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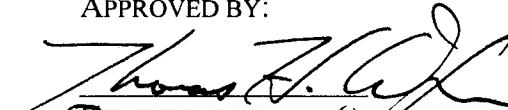
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**CHARCOAL IN PONDEROSA PINE ECOSYSTEMS OF WESTERN MONTANA:
DECOMPOSITION, MINERALIZATION, AND QUANTIFICATION**

BY
VALERIE J. KURTH
B.A. MACALESTER COLLEGE, 1999

PRESENTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
MASTERS OF SCIENCE
THE UNIVERSITY OF MONTANA
2004

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Charcoal in ponderosa pine ecosystems of western Montana: decomposition, mineralization, and quantification

Advisor: Thomas H. DeLuca 

Fire-deposited charcoal potentially plays a major role in ecosystem processes due to its ability to sorb organic compounds and its highly porous nature. Recent studies suggest that charcoal's influence is broad encompassing in soil processes; it has the capacity to enhance microbial community development, alter soil C:N ratios, and alleviate the effects of inhibitory secondary organic compounds. The purposes of the two studies reported here were to investigate approaches for charcoal quantification and to examine the influence of charcoal on litter decomposition in ponderosa pine ecosystems. Traditional methods for charcoal quantification were found to greatly underestimate soil charcoal content; however, it was observed that the Walkley-Black method for determination of organic C was not sensitive to charcoal C. The method estimated about 80% of soil charcoal, which is an improvement over current methods, and appeared to be most reliable at soil charcoal contents equal to or greater than 0.5% (w/w; C as charcoal). This work illustrates the difficulties in establishing a standardized method and presents Walkley-Black as a viable rapid alternative. The influence of charcoal on litter decomposition was studied for two common plant species (*Pinus ponderosa* and *Arctostaphylos uva-ursi*) by using complementary field and laboratory incubations where litter was amended with various rates of charcoal. Response variables measured for the litter included mass lost, total C, N, and lignin, extractable free phenols, net N mineralization and CO₂ evolution. Charcoal was found to enhance the decomposition of *P. ponderosa* litter and to facilitate microbial breakdown of lignin for *A. uva-ursi* litter. This investigation helps clarify charcoal's capability to influence ecosystem processes. Future research should further elucidate the influence of charcoal on C and N cycles and predictions should be based on soil charcoal contents that can be estimated by Walkley-Black.

ACKNOWLEDGEMENTS

I would like to thank my advisor and committee chair, Tom DeLuca, for his exceptionally generous amounts of advice, enthusiasm and encouragement. I would also like to thank my committee members, Ron Wakimoto and Ray Callaway for their input and assistance. I received invaluable field assistance from Sarah Pierce, Treg Christopher, Mark VanderMeer, Marnie Criley, Martin Twer, and Ari Jewell. Lab support and advice was also given by Tricia Burgoyne, Meagan Conry, Rachel Brimmer, Derek MacKenzie, and Michael Gundale. Technical input was also provided by Heiko Langner. This research was partially funded by a grant from USDA McIntire-Stennis program.

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PREFACE

Fire is a primary form of natural disturbance in ponderosa pine (*Pinus ponderosa*) ecosystems of the inland northwest and is widely recognized for its importance in forest nutrient cycles (Neary et al., 1999). Fire volatilizes much of the forest floor and causes a pulse of N mineralization, both of which contribute to the promotion of forest primary succession. In temperate forests where N is considered a limiting nutrient, the process of fire is integral in maintaining forest health and productivity. Although much is understood about fire's direct and indirect role in forest nutrient cycling and succession, there is a significant gap in our knowledge when we consider the biochemical role of charcoal in soils. Charcoal is perhaps one of fire's most eminent legacies: it is known to be highly recalcitrant in soils and its biochemical properties make it a prime candidate for influencing ecosystem processing (Zackrisson et al., 1996). Therefore, it is imperative that we consider its potential role in nutrient cycling and determine an accurate and rapid method to quantify its content in soils.

Charcoal has been demonstrated to enhance soil productivity in a variety of ways. For example, *terra preta* (black earth) soils in the Amazonian rainforests of Brazil have demonstrated that years of anthropogenic charcoal deposition by indigenous peoples has resulted in pockets of exceptionally fertile soil (Glaser et al., 2002). This soil is characterized by higher exchange capabilities, base saturation, and nutrient availability than the surrounding soils in the same region (Zech, et al., 1990). Other properties of the *terra preta* phenomenon are discussed by Glaser et al. (2004) and include sustainable soil fertility management and C sequestration in light of recent global climate change concerns.

Related examples demonstrative of charcoal's unique nature exist for multiple natural ecosystems. Charcoal has been found to facilitate seedling establishment through phenolic adsorption by which both N availability and seedling N uptake were enhanced (Wardle et al., 1998). In Californian chaparral ecosystems, charcoal added to soil in the form of charred wood or charate (powdered) was found to stimulate germination for several native plant species (Keeley et al., 1985; Keeley and Pizzorno, 1986; Keeley, 1987; and Thanos and Rundel, 1995). Other research demonstrate charcoal's ability to enhance the biological process of nitrification in boreal forests (DeLuca et al., 2002; and Berglund et al., 2004).

It is thought that much of charcoal's effectiveness is due to its biochemical properties. Charcoal's ability to adsorb organic compounds, such as phenols released by plants and decomposing litter, has been exploited for centuries, from use in water purification to human ingestion in cases of accidental poisoning. In soils, this ability to sorb phenolic compounds could significantly alter many ecosystem processes; phenols have been implicated in enhancing humus formation and N immobilization, as well as inhibiting N mineralization and litter decomposition (Horner et al., 1988; Kuiters, 1990). Furthermore, some of the compounds sorbed to charcoal are known to be allelopathic (Callaway and Aschehoug, 2000; Bais et al., 2002), and thus, the regular deposition of charcoal by fire may alleviate the negative effects imposed by phenols.

Charcoal may also have biological effects on soils. Soil microbial communities are positively affected by the presence of charcoal; in fact, charcoal particles appear to act as foci for microbial activity for two reasons. The adsorbed C serves as a food source for microbes while charcoal's highly porous structure provides microhabitats that

facilitate predator-prey relationships (Ehrhardt and Rehm, 1985; Zackrisson et al., 1996; and Pietikäinen et al., 2000).

Although research has clearly demonstrated charcoal's capacity to influence ecosystem properties, surprisingly little is understood about its biochemical potential, especially in fire-prone ponderosa pine ecosystems of western Montana. Historically, these forests experienced regular burn intervals of approximately 13 to 50 years (Arno et al., 1995), but this regime has been greatly altered over the last century as a result of active fire suppression and forest management activities in western Montana. This makes it even more imperative that we understand the role of fire-deposited charcoal because the information gained will be useful to both ecologists and land managers of the region.

This thesis strives to narrow the existing knowledge gap on the ecological function of charcoal using two distinct approaches. The first chapter details the difficulties associated with current methods of quantifying charcoal in soils and offers a viable rapid alternative. Effective quantification of charcoal in soil has broad application; for the most part, efforts to predict charcoal's effects are deteriorated if no attempt at quantification can be made. Furthermore, this knowledge will prove useful to fire and ecosystem ecologists, as well as paleoecologists looking to reconstruct fire histories. The second chapter of this work specifically examines one of the many ways charcoal could influence ecosystem processes: litter decomposition. This was chosen as a focus because, to date, our understanding of the associations between charcoal and decomposition are poor (Zackrisson et al., 1996 and Wardle et al., 1998). Although it is logical to conclude that by encouraging microbial colonization, charcoal would enhance the decomposition of associated litters, this has not been demonstrated or applied broadly. In this study, we

investigated whether charcoal had the potential to alter the decomposition rates of two types of litter. In addition to monitoring the effect of charcoal on mass loss rates, we monitored changes in total N, C, lignin content, and extractable free phenolics. The effect of charcoal on litter decomposition is discussed in the context of ecosystem wide processes.

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CHAPTER 1. A METHOD FOR QUANTIFYING CHARCOAL IN FOREST SOILS

ABSTRACT

Traditional methods for estimation of charcoal in soils are can be tedious, expensive, and are not necessarily quantitative; however, a standard quantification method would be a great benefit to ecologists interested in charcoal's biochemical properties or its role in carbon (C) storage. Our research objective was to establish a relatively straightforward and accurate method for charcoal quantification in soil. Laboratory investigations suggested that current techniques underestimated charcoal by as much as 70%. Subsurface soil samples were taken from areas not likely exposed to charcoal deposition and amended with a range of charcoal contents (laboratory produced) and organic matter (EKO Kompost). Samples were analyzed for organic C using the Walkley-Black method and it was apparent that this method was not sensitive to charcoal C. Total C was measured by combustion and percent charcoal was calculated by subtracting organic C from total C and incorporating a simple multiplier. This method estimates about 80% of soil charcoal, which is an improvement over current methods, but it is most reliable at charcoal contents equal to or greater than 0.5% (w/w) charcoal. It should be considered as a viable alternative for estimating soil charcoal content.

INTRODUCTION

Fire is a fundamental form of natural disturbance in most forested ecosystems; however, there are significant gaps in our ecological understanding of its consequences. Fire promotes the recycling of nutrients by clearing out the forest floor and causing a pulse of (N) mineralization, both of which contribute to the initiation of primary succession. Although much is understood about the impact of fire on nutrient cycling and successional processes, our current knowledge of one of fire's most eminent legacies, charcoal, is extremely limited. This is unfortunate because charcoal has the potential to be extremely influential in soil ecology and fertility due to its ability to sorb organic compounds and its inherently porous nature (Zackrisson et al., 1996).

Examples of charcoal's unique properties exist for many different ecosystems. Hundreds of years of anthropogenic charcoal deposition in the Brazilian Amazon has resulted in pockets of exceptionally fertile soil (*terra preta*) within an area typically

characterized by poor nutrient availability (Glaser et al., 2002). Recent work in the boreal forest of Sweden has demonstrated wildfire-deposited charcoal's high persistence in soils as well as its facilitative effects on nitrification and microbial activity (Zackrisson et al., 1996; DeLuca et al., 2002; Bergland et al., 2004). Charcoal has also been shown to enhance seedling establishment and productivity, presumably due to its ability to sorb inhibitory secondary metabolites released in plant litter (Wardle et al., 1998). In agriculture, black carbon deposits from the burning of crop residues has been shown to sorb significant amounts of the pesticide diuron, suggesting that charcoal's function may extend well beyond the general realm of ecology (Yang and Sheng, 2003).

Unfortunately, understanding of charcoal's ecological properties is limited by the lack of an appropriate method for quantifying charcoal content in soils. Current methods for quantifying charcoal in soil are tedious, expensive and often inaccurate. Furthermore, each method is directed towards answering one specific research objective (i.e. fire history reconstruction), and therefore, a standardized and widely accepted charcoal quantification method has not yet been developed. The purpose of this research was to develop a method for estimating charcoal content in soil that is rapid, accurate and relatively inexpensive.

LITERATURE REVIEW

Charcoal has been shown to be highly persistent in soils; it can remain in the soil solum for hundreds of years (Zackrisson et al., 1996). However, estimation of charcoal content of soil has proven to be an exceptional challenge as charcoal occurs in a large range of size fractions, and, depending on the thermal conditions that produce it, its

chemical characteristics can differ widely. These factors make charcoal particles extremely variable in nature, and, therefore, the development of a method that is rapid and reliable for estimation has proven to be remarkably difficult (see Schmidt and Noack, 2000).

