Figure 15). Again, the numerous links of TOC to seasonal changes in meteorological parameters were discussed in Chapter V and can be seen in Figure 7. As discussed in Chapter VI (concerning TOC between pine and CG *pyC*), if we consider that TOC is mainly controlled by inputs from an outside source (and therefore effect the entire study area), it is not surprising that there is no statistical difference between TOC% in the *pyC* from burned and unburned sites. Again, if the DOC from

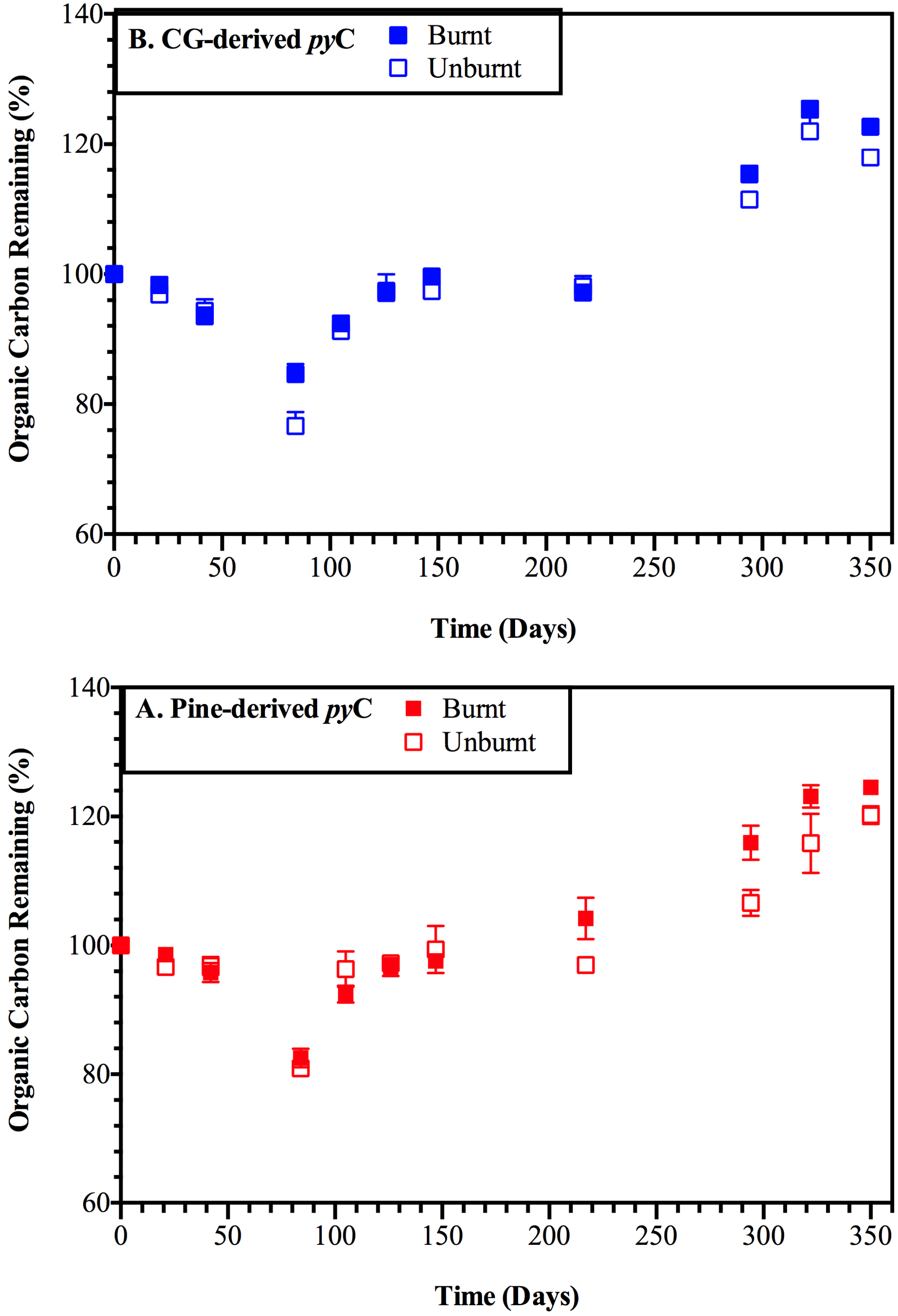

Figure 15. Changes in TOC% of both pine-derived *py*C (A) and CG-derived *py*C (B) in burnt and unburnt sites

outside sources could be removed from the *pyC*s, then changes in TOC% would be more meaningful for determining differences in *pyC* alteration between burned and unburned sites. As of now, this is beyond the scope of this paper and should be considered for future analysis.