Charcoal is commonly analyzed as a paleoecological tool to reconstruct fire histories. Sediment cores are typically collected from lakebeds or alluvial fans and charcoal concentrations analyzed in any number of ways, including sieving, pollen slide thin sections, and chemical assays (Whitlock and Millspaugh, 1996; Laird and Campbell, 2000). Tree species can sometimes be identified using scanning electron microscopy (SEM) and fire dates can be estimated using ^{14}C dating techniques. However, these paleoecological methods are not appropriate for determining actual charcoal content of soil because they tend to focus on macroscopic particles ($>125\ \mu\text{m}$) that can be easily sorted out of the sediment. Hence, the fine charcoal fraction in soils is often overlooked and these estimates are not necessarily quantitative.

This lack of precise quantification is apparent in a pedoanthracological method developed by Carcaillet and Thinon (1996). In this method, soils are passed through a series of sieves ranging from 5 to 0.4 mm mesh size followed by further extraction through a laevigation apparatus. Finally, hand sorting and identification of particles is done with a microscope. The extremely fine charcoal particles are often overlooked in this method because they are basically immeasurable and levels are thought to be “insignificant” in soils (Carcaillet and Talon, 2001). Furthermore, fine particles can be transported farther after a fire (Whitlock and Millspaugh, 1996), and are therefore not relevant for reconstructing fire histories.

The questions of the significance and logistics of quantifying fine charcoal particles in paleoecological methods is of particular interest in light of recent work demonstrating that the majority of soil charcoal is found in the finest particle size fraction (Skjemstad et al., 2002; Tinner and Hu, 2003). Furthermore, given its inherent high surface area and C purity, it is very likely that the fine fraction is of higher biological significance than the coarser fragments that are easier to account for in soil. The fine fraction is also undoubtedly overlooked in methods that exploit the low density of charcoal because the fine particles do not float in water (Magid et al., 2002). Regardless, many of these methods are appropriate for reconstructing fire histories since particles ≥ 0.5 mm are generally good indicators of past fires (Ohlson and Tryterud, 2000). However, when the objectives are quantifying char or determining its biological importance these existing methods are inadequate.

Probably the most common method for determining charcoal is initial removal of non-char organic matter by boiling samples in nitric acid (Tolonen, 1986). This method is widely accepted by both fire paleoecologists (e.g. Singh et al., 1981; Swain, 1973) and ecosystems ecologists (e.g. Zackrisson et al., 1996) for quantifying charcoal abundance. The acid essentially digests other forms of organic C, removing adhering humic materials that may be confused with charcoal, as the charcoal itself is relatively resistant to acid digestion. Drawbacks of this technique are discussed by Laird and Campbell (2000) and include inconsistent gravimetric results where charcoal particles are small enough to pass through the filter paper (Rhodes, 1998) or when total charcoal content of soils or sediment are exceptionally small and produce high variation (Novakov et al., 1997). Other possible sources of error include contributions from fossil fuel combustion near

urban areas (Griffin and Goldberg, 1975; Winkler, 1985) and nitric acid inadvertently digesting particles with low C contents (MacDonald et al., 1991). Laird and Campbell's (2000) research also demonstrated the ability of charcoal to adsorb impurities within nitric acid, resulting in charcoal overestimations.

Another twist on the nitric acid digestion was suggested by Winkler (1985). This method uses a nitric digestion to remove organic materials, theoretically leaving only elemental carbon (charcoal) and mineral particles. This is followed by loss on ignition in a muffle furnace to calculate the amount of charcoal present. This method was closely correlated with values obtained from visual counting techniques and therefore, is important from a paleoecological standpoint; however, for the reasons discussed above, it may not serve for complete quantification or biochemical application. A variation of this method was proposed for marine sediments such that adjustments were made for the potentially high CaCO_3 content of marine sediments (Verardo, 1997). In this variation, specially designed aluminum sample cups were used in the nitric acid digestion to remove CaCO_3 , coal, pollen, and humic acids and total C was measured with an elemental analyzer. It is unlikely that this approach would be necessary for analyzing typical forest soils unless they contained high levels of carbonates.

Kuhlbusch (1995) presented a different approach for quantifying soil black carbon. His method was directed at examining residues from vegetation fires with the ultimate goal of collecting data that could be applied to global carbon cycling. Samples are extracted with HNO_3 and HCl and then analyzed for C and H on an elemental analyzer. However, it is not completely clear whether or not the HNO_3 would cause complications such as those discussed above.

Other chemical digestions have also been suggested. Rhodes (1998) found that weak hydrogen peroxide partially digests organic materials and bleaches the color out without attacking charcoal particulates. Thus, the dark charcoal particles were easily counted using a microscope. The value of this method is that charcoal can be enumerated but by using a weak reagent, the integrity of the charcoal is not compromised.

Carbonaceous particles, such as soot from fossil fuel combustion, have also been quantified, mainly in lake or marine sediments. Griffin and Goldberg (1975) used a series HF, HCl, and peroxide digests followed by an infrared analysis to estimate elemental carbon in marine sediments. Particulates from fossil fuel combustion were not differentiated from charcoal particles; however, it was suggested that particles that originated from coal or oil burning were distinctly spherical in shape compared to particles from forest fires. Another method used by Renberg and Wik (1985) on lake sediments from Sweden uses a prolonged peroxide digestion followed by counting particles with a stereomicroscope, but a major disadvantage of this approach is the time-consuming nature of the counting procedure. A modification of Griffin and Goldberg's methods, as well as an improvement on Renberg and Wik's was proposed by Rose (1990). This method used a more aggressive digestion that decreased processing time and was more sensitive to fine particles than both of the previously discussed methods. Also, this newer method was specifically aimed at counting those particles that were emitted as a result of fossil fuel combustion.

A different approach to quantifying black carbon in soils is based on its oxidative degradation properties produced during acid digestion (Glaser et al., 1998). In this approach, polycyclic or substituted aromatic centers are converted to

benzenepolycarboxylic acids (BPDA) during HNO₃ digestions, and these compounds are used as markers for black C. Polyvalent cations are removed by cation exchange resins and BPDA are analyzed by gas chromatograph. Since this method appears to provide an accurate estimation of black C for artificially charred materials, it was applied to Terra Preta soils in the Brazilian Amazon (Glaser et al., 2000).

A method introduced by Skjemstad et al. (1993) uses a combination of high-energy ultraviolet (UV) photooxidation, and nuclear magnetic resonance (NMR) spectroscopy. The photo-oxidation was demonstrated to destroy a variety of organic materials commonly found in soils; however, physical protection of organic matter by soil microaggregates was found to be a potential problem. In an ensuing investigation, a stronger photo-oxidizer was used and the chemical compositions were examined using solid state ¹³C CP/MAS NMR (Skjemstad et al., 1996). With the NMR results, fine fraction (<53 μm) charcoal abundance was approximately estimated to be around 88% in the Australian soils sampled. A more quantitative approach was developed for estimates using the aryl content of the fine soil fraction (Skjemstad et al., 1999) and it was found that nearly all of the charcoal in Australian soils is found in the fine particle fraction. Similar results were reported for U.S. agricultural soils (Skjemstad et al., 2002) in which fine fraction charcoal ranged from 1.8 to 13.6 g C kg⁻¹, suggesting that charcoal is highly recalcitrant and represents a significant portion of C dynamics in soils. The biggest disadvantage to the photooxidation-NMR method is that it is both extremely time consuming and prohibitively expensive, and therefore, it is likely that many laboratories would lack both the financial and equipment resources to run the procedure.

It has been suggested that the Walkley-Black method for determining soil organic carbon is unaffected by the presence of elementary carbon, such as charcoal or coal (Piper, 1944). The Walkley-Black method uses a dichromate digestion followed by either colorimetric or titrimetric analyses of organic C. A variation on this method, developed by Heanes (1984), uses an externally applied heat source and provides a highly accurate estimation of total organic C without interference from carbonates. Skjemstad and Taylor (1999) compared the Walkley-Black and the Heanes methods, to results obtained with a LECO carbon analyzer. They amended Australian soils with charcoal produced from various plant material, including straw, eucalyptus leaves, and eucalyptus wood. The Walkley-Black method detected considerably less charcoal than the Heanes and wood-based charcoal was found to be more susceptible to oxidation than leaves or straw. They suggest that neither method was very reliable in charcoal detection since naturally produced charcoal has a great amount of variability in its chemical nature.

Kerven et al. (2000) also compared Walkley-Black, Heanes, and C by combustion with respect to charcoal oxidation. Their results were slightly different than those of Skjemstad and Taylor (1999) in that the Walkley-Black measured C in less than 10% of the charcoal, the Heanes in 85-96%, and the combustion analyzer in 97-106%. They concluded that the unheated dichromate digestion (Walkley-Black) does not significantly attack charcoal C. This suggests that Walkley-Black may be used to effectively estimate char content in soils by subtracting its organic C content from the total C content of the soil.

It is clear from the preceding discussion that a wealth of analytical and visual approaches exists for quantifying charcoal content in soils and sediments. However,

because each method is based on serving one specific objective, such as fire history reconstruction or charcoal quantification, and standard reference materials do not exist, it is difficult to draw direct comparisons among them (Schmidt and Noack, 2000).

Furthermore, to date, there is no widely-accepted method that is proven to be inexpensive and accurate for estimating the charcoal content of soils.

The purpose behind this work is to develop a method for estimating soil charcoal because it is difficult to predict the effects of fire-deposited charcoal without an accurate estimation of how much exists in the soil. The objectives of this series of experiments were:

(1) Determine charcoal C recovery from soils amended with charcoal and organic matter using conventional nitric acid and peroxide digests, and the Walkley-Black method.

(2) Develop and evaluate a rapid and accurate method for quantifying wood charcoal in mineral soil.

(3) Apply this method to soils with unknown charcoal levels.

MATERIALS AND METHODS

Subsurface soil was collected from three different sites in the Missoula Valley that were thought to have minimal amounts of charcoal in them (Table 1.1). All soils were collected at a depth of 60-120 cm to avoid potential contamination by surface char. The soils were air dried and sieved to 2 mm.

Table 1.1 Collection sites and soil subgroups for each soil used to test methodology.

Site	Subgroup
Deer Creek	Udic Haplustepts
Pattee Canyon	Typic Eutrocryalfs
Rattlesnake	Calcic Argixerolls
Willow Creek	Lithic Dystrustepts

Charcoal was produced in a muffle furnace at 450°C from Douglas-fir wood. Pieces were ground into a range of sizes using an apple grinder.

Soils were amended with humic materials generated from commercially available compost (EKO Compost; Missoula, MT). Compost was sieved to 2 mm to remove any coarse woody materials. Soils were amended with either 10% organic matter (w/w) or 1% charcoal (w/w) or both for the preliminary digestions. Non-amended soil and undigested controls were also included. Three replicates were used from each treatment.

Charcoal Recovery in Nitric Acid and Peroxide Digestions

Five grams of each treatment mixture were placed in a digestion tube with 20 ml of HNO₃. The acid was used in two different concentrations: 10M and 11.1M (70%). Samples were placed in the digestion block and digested at 95°C for 24 hours. After digestion, the tubes were allowed to cool and then NaOH, in concentrations equal to the corresponding acid, was added to neutralize the solutions.