Changes in R_{50} of pyCs Located in Burnt and Unburnt Sites.

In the CG *pyC*s, differences in site treatment had a significant effect on the overall recalcitrance of the buried *pyC*, accounting for 13% of the total variation in R_{50} (Table 8). In the pine *pyC*s, however, differences in site treatment did not have a significant effect on the overall recalcitrance of the buried *pyC*, accounting for only 6% of the variation in R_{50} values. This shows that changes in relative recalcitrance in CG *py*C's is more likely to vary in with changes in site treatment than in pine *py*Cs. As in most cases, time had a significant effect on overall recalcitrance of *pyC* regardless of feedstock, accounting for 67% of the variation in CG *pyC*s and 38% of the variation in pine *pyC*s.

Table 8

Two way ANOVA analysis of the effects of time and site treatment (burnt vs. unburnt) on changes in R_{50} of cordgrass and pine derived pyC

	Source of Variation		
Feedstock	Time	Site Treatment	Interaction
	% of variation		
CG	67.14 ****	12.97 *	3.494 ^{ns}
PI	37.82 ****	6.077 ^{ns}	5.369 ^{ns}

*,**,***,**** significant at the 0.05, 0.01, 0.001, and 0.0001 level, respectively

^{ns} not significant

As discussed in Chapter VI, R_{50} values give a much clearer view of overall recalcitrance of the *pyC*s than TOC, as the changes occurring in the original *pyC* material were not masked by the foreign DOC that had accumulated on the *pyC* surface over time. Figure 16A shows an overall decreasing trend in R_{50} of the pine *pyC* in both the burned and unburned zones (this general decrease occurs as microbes utilize the more labile fraction of the pine *pyC*, as previously discussed in Chapter VI). However, there is a notable difference in the rate at which this decrease occurs, however, between the two zones. The R_{50} of the pine *pyC* starts at an initial value of ~ 0.522 in both the burned and unburned site, and decrease linearly as time progresses. In the burned sites, the R_{50} values of the pine *pyC* drop to a value of 0.518 by day 147, and to a value of 0.512 by the end of the study period. The R_{50} values of the pine *pyC* in the unburned sites dropped more slowly, reaching a value of 0.521 by the end of the study period. The faster rates of R_{50} decrease seen in the burned pine *pyC* was attributable to the presence of a microbial community that is more adapted to the utilization of pine *pyC* as a source of C. These microbes are used to seeing the more aromatized structures that are present in the *pyC* and have adapted some means of metabolizing the C from the *pyC* structure (albeit at a slow rate). The microbes in the unburned zones, on the other hand, have presumably never encountered *pyC*, and therefore have not developed a means of utilizing the more aromatic portions of the pine *pyC*. This manifests itself in the lower rate at which the R_{50} drops in the pine *pyC*.

Figure 16B shows an overall increase in the R_{50} values of the CG *py*C in both the burnt and unburnt sites (this increase occurs as microbes cannot utilize the more recalcitrant portions of the CG *py*C, and must utilize the more labile fractions of the

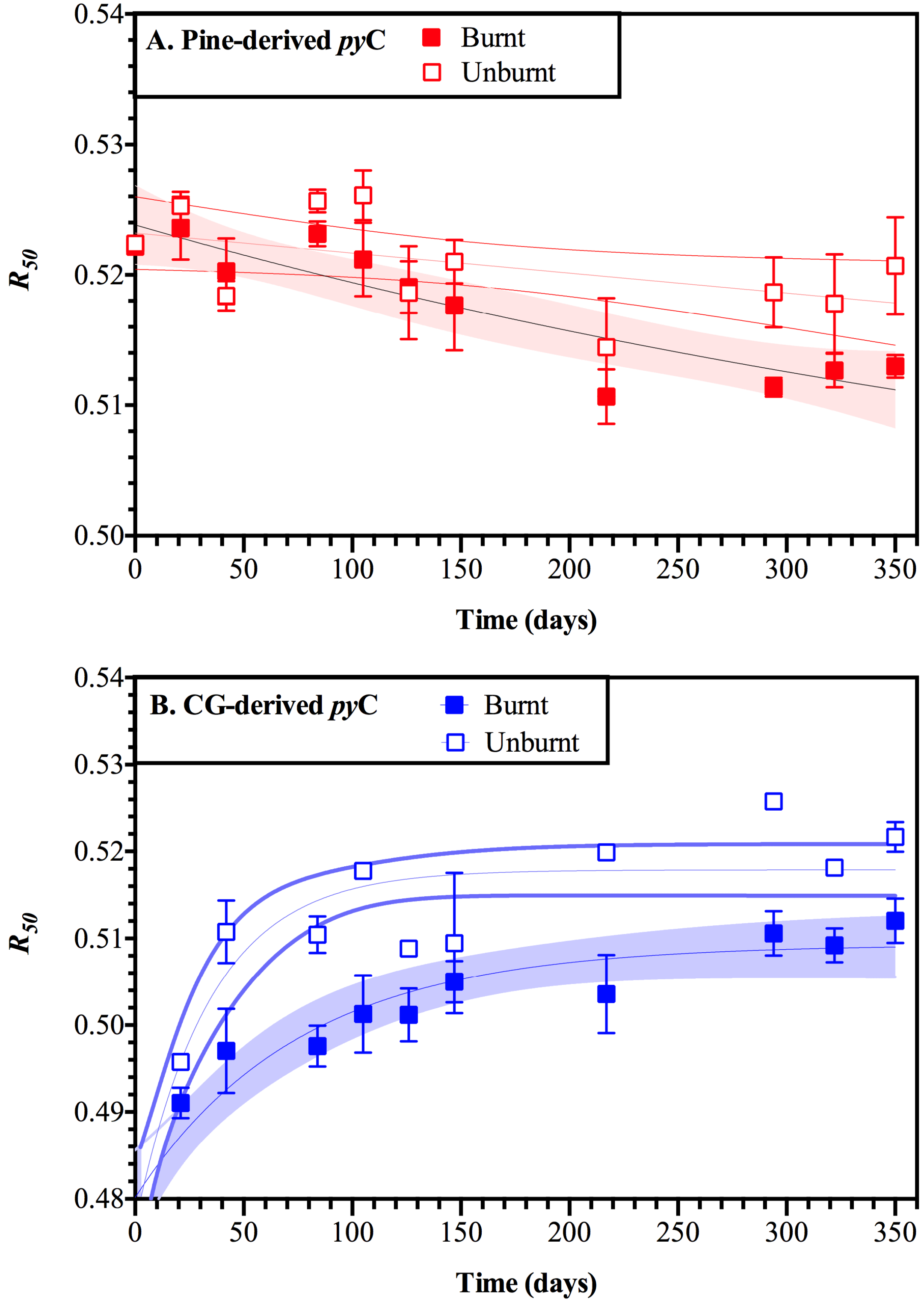

Figure 16. Changes in R_{50} of both pine-derived *py*C (A) and CG-derived *py*C (B) in burnt and unburnt sites.

CG *pyC*, as previously discussed in Chapter VI). There is, again, a noticeable difference at the rate of which this increase occurs between the burnt and unburnt sites. The R_{50} of the CG *pyC* starts at an initial value of 0.477, and increase logarithmically as time progresses. In the burnt sites, the R_{50} of the CG *pyC* rose to a value of 0.50 by day 105 and reached a value of 0.51 by the end of the study period. The R_{50} values of CG *pyC* in the unburned zone rose more quickly, and eventually to a higher value, reaching 0.517 by day 105 and 0.52 by the end of the study period. Much like with the pine *pyC*, the different rate at which R_{50} changes between the burnt and unburnt sites can be associated with the different microbial communities found at each site variation. Previous studies have shown that burnt soils treated with *pyC* have a larger, yet less diverse microbial community (Khododad et al., 2011). This means that, in the burnt zones there is a larger portion of the community that is adapted to utilizing the more recalcitrant fraction of *pyC* as a carbon source, and a smaller portion of the community that must settle with utilizing the more labile fraction of the *pyC*. Alternatively, in the unburnt zones, there is a greater portion of the microbial community that utilizes the more labile fractions of the *pyC* and a smaller portion of the microbial community that utilizes the more recalcitrant fractions of the *pyC*. The fact that CG *pyC* has a higher ratio of labile material within its structure, and that a higher portion of the microbes in the unburnt zones are willing to utilize this labile material, manifests itself in the more rapid change in R_{50} in the unburnt CG *pyC*s.