The neutral solutions were then filtered using glass fiber filters. The filtrate was rinsed with deionized (DI) water and then 50% methanol to dissolve out any remaining contaminants. The methanol was then rinsed out with DI. Filtrate was carefully scraped off the filter and dried overnight at 100°C. The dry material was ground and homogenized using an agate mortar and pestle and the samples analyzed by dry combustion for total C and N (Fissions EA 1100).

Laboratory produced charcoal was also partitioned into 4 different size classes: >250 μm ; 106-250 μm ; 50-106 μm ; and <50 μm . Three replicates of one gram of each class were digested in 5 ml 10M HNO_3^- at 95°C for 24 hours. Samples were neutralized with 5 ml of 10M NaOH, filtered with pre-weighed glass fiber filters, dried at 100°C, and weighed.

Laboratory charcoal produced at a range of temperatures in the muffle furnace was also investigated. The temperatures used were: 300°, 400°, 500°, 600°, 700°, and 800°C. The samples were digested, filtered, and weighed as described above.

Hydrogen peroxide digestions were performed on charcoal produced at 400° and 800°C and EKO Kompost. One gram of sample was combined with 20 ml of H_2O_2 for 30 days. Samples were filtered with pre-weighed glass fiber filters, dried, and weighed.

Walkley-Black Organic C

Preliminary Experimentation

Five gram soils samples from each of the sites described above were amended with 10% organic matter (w/w) or 1% charcoal (w/w) or both and then shatterboxed. The Walkley-Black method for estimating organic C was performed on 0.5 g of each sample (n = 3). Samples were digested in 10 ml of potassium dichromate and 20 ml of sulfuric acid for ½ hour. Then 200 ml of DI was used to dilute them and each was centrifuged for 10 minutes to settle the particulates. Samples were read on the spectrophotometer at 590 λ and converted to % organic carbon using a standard curve.

Walkley-Black Assessment

An additional analysis was performed to attempt to assess the accuracy of the new method at a range of different charcoal concentrations. Three soils (Rattlesnake, Deer

Creek, and Pattee Canyon) were combined in factorial with seven rates of C as charcoal. Larger soil samples (20 g) were combined with the appropriate amount of charcoal to result in mixtures of 5, 2, 1, 0.5, 0.1, 0.05, and 0 percent (w/w) C as charcoal. Inorganic C was determined using a coulometer for soils that had visible reactions with 5% HCl. Each mixture was shatterboxed and then analyzed for organic C by Walkley-Black as described above.

The analysis was also performed on soils containing unknown amounts of charcoal that were collected in the summer of 2003 from the Selway-Bitterroot Wilderness Area. These samples were collected from a variety of sites which had undergone various wildfire regimes; from zero to three or more burns over the last 120 years.

All the samples described above were also analyzed by combustion for total C. The amounts of organic and inorganic C were then subtracted from the total C to yield an estimate of charcoal.

Statistical Analysis

Analysis of Variance was conducted on all normal data sets (as determined by Levene's test of homogeneity of variances) and Tukey's HSD was performed where necessary to determine significant differences (SPSS 10.0 for Windows). Nonparametric data was analyzed using Kruskal-Wallis and significant differences were determined by Dunnett's C. Linear regression was used to look for correlations between percent of added C as charcoal and the percent recovered by Walkley-Black. Correlations were also determined for the Selway-Bitterroot soils by correlating the amount of charcoal estimated and the time since the last fire.

RESULTS

Charcoal Recovery from Traditional Chemical Digestions

Nitric acid digestions resulted in a noted loss of C as char. The 10M nitric acid digestions recovered more charcoal than the concentrated 11.1M acid, although the concentrated acid eliminated more of the organic matter than the 10M (Figures 1.1 and 1.2). Neither one adequately digested the organic matter and both resulted in partial digestion of the charcoal. The hydrogen peroxide digestion had the best charcoal recovery at approximately 90%, but it also failed to oxidize nearly 50% of the organic material.

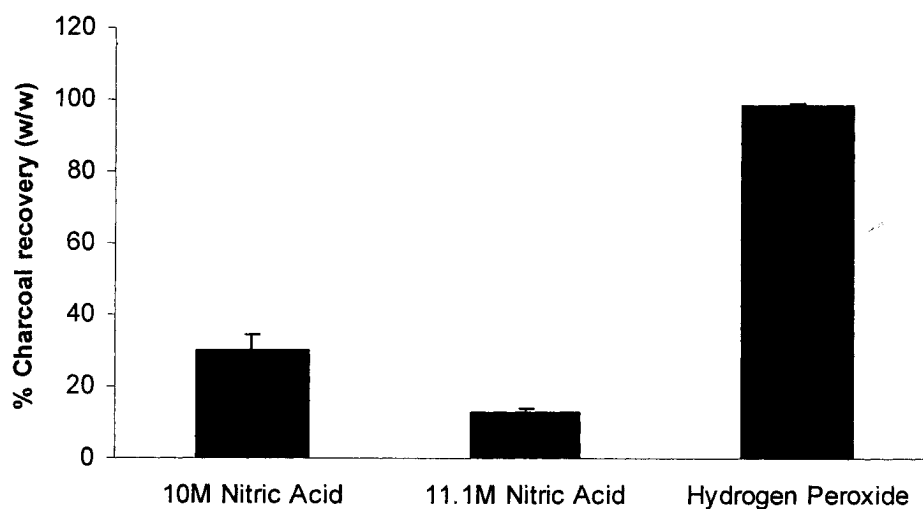


Figure 1.1 Percentage of charcoal recovered from digestions in 10M HNO₃, 11.2 M HNO₃, and H₂O₂. Bars = 1 SE; n = 3.

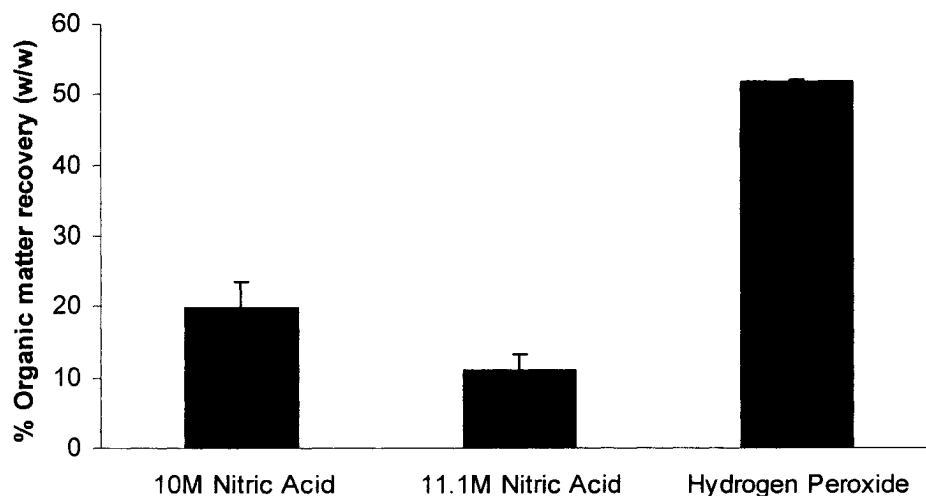


Figure 1.2 Percentage of organic material recovered from digestions in 10M HNO₃, 11.2 M HNO₃, and H₂O₂. Bars = 1 SE; n = 3.

Charcoal particle size also greatly affected recovery in 10M nitric acid digestions (Figure 1.3). The fine fraction showed far greater recoveries than the three coarse fractions, although none of them produced very sizable recoveries.

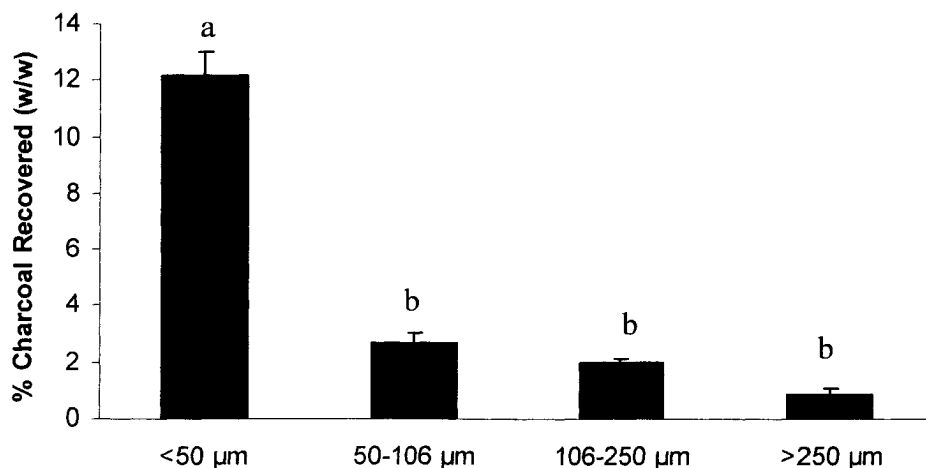


Figure 1.3 Percent charcoal recovered from 10M nitric acid digestions when charcoal was divided into four different size fractions. ANOVA: $F_{\text{size}} = 107.993$; $df = 11$; $p < 0.0001$. Letters indicate significance differences at 0.05 (Tukey's LSD); Bars = 1 SE; n = 3.

The temperature at which the charcoal was produced greatly influenced the recovery rate in 10M nitric acid digestions (Figure 1.4). The acid digestions attacked a greater percentage of the charcoal when produced at 300° and 400°C while 500° charcoal was recovered at more than 50%. Greater than 100% recoveries were recorded for 600°-800° charcoal, suggesting that this particular charcoal may have sorbed impurities from the acid.

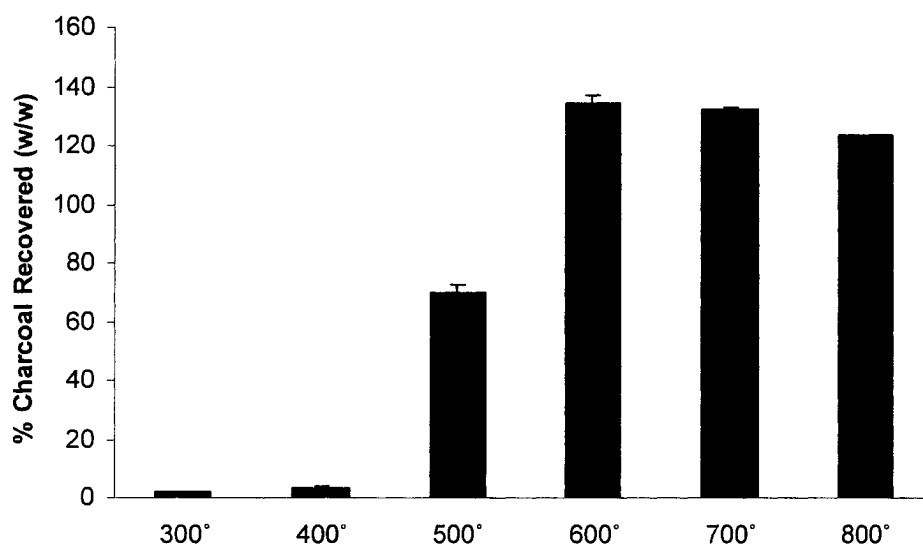


Figure 1.4 Percent of charcoal recovered from 10M nitric acid digestions based on charcoal produced in the muffle furnace at a range of temperatures. Kruskal-Wallis: $df = 17$; $p < 0.01$; bars = 1 SE; $n = 3$.