Fungal biomass (ergosterol)

As mentioned in Chapter VI, fungi are a primary utilizer of *pyC* in forest soils and can be considered a reliable marker for general microbial biomass within *pyC* material.

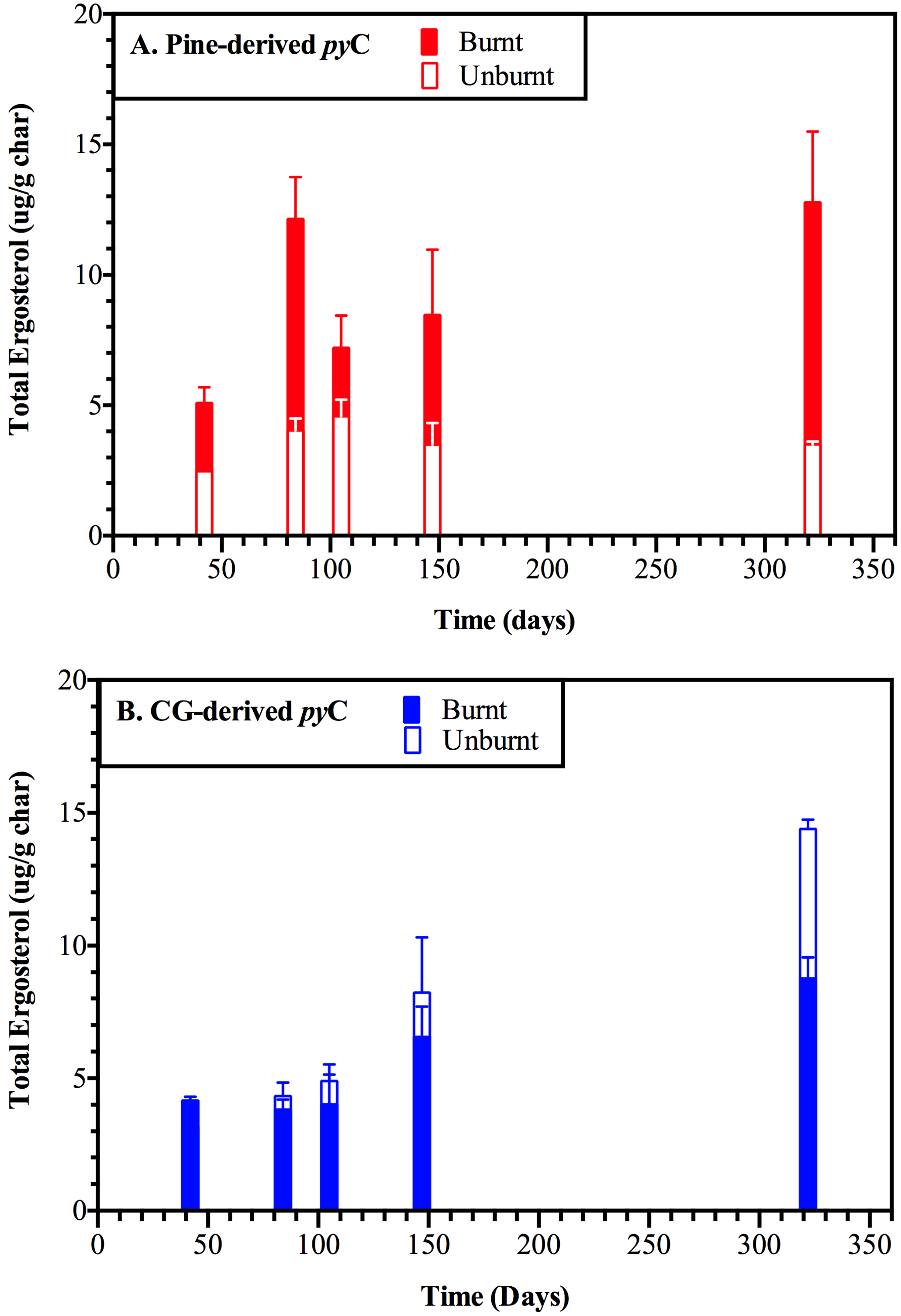

Figure 17. Changes in total ergosterol of both pine-derived *py*C (A) and CG-derived *py*C (B) in burnt and unburnt sites.