Walkley-Black Charcoal Estimations

Preliminary Experimentation

The preliminary data collected using the Walkley-Black organic C method resulted in charcoal estimates that were from 30 to 60% accurate (Figure 1.5). These estimates were also accompanied by a high amount of variability. The addition of organic matter in the amended samples did not appear to influence the accuracy of the charcoal estimate (Figure 1.6).

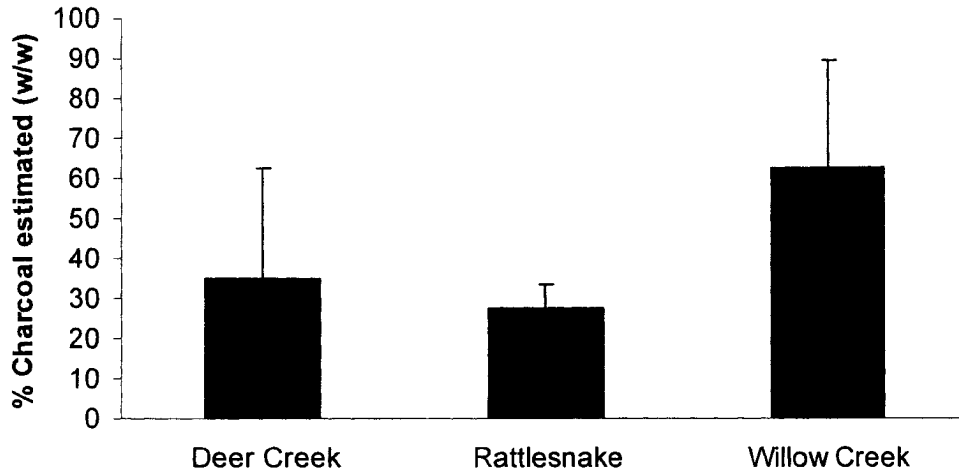


Figure 1.5 Percent charcoal estimated by using the Walkley-Black method for three different soils amended with charcoal alone. ANOVA: $F_{\text{soil}} = 0.902$; $df = 8$; $p < 0.5$. Bars = 1 SE; $n = 3$.

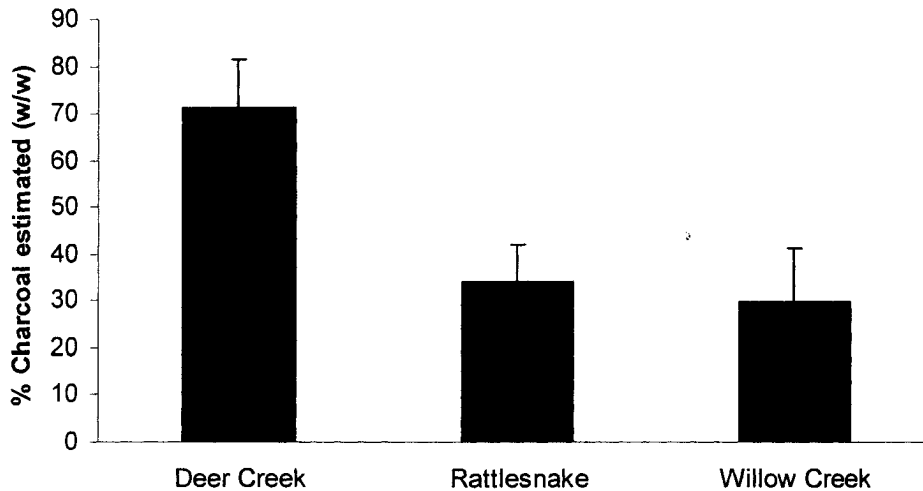


Figure 1.6 Percent charcoal estimated by using the Walkley-Black method for three different soils amended with both organic matter and charcoal. ANOVA: $F_{\text{soil}} = 9.411$; $df = 8$; $p < 0.05$. Bars = 1 SE; $n = 3$.

Walkley-Black Assessment

In general, charcoal estimates were relatively high using the Walkley-Black method for all three of the soils tested (Figure 1.7). For those samples amended with at least 0.5% C as charcoal (w/w), approximately 80% of the charcoal was accounted for by

the method. Percent recoveries were somewhat less reliable for the two smallest amounts of added C as charcoal (0.05 and 0.1% w/w).

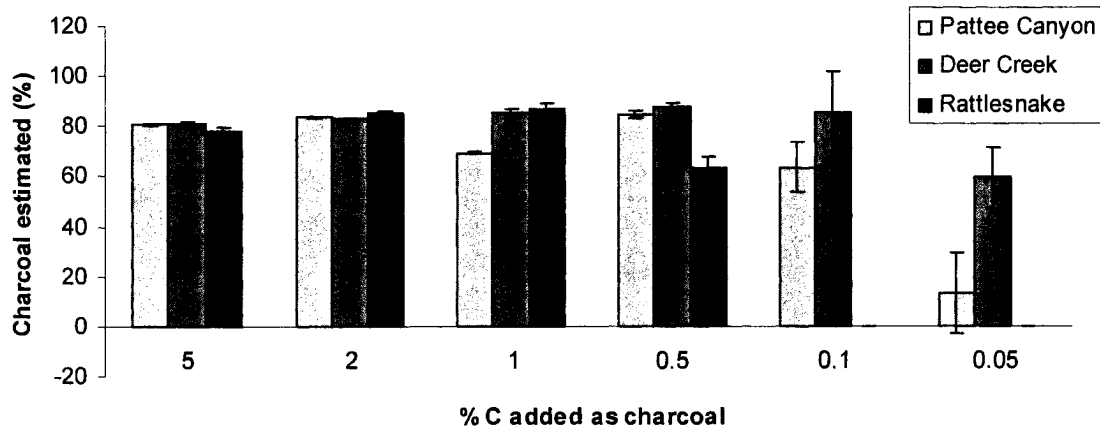


Figure 1.7 Percent charcoal estimated using the Walkley-Black method. The three soils were amended with between 0.05 and 5% C added as charcoal (w/w). Bars = 1 SE; n = 3.

The percent C added as charcoal showed a strong linear correlation with the amount estimated by the Walkley-Black method ($R^2 = 0.9969$; Figure 1.8). This correlation was slightly stronger when only the higher percentages of added C as charcoal (those with less than 0.5% added) were included in the analysis ($R^2 = 0.9975$; Figure 1.9). The linear regression coefficient was the weakest when only the lower percentages of added C were considered ($R^2 = 0.9606$; Figure 1.10).

Using the equation of the line produced by the strongest regression (Figure 1.9), a multiplier of 1.24 can be extracted by solving for x . The final equation that can be applied to soils with unknown charcoal contents is:

$$(\text{Total C} - \text{Walkley Black C}) * 1.24 = \text{Charcoal C}$$

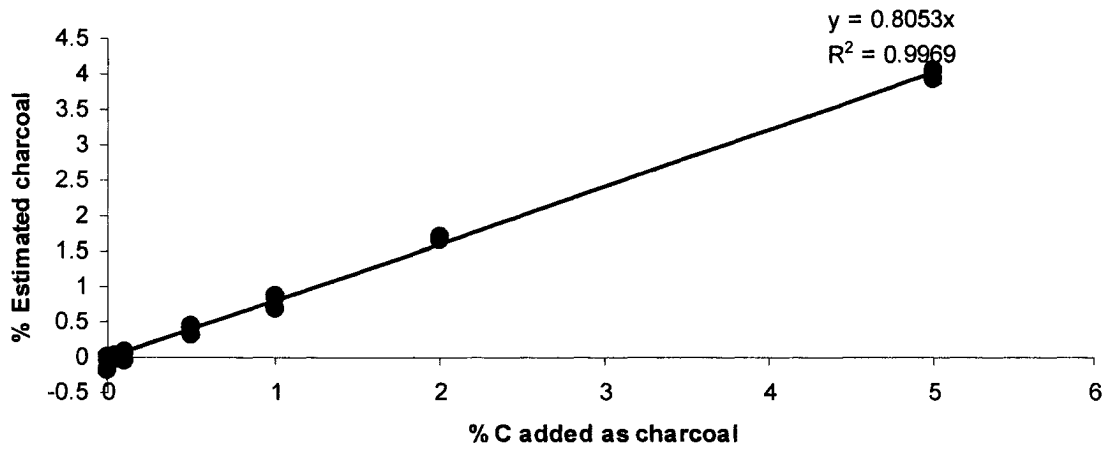


Figure 1.8 Relationship between % C added as charcoal and % estimated charcoal for 0-5% C (added as charcoal w/w). Bars = 1 SE; n = 3.

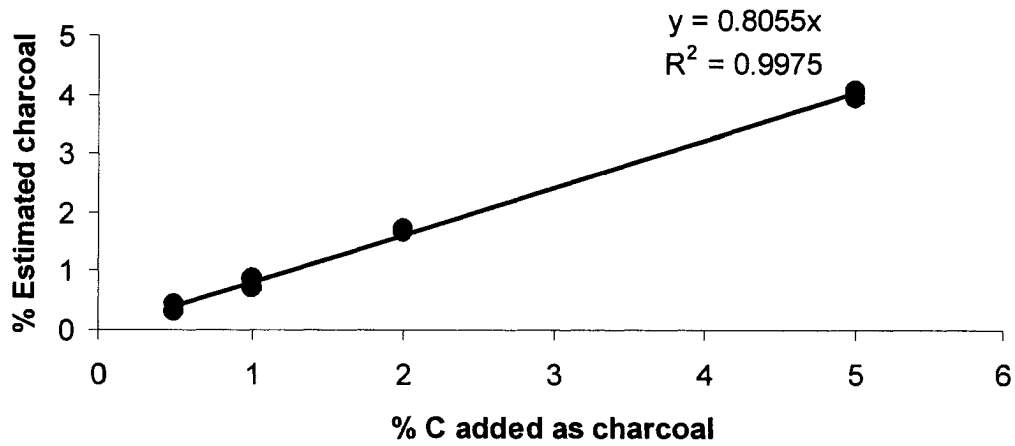


Figure 1.9 Relationship between % C added as charcoal and % estimated charcoal for 0.5-5% C (added as charcoal w/w). Bars = 1 SE; n = 3.

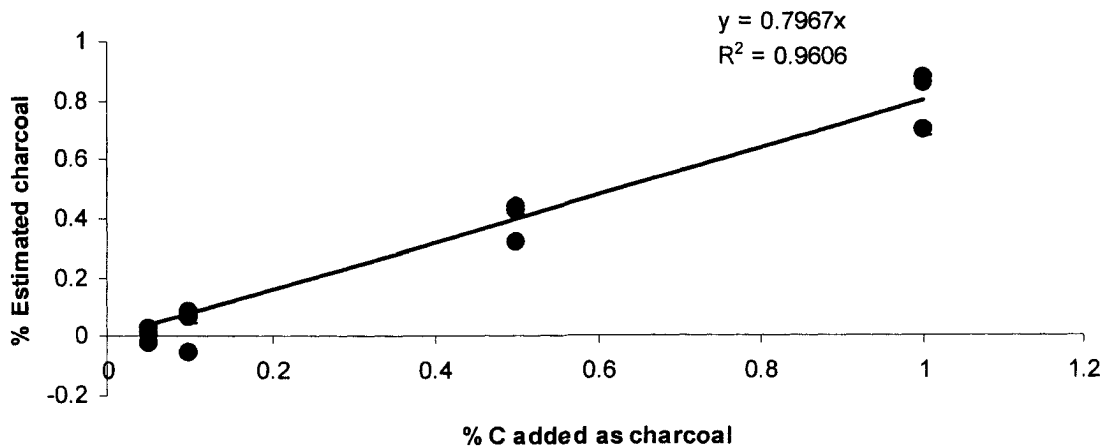


Figure 1.10 Relationship between % C added as charcoal and % estimated charcoal for 0.05-1% C (added as charcoal w/w). Bars = 1 SE; n = 3.

Estimated charcoal contents for the four Selway-Bitterroot sites were computed using the Walkley-Black method and the equation generated above. Charcoal contents were not significantly correlated with time since last fire (Pearson's correlation coefficient = -0.025 ; $p < 0.5$; $df = 119$; $n = 12$) and no particular pattern was observed between charcoal content and time (Table 1.2; Figure 1.11). Charcoal estimates varied widely between sites and fire regimes; for sites that had not burned in more than 120 years (0 burn), charcoal estimates ranged from 0.10-0.40% while those that experienced three or more burns in the last 100 years (3 burn) ranged from 0.14-0.53%.

Table 1.2 Burn ages for Selway-Bitterroot Wilderness Area study sites.

Site	Number of Fires	Years
23 Mile	1	1910
	3	1910, 1934, 1992
Moose Creek	1	1910
	3+	1910, 1934, 1981, 1987
Mackay Bar	3	1919, 1960, 1987
Whitewater Ranch	3	1919, 1933, 1988

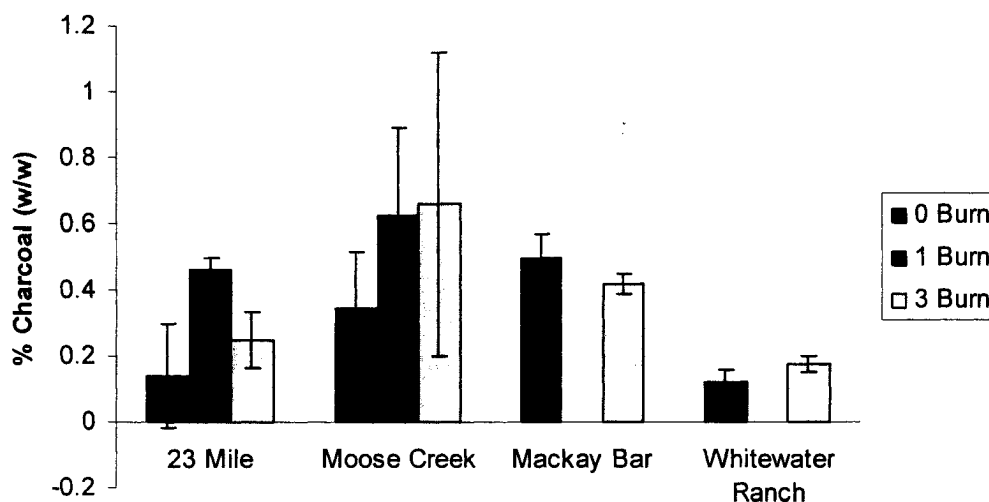


Figure 1.11 Charcoal estimates for sites located in the Selway-Bitterroot Wilderness Area. Pearson's correlation coefficient = -0.025; $p < 0.5$; $df = 119$; bars = 1 SE; $n = 12$. 0 Burn sites had not experienced fire in more than 120 years; 1 Burn sites had experienced one fire in the last 100 years; and 3 Burn sites had experienced three or more fires in the last 100 years.

DISCUSSION

The traditional method for estimating charcoal, nitric acid heated digestion, also oxidizes some C in charcoal, particularly particles of coarse sizes and those produced at relatively low temperatures. Charcoal recovery was somewhat low regardless of the strength of the acid used and both acid concentrations failed to completely oxidize all the organic matter. We speculated that the acid attacks the less pure forms of charcoal which was what led us to experiment with different size and temperature charcoal fractions.

Charcoal produced at a variety of temperatures in the muffle furnace was tested because it has been demonstrated that higher temperature charcoals have higher aromaticity and adsorption potential (Glaser et al., 2002). Charcoal produced at 300° and 400°C was virtually irrecoverable using a 10M nitric digestion while much greater recoveries were observed for 500°C charcoal and higher. In fact, 600°, 700°, and 800°

charcoal all resulted in greater than 100% recoveries, which can be explained by the stronger sorption capacity of these three charcoals. It is likely that the higher temperature produced charcoal was actually able to sorb inorganic impurities from the nitric acid and this would explain the greater than 100% recovery rates. This phenomenon was also observed by Laird and Campbell (2000).

The charcoal used in the previous nitric acid digestions was all produced at 450°, so it is possible, based on the results from the temperature digestions, that higher recoveries would have been maintained if charcoal produced at 600° C or higher had been used. It is also plausible that higher charcoal recoveries would have been observed throughout this research if higher temperature charcoal had been used; however, it was the objective of this research to mimic natural fire conditions as accurately as possible and subsurface temperatures in most forest soils rarely exceed 600°C for any length of time (Hungerford et al., 1991)

The size fraction experiment yielded somewhat unexpected results. We originally speculated that the finer particulates were being digested because their small size would theoretically facilitate their consumption by acid. Although all size fractions had relatively low charcoal recovery rates, the fine particle size fraction had the greatest recovery. These results are consistent with those of Skjemstad and Taylor (1999) in which smaller charcoal particles (i.e., those with larger surface areas) were recovered at higher rates than larger ones, presumably due to larger surface areas. They demonstrated this with a chromic acid digestion; however, the theory behind is applicable to various types of chemical digestions. It is apparent that particle size fractions with high surface

areas are more resistant to attack by dichromate because they contain fewer organic impurities.

Skjemstad and Taylor (1999) also found that the plant origin of the charcoal affects the amount of C in the charcoal and, as a consequence, the rate of recovery for Walkley-Black (chromic acid digestion). When wood charcoal was compared to various charcoals produced from leaves and straw, it was found to contain the lowest amount of C and had the lowest rate of recovery. The C content of charcoal appears to have a strong influence on the percent of charcoal recovered in various digestion techniques.

Charcoal C content was also a proposed explanation offered by MacDonald et al. (1991) when no significant correlation was observed among analyses for microscopic charcoal, digestion-combustion charcoal, and macroscopic charcoal. They concluded that, because naturally produced charcoal varies considerably in C content, the charcoal identified optically may have actually been removed by the acid digestion because of relatively low C contents, resulting in little correlation among the estimates.

Charcoal was greatly resistant to digestion by hydrogen peroxide with nearly 100% of the charcoal being recovered. However, the peroxide left nearly half of the organic matter intact, thus, it appears that this method did not aggressively attack the organic C and would not serve to eliminate organic matter. Furthermore, I allowed 30 days for oxidation of one gram of either charcoal or organic matter with periodic additions of peroxide, and this amount of time is simply unfeasible under most circumstances.

Kerven et al. (2000) suggested that the dichromate oxidation used in the Walkley-Black method for organic C analysis failed to attack charcoal. Based on this information,

we speculated that charcoal C could be estimated by subtracting organic C by Walkley-Black from total C by combustion. This technique was tested preliminarily on three different soils amended with charcoal and organic material. Inconsistent results were observed which suggested that the small soil sample of 5 g was insufficient in size to eliminate variability.

Following the preliminary investigations using Walkley-Black, larger soil samples (20 g) were amended with a range of charcoal contents in an effort to define the reliability of this method. The amount of charcoal estimated for % C amendments of 0.5 to 5% (w/w) was approximately 80% (Table 1.3). However, the % C amendments below 0.5% (w/w) had relatively poor and variable charcoal estimation capacity.

Table 1.3 Mean charcoal content estimated by the Walkley-Black method and percent recovery rates for each charcoal amendment.

Percent C Added as Charcoal	Walkley-Black Estimation (%)	Charcoal Estimated (%) of % C Added
5	4.00	80.0
2	1.68	84.0
1	0.81	80.6
0.5	0.39	78.6
0.1	0.03	49.7
0.05	0.004	24.1

The amount of charcoal estimated by the method was well correlated with the amount of C added as charcoal as determined by the regression analysis. It was expected that at some point along the regression, the method would inevitably lose some of its power. Further analysis showed a weakening of the line when only the smaller charcoal estimates were included (those < 0.5% C w/w). Based on this information, a regression using only charcoal estimates $\geq 0.5\%$ C was performed (Figure 1.8). Using the equation of the line produced, a multiplier of 1.24 was calculated by solving for x . The final equation that can be applied to soils with unknown charcoal contents is:

$$(\text{Total C} - \text{Walkley Black C}) * 1.24 = \text{Charcoal C}$$

This equation was applied to soils of unknown charcoal contents collected from four sites in the Selway-Bitterroot Wilderness Area. Charcoal contents were not correlated with time since last fire. These results suggest that charcoal is highly persistent in ponderosa pine/Douglas fir forest soils and is consistent with findings reported by Zackrisson et al. (1996) for boreal forest soils.

It is evident from the analyses that a small portion of charcoal is probably being oxidized by the dichromate in the Walkley-Black procedure. This is equable with Skjemstad and Taylor (1999), who estimated that approximately 10% of the soil charcoal was oxidized during the dichromate reaction. Regardless, consistent estimates of around 80% of the soil charcoal can still be acquired using Walkley-Black for soils that contain at least 0.5% C as charcoal. For soils that contain less than 0.5% C as charcoal, it is likely that the method may be refined by increasing the soil sample size from 20g to 50g. In this way, variability will be reduced and the method's accuracy at these lower percentages will be increased.

The source of variability in this method stems from it being spectrophotometric. This provides for a relatively easy and rapid procedure; however, as a result, precision is sacrificed to some extent. For this reason, it is imperative that all other sources of variation be held as tightly as possible. In our lab, the Walkley-Black was standardized using standard soils to ensure the closest possible estimate of organic C. In soils that had visible reactions with HCl (>1% carbonates), inorganic C analysis was performed with a high precision coulometer. Finally, in the analysis for total C, a combustion analyzer was used to maintain the highest possible precision.

CONCLUSIONS

The work reported here clearly demonstrates the lack of accuracy and consistency in current methods used to evaluate charcoal contents of forest soils. Chemical digestions, using such acids as HNO₃ or H₂O₂, invariably fail to completely digest organic material and commonly eliminate charcoal particles in the process of removing organic matter. Furthermore, once removal of adhering organics takes place, we still lack an efficient method for enumerating the remaining charcoal. Research that relies on HNO₃ digestions will continue to underestimate charcoal contents of soils because of charcoal oxidation. The implications of this oversight could be widespread in light of heightened interest in forest fires ecology, C sequestration and global warming.

The majority of the difficulty in identifying a widely acceptable method lies in the fact that naturally-produced charcoal is chemically quite variable and depends largely on the temperature at which it is produced. Impurities, as demonstrated in this research, largely are present in coarse charcoal particles with lower surface areas and those produced at temperatures less than 600° C. The presence of impurities in naturally produced charcoal causes it to be oxidized in the process of eliminating organic matter and eventually leads to underestimation of charcoal. However, methods that do not use a chemical digestion, i.e. those that rely primarily on optics, invariably fail to identify the fine particle size fraction of charcoal. Those methods that do appear to be highly quantitative for char estimation, such as photooxidation and NMR spectroscopy (Skjemstad et al., 1999), are generally not feasible due to their excessive financial expense. In the interest of time and financial efficiency, we have chosen a simple

chemical technique that will not neglect the fine charcoal particulates common to forest soils.