Ergosterol levels within the *py*C were measured at 5 points over the course of the study period and can be seen in Figure 17. In general, ergosterol levels increased with time across all site treatments and *pyC* types (with the exception of pine *pyC* in the unburned zone). Figure 17A, shows that for the pine *pyC*s, ergosterol levels were generally higher in the burnt areas, nearly doubling (and in some cases, tripling), the ergosterol levels found in the unburnt areas. This data supports the argument made for the differences in changes in R_{50} for the pine *pyC*s, as we see more fungal biomass in the burnt areas where microbial communities are adapted to the presence of the pine *pyC*. Figure 17B shows the opposite trend occurring with the ergosterol levels in the unburnt sites, where, as time progresses, ergosterol levels in the unburnt CG *pyC*s become increasingly higher as time progresses when compared to the ergosterol levels in the burnt CG *pyC*'s. This data again supports the argument made for differences in changes of R_{50} for the CG *pyC*'s, as there is a higher portion of fungal biomass in the unburnt zones that must settle for the more labile CG material.

Changes in pH of *py*Cs Located in Burnt and Unburnt Sites

When the comparison between groups B1:B3 and U1:U2 is considered, site treatment has a significant effect on the pH of the buried pyC regardless of feedstock (Table 9). In this case, the percent of variation attributable to differences in site treatment was 6 and 4% for CG and pine pyCs respectively. When considering the comparison between groups B2:B4 and groups U1:U2, site treatment had only had a significant effect on the pH of buried CG pyCs, and was not significant when considering pine pyCs. Time again had a significant effect on pH of all pyCs, regardless of feedstock or groups compared. When comparing burnt groups B1:B3 to the unburnt group, time accounted for

78 and 73% of changes to pH for CG and pine pyCs, respectively. When comparing burnt group B2:B4 to the unburnt group, time accounted for 76 and 81% of changes to pH for CG and pine pyCs, respectively. This shows that changes in relative parameters at each site over time were the primary causes of the change seen in pH.

Table 9

*Two way ANOVA analysis of the effects of time and site treatment (burnt vs. unburnt) on changes in pH of cordgrass and pine derived py**C***

	Source of Variation		
Comparison by group	Time	Site Treatment	Interaction
(B1,B3) : (U1,U2)	-------------------------- % of variation ----------------------		
Feedstock			
CG	77.87 ****	5.558 ***	6.682 ****
P	72.91 ****	4.014 ***	3.842 *
(B2,B4) : (U1,U2)			
Feedstock			
CG	75.88 ****	10.98 ****	5.828 ****
P	80.88 ****	0.483 ns	3.433 **

*,**,***,**** significant at the 0.05, 0.01, 0.001, and 0.0001 level, respectively

ns not significant

The pH in pine and CG *pyC*s follows the same general trend discussed in previous chapters (Figure 18). pH at all sites remains relatively stable in the 6.4-6.8 range for the majority of the study period, only dropping to a range of 5.0-5.5 during the period from day 217 to 294 (Figure 18A). Although all sites follow the same trend, there is a statistically significant difference between pH of the pine *pyC*s of the burnt group B1:B3 and the unburnt sites. This small difference is mostly like the result of different metabolic