The Walkley-Black method uses a “heat of reaction” dichromate oxidation which appears to not attack a significant amount of the soil charcoal. It is a measurement of soil organic C that can be subtracted from total C to get a relatively accurate estimation of soil charcoal content. The research presented here demonstrates its ability to yield strong estimates of approximately 80% for soils that contain at least 0.5% C as charcoal. Furthermore, it is apparent that using Walkley-Black for estimating soil charcoal content fits our original methodological criteria extremely well: it is rapid, inexpensive and accurate for the soils in which it was tested.

It is apparent from this research that traditional methods for quantifying charcoal in soils are either prohibitively expensive or lead to significant underestimations. Furthermore, many are exceedingly time consuming and tedious to perform. The chemical method presented in this paper offers an alternative that estimates charcoal contents consistently while maintaining ease and simplicity of performance.

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CHAPTER 2. THE INFLUENCE OF CHARCOAL IN LITTER DECOMPOSITION IN PONDEROSA PINE ECOSYSTEMS OF WESTERN MONTANA

ABSTRACT

Fire-deposited charcoal has the potential to be influential in many ecosystem processes; however our current understanding of its precise role is limited, especially in western ponderosa pine forests. Parallel laboratory and field incubations were undertaken to clarify charcoal's effect on litter decomposition in two common plant species of western Montana: *Arctostaphylos uva-ursi* (kinnikinnick) and *Pinus ponderosa* (ponderosa pine). Response variables measured included mass lost, net N mineralization, soil respiration, extractable phenolic content, and total N, C, and lignin content. Charcoal significantly increased rate of decomposition for *P. ponderosa* and affected extractable free phenols and the lignin and N contents of *A. uva-ursi*. Based on these results, we conclude that charcoal can have important effects on organic matter decomposition.

INTRODUCTION

Currently, little is known regarding the direct effects of fire-deposited charcoal on soil processes. However, given its noted chemical and biological properties, it has the potential to play a major role in ecosystem processes, including nutrient cycling.

Charcoal's ability to sorb organic compounds may alter soil chemistry by shifting C:N ratios, or potentially alleviating the inhibitory effects of allelochemicals. Furthermore, its highly porous nature may provide microsites for soil organisms while the sorbed carbon can serve as a food reserve. Because the presence of charcoal could facilitate soil decomposer communities, it is plausible that decomposition rates in ecosystems that regularly undergo fire events will be enhanced by routine charcoal deposition.

To date, little evidence exists that specifically connects charcoal to litter decomposition rates. Existing research suggests that charcoal serves as foci for microbial activity due to its high porosity and ability to sorb carbon compounds (Ehrhardt and Rehm, 1985; Zackrisson et al., 1996; Pietikäinen et al., 2000). However, microbial activity is not a reliable indicator of decomposition since correlations between microbial

biomass and decomposition rates are typically inconsistent (Zackrisson et al., 1996). Regular fire return intervals have been linked to faster decomposition in boreal forests (Wardle et al., 1997), but charcoal's effect on decomposition rates is unpredictable (Zackrisson et al., 1996; Wardle et al., 1998).

With the purpose of clarifying charcoal's role in litter decomposition, the research reported here specifically examined decomposition in the ponderosa pine/Douglas-fir forests of western Montana. Parallel laboratory and field investigations were designed to complement each other and to shed light on charcoal's influence on decomposition and nutrient mineralization. Laboratory-produced charcoal was added to two common litter types of these ecosystems. It was hypothesized that the added charcoal would facilitate more rapid decomposition, and as a result, affect nutrient release and mineralization.

Wildfires are the primary disturbance mechanism for initiating the recycling of nutrients in many ecosystems. Historically, the ponderosa pine/Douglas-fir forests of western Montana experienced regular fire return intervals of between 13 and 50 years (Arno et al., 1995). Fires clear the forest floor of accumulated organic matter and promote a large pulse of inorganic N, both of which contribute to the establishment of pioneer plant species. Although the importance of nutrient cycling with respect to fire is widely recognized (see review by Neary et al., 1999), current research has yet to establish a clear relationship between fire and decomposition rates. Furthermore, the role of fire-deposited charcoal has long been overlooked in studies in the western United States. Given charcoal's porous nature and capacity to sorb inhibitory organic compounds, it has the potential to function significantly in such processes as litter decomposition and nutrient cycling.

Many soil and climatic factors are known to influence decomposition rates. A review by Berg (2000) emphasizes the extreme temporal and spatial variability in natural systems that make it difficult to formulate general tendencies. Generally speaking, climate (primarily temperature and precipitation) has the strongest influence on decomposition rates; whereas litter quality is more important on localized levels. In arid and semiarid environments, moisture is usually the main limiting factor for decomposition (Fioretto et al., 1998). However, where moisture levels are sufficient, temperature takes precedent, but this pattern is more pronounced in cooler climates (Kirschbaum, 1995). In general, two stage decomposition patterns are often observed: the early stage eliminates soluble and easily broken-down material; the late stage is when the more recalcitrant material, such as lignin, accumulates in greater amounts (Berg and Staaf, 1980). The amount of time taken for each stage is dependent on biotic and abiotic factors.

There is evidence in some forest ecosystems that the absence of fire, either through inadvertent exclusion or active fire suppression, causes decomposition rates to be extended. Wardle et al. (1997; 2003) conducted a unique series of experiments on an island archipelago in northern Sweden. First, they demonstrated through charcoal C dating and tree fire scar analysis that the larger islands experienced fire far more frequently than the smaller ones, presumably due to their larger area increasing their likelihood of being struck by lightning. Then they measured a variety of ecosystem processes including decomposition rates. Their results show that not only was decomposition on the small islands suppressed by poor litter quality, but that litter from single source island degraded much more slowly on the small islands than the large ones.

This suggests that in the absence of fire, decomposition is inhibited by apparent shifts in soil microbial communities in boreal forests. Although this research demonstrates a clear connection between decomposition and fire return intervals, the potential involvement of charcoal in such studies has yet to be determined.

Other work conducted in the area of fire effects on decomposition rates is contrasting or unclear. In a field study of ponderosa pine decomposition, Monleon and Cromack (1996) found that decay rates were slightly faster on unburned control plots than plots that had experienced prescribed fire. This trend was evident up to twelve years post-fire. Similar results were observed in Australia, where various eucalyptus species decomposed slower in burned than unburned areas (Raison et al., 1986). The fire reduced understory vegetation and caused a reduction in shade and moisture content which was suggested to explain the observed results.

Although unpredictable trends are often found between microbial biomass and decomposition rates (Zackrisson et al., 1996), it is evident that charcoal has the ability to enhance microbial activity in soil and humus (Pietikäinen et al., 2000). The porous structure of charcoal facilitates organismal predator-prey interactions by providing additional microhabitats. Furthermore, because charcoal sorbs organic compounds, it provides a C source for microbes. The results of Zackrisson et al. (1996) suggest that microbial activity is enhanced by the presence of charcoal due to its micropores and sorbed C. Additionally, particles that have become saturated with C compounds over time have the ability to become “reactivated” by microbes, who can also serve to delay saturation. Activated C produced similar results by sorbing phenolic compounds, which then served as a carbon source for soil microbes (Ehrhardt and Rehm, 1985). Pietikäinen

et al. (2000) further supported this evidence with findings that demonstrated charcoal's ability to serve as a microhabitat for microbes.

Litter quality, including lignin and N content, are often strong indicators of decomposition rates; however, these indices have not been examined in relation to charcoal. Many studies have demonstrated the reliability of lignin concentration as a predictor of decomposition rates (Taylor et al., 1991; Rutigliano et al., 1996; McTiernan et al., 2003; Sariyildiz and Anderson, 2003). High initial litter N contents have been associated with retarded decomposition rates (Berg, 2000) and have been negatively correlated with lignin loss rates (Rutigliano et al., 1996). A further complication is that litter quality, including lignin content, from site to site often varies even among the same plant species due to soil chemistry differences (Sanger et al., 1996). Therefore, it is difficult to predict decomposition losses because of complex site and species variability.

The phenolic content of litter also has a significant influence on decomposition rates in many ecosystems and some research suggests that phenolics exert a stronger influence than N or lignin content (Palm and Sanchez, 1990). A review by Hättenschwiler and Vitousek (2000) details several reasons for their profound influence, including litter quality effects, microbial community changes, and inhibition of nitrifiers and/or mycorrhizal infection. Soil microbial degradation of these compounds can effectively reduce the concentration of soil phenols and even eliminate the inhibitory effects (Domínguez, 1994). Kuiters (1990) also reviewed evidence of phenolic compounds exerting negative influences on soil productivity and nutrient mineralization. A higher amount of phenols released by decomposing litter leads to lower organic matter turnover, and eventually causes a reduction in overall nutrient mineralization rates.

Litter phenolic content has also been implicated in regulation of litter N release, both in amount and type of N. Northup et al. (1995) observed N release of highly decomposed pine needles in California pygmy forest. As litter phenolic content increased, the litter release of dissolved organic N also increased while the release of mineral N (as NH_4^+) decreased. It is apparent that the phenolic compounds in needle litter exert some control over amount and type of N released and thus, they can suppress the amount of substrate available for nitrification. Phenolics also promote and regulate humus development, and in this way, they are able to conserve nutrients within the organic layer. Therefore, phenolics have the capacity to play a large part in nutrient regulation in many forested ecosystems.

The importance of fire disturbance ecological processes in forested ecosystems is beginning to be recognized. However, the function of fire-deposited charcoal has yet to be clearly established. Charcoal's potential to affect ecosystem processes such as litter decomposition is undeniable; therefore, current research on fire effects is not complete unless the role of charcoal is taken into consideration.

PURPOSE AND OBJECTIVES

The purpose of this series of experiments was determine if charcoal deposited by fire influences decomposition rates and nutrient dynamics in the forest floor of ponderosa pine ecosystems. Compatible field and laboratory trials were undertaken to determine the influence of charcoal on:

- 1) Litter decomposition as measured by mass lost.

- 2) Nitrogen release, loss of lignin, and presence of extractable free phenolics.
- 3) Net nitrification, ammonification and CO₂ evolution.

MATERIALS AND METHODS

Field Incubation

Study Site Description: This study took place at Lubrecht Experimental Forest, 45 miles west of Missoula, Montana. The climate in this part of western Montana is generally warm and dry. Mean annual precipitation is approximately 45 cm with roughly half of that falling in the winter as snow (Nimlos, 1986). The forest is characterized by *P. ponderosa* and *Pseudotsuga menziesii* with an understory dominated by *Calamagrostis rubescens* and *Arctostaphylos uva-ursi*. The soils are shallow poorly developed Udic Dystrustepts with a 4 cm organic horizon. For this study, a mature ponderosa pine stand was selected such that most of the trees were medium to large in size and 80 to 125 years in age.

Methodology

A buried bag technique was employed to assess field decomposition rates wherein litter is placed in a nylon mesh bag and decomposition estimated by the rate of mass loss from the bag over time.

Fresh litter was collected from ponderosa pine (*Pinus ponderosa*) and kinnickinick (*Arctostaphylos uva-ursi*) in Pattee Canyon recreation area in April of 2003. Ponderosa pine is a dominant tree in these pine ecosystems and its needles comprise the majority of the forest floor material. Kinnickinick is a common ericaceous understory

shrub. Litter was dried a minimum of 24 hours at 40° C and both needles and leaves were removed from their stems. A small subset of each litter type was shatterboxed and analyzed for total C and N using a dry combustion analyzer (Fissions EA 1100). Another subset was stored for extraction and analysis of free phenols.