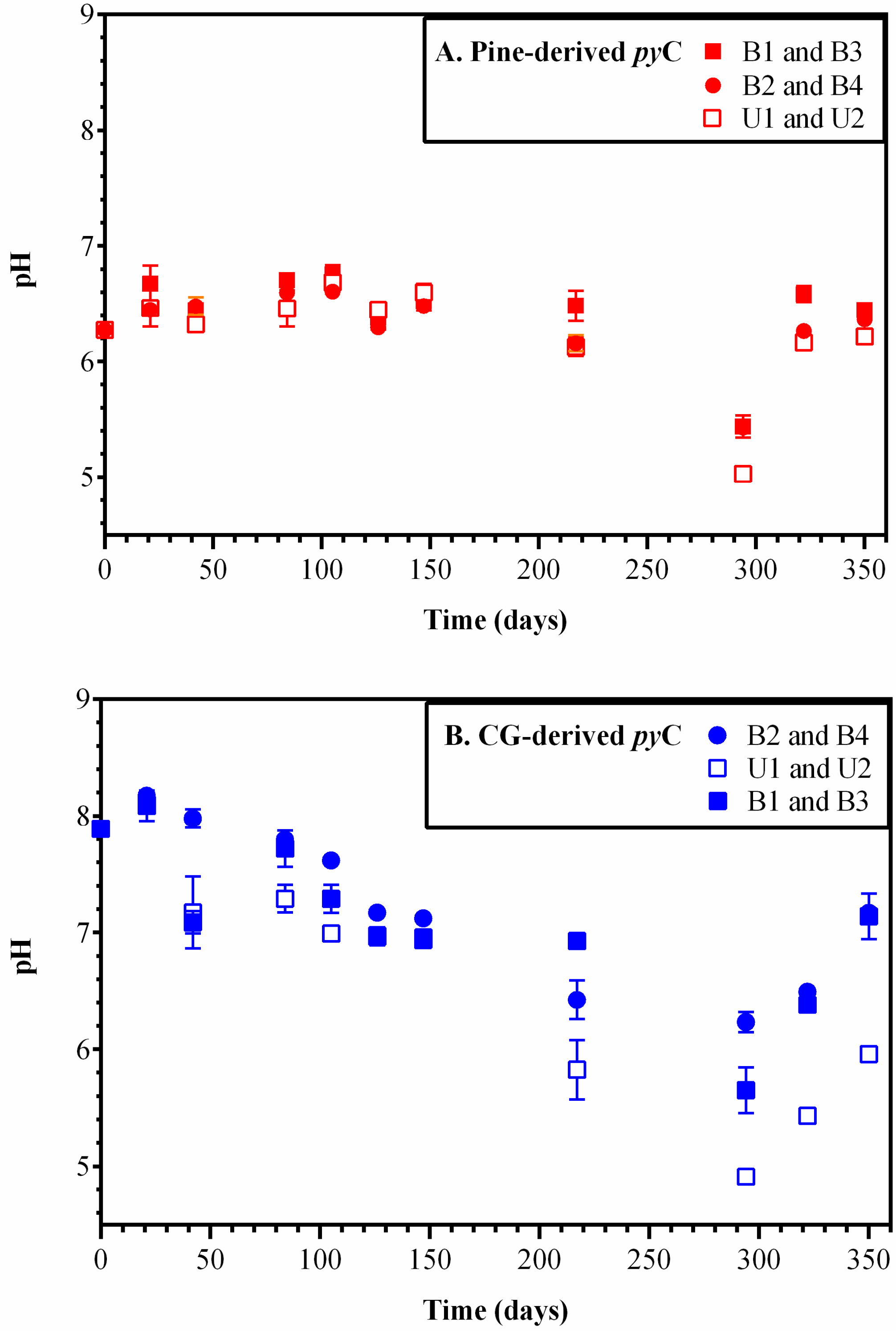

Figure 18. Changes in pH of both pine derived *py*C (A) and CG-derived *py*C (B) in burnt and unburnt sites.

pathways utilized by the different microbial communities present in the burnt and unburnt zones. Because the two communities are utilizing different components of the *pyC*, they will have different waste products. These waste products could potentially be the reason for the difference in pH that was encountered. Much like as was seen in the pine *pyC*s, the trend in pH for CG *pyC*'s in both the burnt and unburnt zone also remained similar throughout the study period, displaying a relative decrease as time progressed (Figure 18B). The burnt zones, however displayed a slightly higher pH than the unburnt zones. The *pyC*'s in the both burnt and unburnt zones displayed an initial pH of 7.9. By day 74, the pH of the *pyC*'s in the burnt and unburnt sites had dropped to 7.8 and 7.2, respectively. By day 294, the pH of the *pyC* in the burnt zones had decreased to the 5.7-6.3 range, while the pH of the unburnt site *pyC*'s had decreased to 4.9. By the end of the study period, the burnt site *pyC*'s had a pH of 7.2, while the unburnt *pyC*'s finished with a pH of only 6. Much like with the pine *pyC*s, the difference between the pH of CG *pyC* in burnt and unburnt sites is most likely attributed to the different waste products generated by the different microbial communities found at each type of site. There is also evidence that significant burning