Charcoal was produced in a muffle furnace at 450° C from Douglas-fir wood. A small subset of charcoal was also shatterboxed and analyzed for total C and N. Pieces of charcoal were coarse ground using an apple grinder and then sieved to 1-mm to generate a size range that would remain in the mesh bags. Charcoal was added to the two different litter types in three rates: high (3 g; 156 g M⁻²), low (1 g; 52 g M⁻²), and a no charcoal control. Additional charcoal-only control bags were used to assess the decomposition rate of the charcoal.

Ten grams of litter was combined with the appropriate rate of charcoal in 1-mm mesh nylon bags (12 x 16 cm). Bags were placed in the field in late April 2003. Bags were arranged in a completely randomized 30 x 30 m block and buried under the organic horizon. Because of the exceptionally dry nature of the summer of 2003, approximately 1 cm³ of distilled water was sprinkled on the forest floor above each bag in early September. Half of the litterbags from each charcoal and litter treatment were collected and placed in individual envelopes in October of 2003, and the remaining bags were collected in May of 2004. Bags were dried at 60° C for 4 days. Dry weights were recorded for mass loss calculations at both collections times.

The litter collected in May was stored for further analysis. A subset of each charcoal and litter treatment was bulked and homogenized in the shatterbox and subsequently analyzed for total C and N, and lignin carbon. Carbon and N were analyzed

as described previously. Lignin was analyzed following the acid-detergent fiber (ADF) – sulphuric lignin technique described by Rowland and Roberts (1994).

An additional subset was extracted for total phenol analysis as described by Northup et al. (1998). A bulked sample of each treatment was ground to 0.2 cm in a Wiley mill and 1 g was extracted with 30 ml of 50% aqueous methanol over a 24 hour period. Extracts were immediately filtered using Whatman #42 filters and were subsequently analyzed for total phenols using the Prussian Blue technique (Stern et al., 1996).

Laboratory Incubation

A parallel laboratory incubation was conducted to evaluate the effects of charcoal on short-term decomposition and nutrient dynamics using the same litter types and mineral soil.

Fresh litter was collected as described previously for *Arctostaphylos uva-ursi* and *Pinus ponderosa*. Litter was dried at 40°C and ground using a Wiley mill to 0.1 cm. Soil was collected from the Pattee Canyon recreation area to a depth of 15 cm and sieved to 2 mm. The texture was sandy clay loam. The water holding capacity of the soil was determined using a pressure plate at 1/3 bar. Charcoal was produced using the same procedure described above.

Soil samples of 100 g (dry weight) were placed in Mason jars and amended with either 1 g of litter (10 g kg⁻¹ soil), 0.3 g of charcoal (3 g kg⁻¹ soil), or both and the materials were mixed thoroughly. Initial NO₃⁻-N and NH₄⁺-N concentrations were determined at time 0 by extracting 30 g of soil with 50 ml of 2M KCl. The extracts were analyzed using a segmented flow analyzer (Mulvaney, 1996)

Jars were placed in an incubator at 30°C for the duration of the experiment. Soil was kept at approximately 60% of its water holding capacity throughout. Evolution of CO₂ was measured four times during the course of the incubation using alkali traps and the titration method (Zibilske, 1994). After 29 days, soils were again extracted for NO₃-N and NH₄⁺-N analysis as discussed above.

Statistical Analysis

Response variables were analyzed by using Analysis of Variance following determination of suitability using Levene's test of homogeneity of variance. Significant differences between treatments were determined using Tukey's LSD. Nonparametric data was analyzed using Kruskal-Wallis and significant differences were determined using Dunnett's C.

RESULTS

Field Incubation

Pinus ponderosa needle litter decomposed significantly faster than *A. uva-ursi* leaf litter for both time periods. When all variables (time, litter, and charcoal) were analyzed together, only litter type was found to have a significant impact on % mass lost. The data was subsequently divided into the two time periods, and it was obvious that the most notable differences were in the data from the year-long incubation period; therefore, all ensuing analyses used only this data set.

When two litter types and the three charcoal treatments were compared together, the effect of litter was type was highly significant ($p < 0.0001$) while the charcoal was less so ($p < 0.1$). Litter type significantly influenced decomposition for both the high and

the low levels of charcoal amendment ($p < 0.0001$ for the high and $p < 0.05$ for the low). Additional patterns were apparent when the litter types were analyzed separately. The charcoal significantly increased the decomposition of *P. ponderosa* needles; however, rates for the *A. uva-ursi* leaf litter were not affected. (Figure 2.1)

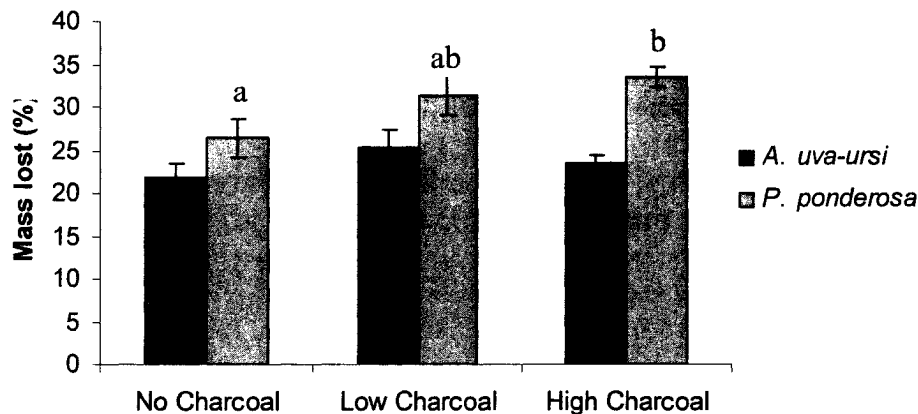


Figure 2.1 Percent mass lost from bags containing *A. uva-ursi* and *P. ponderosa* litter after one year field incubation combined with different rates of charcoal. ANOVA, $F_{\text{charcoal}} = 2.942$; $df = 52$; $p < 0.01$. Letters indicate significance at 0.05 for *P. ponderosa* needle litter (Tukey's LSD); bars = 1 SE; $n = 10$.

The charcoal amendments influenced *A. uva-ursi* litter decomposition with respect to lignin, N and extractable free phenolics. Lignin content decreased with increasing charcoal amendment and the greatest difference was observed between the low charcoal treatment and the no charcoal control (Figure 2.2). Charcoal also affected the N concentration of the *A. uva-ursi* litter. Significantly less N was found remaining in the charcoal treatments (Fig. 2.3). The high charcoal treatment resulted in higher C:N ratios ($p < 0.01$) and greater extractable free phenolics ($p < 0.05$) in the *A. uva-ursi* litter ($p < 0.01$). The lignin:N ratio declined with increasing rates of charcoal ($p < 0.1$; Table 2.1)

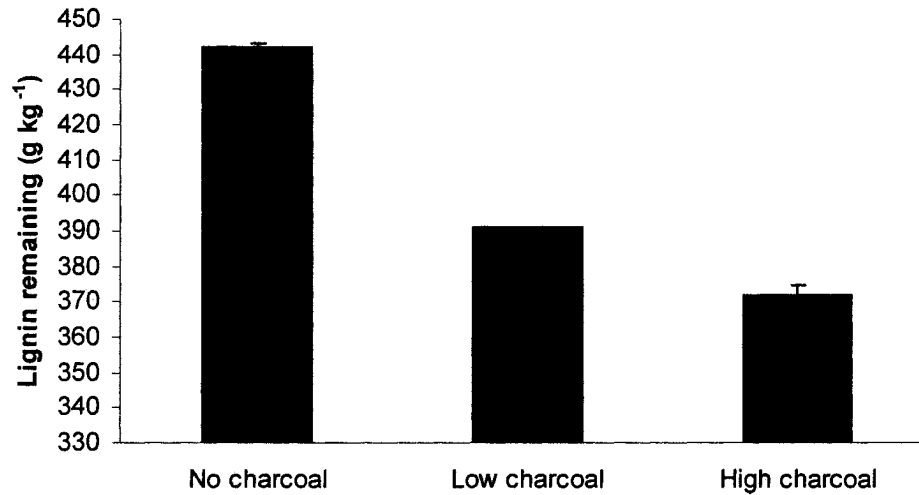


Figure 2.2 Lignin remaining in the *A. uva-ursi* leaf litter after 1-year field incubation. Kruskal Wallis: $df = 8$; $p < 0.05$; bars = 1 SE; $n = 3$.

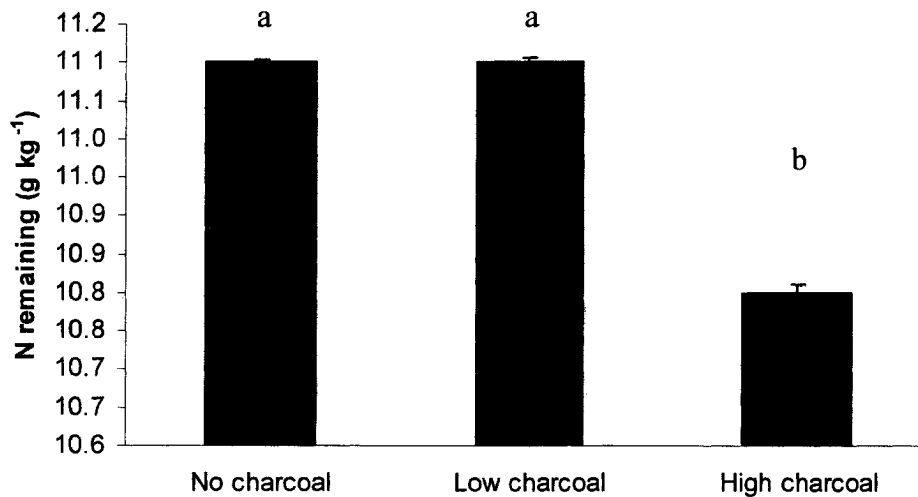


Figure 2.3 Nitrogen remaining in *A. uva-ursi* leaf litter after 1-year field incubation. The initial N content of the *A. uva-ursi* litter was 11.5 g kg^{-1} . ANOVA: $F_{\text{charcoal}} = 4.981$; $df = 8$; $p < 0.05$; $n = 3$. Letters indicate significance at 0.05 (Tukey's LSD); bars = 1 SE.

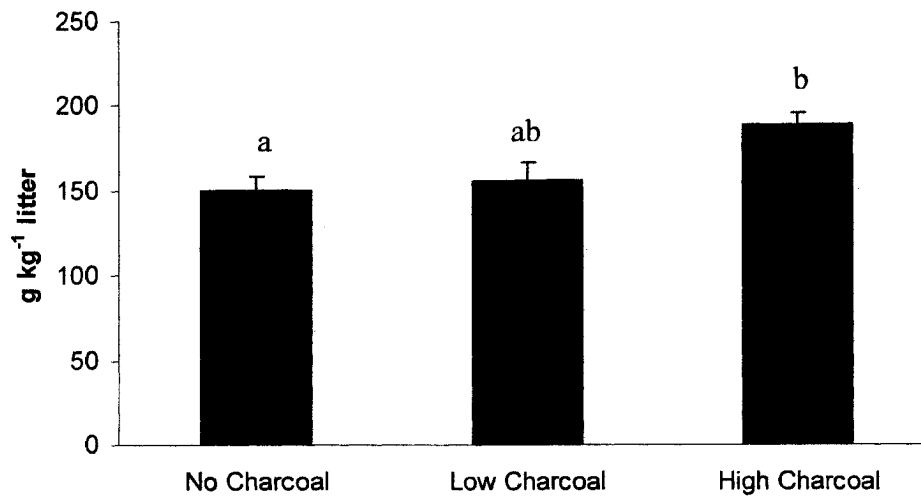


Figure 2.4 Extractable free phenolics remaining in *A. uva-ursi* litter (g kg⁻¹) after 1-year incubation. The extractable free phenolic content of the untreated *A. uva-ursi* litter was 1880 g kg⁻¹ litter. ANOVA, $F_{\text{charcoal}} = 5.703$; $df = 8$; $p < 0.041$. Letters indicate significance at 0.05 (Tukey's LSD); bars = 1 SE; $n = 3$.