Concluding Remarks

The more rapid decline of *R50* values of pine *pyC*s in the burnt sites supports the hypothesis that microbes in burnt areas will degrade *pyC*'s at a faster rate than *pyC*'s in the unburnt zone. This was attributed to the ability of microbes in the burnt zone to utilize C from the pine *pyC*, whereas microbes in the unburnt zone are not adapted to the presence of *pyC* and therefore have little to no means of metabolizing it. In the case of the CG *pyC*'s, however, the R_{50} values of the *pyC*'s changed more rapidly in the unburnt sites than in the burnt sites, which is the opposite of what we hypothesized. This trend was attributed to: 1) the restriction of microbial communities in both sites to only utilizing the more labile fraction of the *pyC* (because it is not native to the study area), and 2) the smaller proportion of microbes in the burnt zones that were that were willing to utilize this labile fraction. Ergosterol data supported both of these arguments, as in the case of the pine *pyC*, there was more ergosterol in *pyC*'s from the burnt zone than in *pyC*'s from the unburnt zone. The opposite was true in the case of the CG *pyC*, where more ergosterol was found in the *pyC*'s from the unburnt zone than in the burnt zone. As well as further research into differences in R_{50} trends between *pyC* in in burnt and unburnt sites, future studies should look into the measurement of TOC% of the *pyC* after removal of DOC from outside sources, as the presumed differences in TOC% between *pyC* in differently treated sites was masked by the large input of DOC that was seen across the study area. A more in depth analysis of the microbial community would also be beneficial to the understanding of *pyC* degradation between *pyC* of different feedstocks.

CHAPTER VIII

CONCLUSION

The recognition of the high resistance of pyrogenic organic carbon (*pyC*) to both abiotic and biotic forms of degradation has led to increased interest in *pyC* as a potential C sink, as well as a strategy for sequestering atmospheric CO_2 to mitigate excess C emissions (Lehmann et al., 2006). Current estimates for C sequestration potential by *pyC* are as high $9.5X10^9$ tons/yr, although a number of varying factors have the potential to cause this estimate to fluctuate. Changes in climate, soil structure, microbial community, and *pyC* feedstock and formation processes have all been shown to effect the degradation of *pyC*. Most studies on these factors and their effects on *pyC* degradation, however, have been conducted in a controlled lab setting where certain environmental factors could not be replicated. This study looked to examine the potential effects that seasonal changes in temperature and precipitation, differences in *pyC* feedstock material, and differences in site treatments (burnt vs. unburnt) would have on the potential degradation of *pyC* in a field setting.

We observed that as seasons change, the potential for DOC from outside sources to adsorb itself to the surface of *pyC* changes as well. During the fall, increases in the amount of leaf and needle litter on the soil surface result in an increase in the total DOC leaching downward through the soil, eventually adsorbing to the surface of pyCs. During the winter, this adsorption of DOC to the surface of pyCs begins to slow as colder temperatures lead to decreasing rates of microbial degradation of this litter, leading to smaller amounts of DOC entering the soil system. As spring arrives with higher temperatures, more DOC is released from the litter as microbial activity increases, leading to another increase in the amount of DOC adsorbing to the pyC surface. We also observed that pine and CG derived *pyC*’s degrade very differently as different portions of the *pyC*’s are utilized by the microbial community. The microbes in the pine forest are able to utilize the more recalcitrant fraction of the pine pyC, and as they remove this recalcitrant fraction, the overall recalcitrance of the pine pyC decreases. In the case of the CG pyC, however, the microbes in the pine forest must utilize the more labile fractions of the pyC, and as this labile fraction is removed, the overall recalcitrance of the CG pyC increases. Finally, we observed *pyC*s degrade differently in burnt and unburnt sites due to the differences in microbial community found in each. For instance, in the burnt sites, the microbial community was better adapted to the utilization of pine pyC material, and was able to degrade this material faster than the microbial community found in unburnt sites.

Further research should include a more intense analysis of the microbial community present in the *pyC*s as well as continuing investigation of the recalcitrance of the different *pyC*s after field aging. The effects of DOC from outside sources was also shown play a role in the overall C dynamics of pyC and should also be considered as a point of emphasis in future work.

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