The litter chemistry analysis for *P. ponderosa* needle litter was more enigmatic than that for *A. uva-ursi* leaves in that the low charcoal treatment had the strongest effect on all three chemical variables examined. For C, N, and lignin contents, the litter treated with high charcoal rates was slightly higher than the no charcoal control litter. However, for all three of these variables, the low charcoal treatment contained substantially higher amounts than the high charcoal and no charcoal control treatments. All three levels of charcoal amendment significantly influenced N accumulation ($p < 0.0001$) and similar patterns emerged when the C:N and lignin:N ratios were analyzed. The C:N ratio was significant at $p < 0.001$ with the low charcoal treatment differing significantly from the control and the high charcoal. The lignin:N ratios also differed significantly ($p < 0.1$) and the low charcoal treatment showed the greatest difference from the control. No observable patterns were found for the extractable free phenolics for the *P. ponderosa* needle litter.

Table 2.1 Litter chemistry values after 1-year field incubation under different charcoal levels. “*” Indicates values different from the others in the group at 0.05 significance; “**” indicates values different from the no charcoal control at 0.05 significance; n = 3 for all values.

Species	Charcoal Treatment	N g kg ⁻¹	C	Lignin	C:N	Lignin:N	Extractable Free Phenolics
<i>P. ponderosa</i>	No Charcoal	14.8	475.2	355	32.18	24.0	49
	Low Charcoal	**15.6	**486.1	*480	*31.17	**30.7	44
	High Charcoal	**15.2	**485.2	369	31.96	24.3	52
<i>A. uva-ursi</i>	No Charcoal	11.1	497.2	442	44.80	39.8	150
	Low Charcoal	11.1	**493.6	**391	44.49	35.3	155
	High Charcoal	10.8	**500.8	372	*46.24	34.4	**188

Laboratory Incubation

Litter type had a significant influence on net ammonification, nitrification and soil respiration; whereas, the charcoal amendment did not significantly alter any of the experimental variables. Both litter amendments increased soil respiration rates and the *P. ponderosa* needle litter did so to a greater extent than did the *A. uva-ursi* leaf litter (Figure 2.5). Net immobilization of ammonium was observed for both litter types while nitrate immobilization was only observed for *A. uva-ursi* litter treated with charcoal.

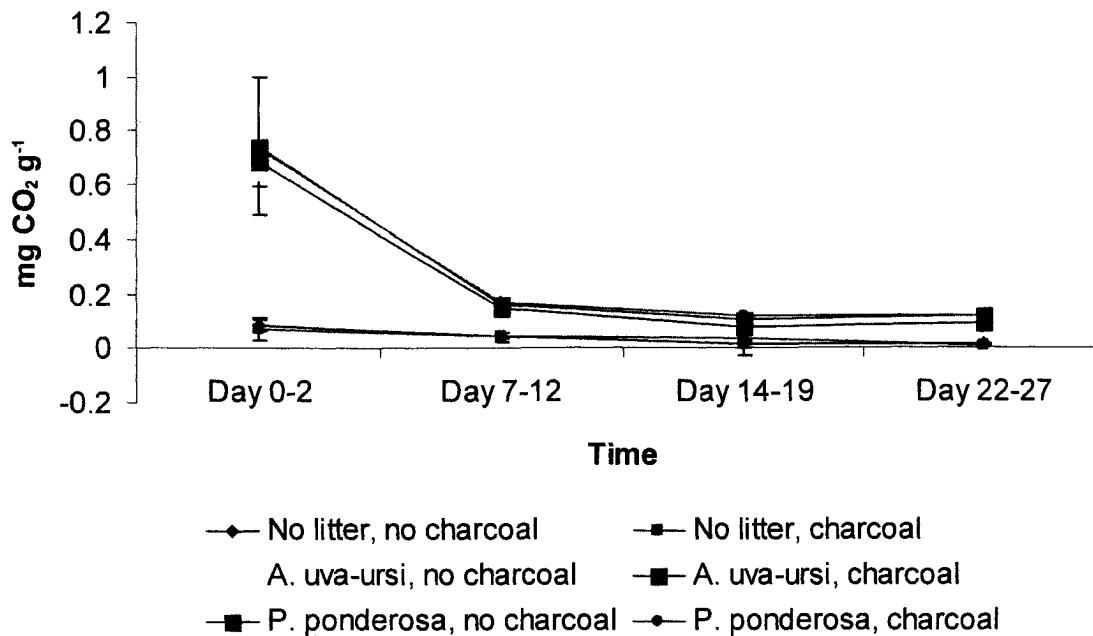


Figure 2.5 Evolution of CO₂ over time for litter and charcoal treatments in laboratory incubation. Bars = 1 SE; n = 5.

DISCUSSION

Decomposition rates are generally controlled by litter lignin and N content. Depending on specific site characteristics and litter type, strong correlations may be found between decay rate and initial lignin content (Fogel and Cromack, 1977; Sariyildiz and Anderson, 2003), initial N content (Berg, 2000), or initial lignin:N ratio (Melillo et al., 1982). Although N is a necessary nutrient for microbial activity, high initial N contents can actually retard decomposition rates because ammonia or amino acids react with lignin to form even more recalcitrant organic N complexes (Nommik and Vahtras, 1982; see review by Berg, 2000). This explains why initial N content is often negatively correlated with lignin decay rates (e.g. Rutigliano et al., 1996).

When comparing the results of this study to other decomposition studies, it is apparent that these commonly used indices are not applicable. One would expect faster

rates of decay for the *A. uva-ursi* leaves than *P. ponderosa* needles based on initial N, lignin, and lignin:N ratio (Table 2.2); however, after one year of field incubation the opposite was observed. A likely explanation for this occurrence is that the field incubation was not long enough and linear correlations for decay rates could not be calculated for one incubation period. It is also possible that the amount of charcoal added altered the microbial community so much that normal prediction indices were not relevant in this study.

Table 2.2 Initial litter quality indices.

	N g kg ⁻¹	C	Lignin	C:N	Lignin:N	Extractable Free Phenolics
<i>A. uva-ursi</i>	11.5	529.4	111.0	45.9	9.6	1880
<i>P. ponderosa</i>	12.0	513.5	138.6	42.9	11.5	516

Another explanation for the litter decomposition trends observed is the relative phenolic contents of the two litter types. In a study of decomposition trends in composted forest plants, Almendros et al. (2000) used ¹³C NMR to examine C patterns in twelve species, including various trees, shrubs, and *A. uva-ursi*. *A. uva-ursi* had the highest level of phenolics for all the species studied. Evidence from Gallet and Lebreton (1995) also points to evergreen shrubs having higher phenolic contents than spruce needles. Coupling the previous findings with the current work, it is logical to contend that the higher phenolic content of the *A. uva-ursi* leaves led to its slower decomposition rate.

Although identification of proximate decomposition controls on the two litter types was not an objective of this study, the evidence presented here demonstrates that litter phenolic content exerted a stronger influence on the first-year decomposition rates

than initial lignin or N contents. This concept of lignin as a key variable in decomposition may be overstated; lignin may simply be “an artifact of its covariance with polyphenol concentration or the inability to segregate polyphenols from lignin in lignin assays” (Northup et al., 1998). Furthermore, lignin assays commonly include extra, non-aromatic materials in the acid-insoluble portion such as lipid biomacromolecules and tannins (Almendros et al., 2000). It is likely that estimates of lignin content contain accessory materials, including phenols, making it nearly impossible to completely attribute retarded decomposition rates to litter lignin content.

The charcoal amendment resulted in higher rates of decomposition for the *P. ponderosa* needle litter but not for the *A. uva-ursi* leaf litter and there are several probable explanations for this observation. Given the predominance of needle litter on the forest floor of these ecosystems, the microbial community is likely to be better adapted to breaking down needle litter. The microbial decomposition rates could also have been retarded by the high levels of phenolic compounds in the *A. uva-ursi* leaf litter. Furthermore, the waxy cuticle of *A. uva-ursi* leaves could inhibit microbial degradation of these leaves.

The differences observed in decomposition rates between the charcoal amended and control litterbags can also be attributed to differences in moisture contents. In dry ecosystems such as ponderosa pine, moisture is extremely limiting to microbially-mediated processes. The addition of charcoal to some of the bags increased the surface area and added multitudes of pore spaces, both of which combine to increase water holding capabilities and the residence times of the moisture within the bags. This could

help explain the charcoal effect on decomposition rates and suggests that charcoal could be especially important to soils in regions where moisture is considered highly limiting.

Although it is not apparent from looking at the mass loss data alone, evidence of microbial response for the *A. uva-ursi* leaf litter is clearly shown in the litter chemistry results. Charcoal amendments resulted in a reduction in the leaf lignin content accompanied by an increase in extractable phenolics. The microbial communities in this litter were enhanced enough by the presence of charcoal to cause increased lignin breakdown. Further evidence of lignin degradation was demonstrated in the corresponding increase in extractable free phenolics. The simplified lignin degradation compounds were recognized by the Prussian Blue technique where normally lignin is too large and complex a molecule to be detected by this method.

Significant charcoal effects were not observed in the laboratory incubation for N mineralization or soil respiration, which is likely as a result of the overwhelming effect of the litter application. The litter amendments increased respiration rates and caused NH_4^+ immobilization. Slight nitrification was observed with the litter and charcoal amendments. Differences in mineralization may be observed over longer term incubations where additional leaching is permitted.

Charcoal had a much stronger effect on C and N chemistry in an earlier laboratory incubation. Soils were amended with charcoal, glycine, and a water extract of *A. uva-ursi* leaves and accumulation of NO_3^- , NH_4^+ , and free phenols were measured. The accumulation of NO_3^- was significantly increased by the presence of charcoal but not glycine, regardless of NH_4^+ accumulation, which indicates that net nitrification is not substrate limited but enhanced by possible changes in C chemistry as influenced by the

presence of charcoal. It is possible that charcoal ties up C that would otherwise promote immobilization. Charcoal also decreased the concentration of free phenols in this incubation, which further suggests its role in alteration of soil C chemistry. The results of this incubation alone warrant further investigations.

CONCLUSIONS

This research demonstrates that charcoal has the potential to greatly alter litter decomposition rates and associated processes. The addition of charcoal to litter facilitated microbial community development in the *P. ponderosa* needle litter and increased the rate of decomposition. It also enhanced the breakdown of lignin and polyphenolics of the *A. uva-ursi* litter as demonstrated by the decrease in lignin content and the increase in extractable free phenolics. However, given that little research has specifically addressed charcoal's role in decomposition, a wealth of questions remain.

Both field and lab incubations should have lasted much longer, but due to logistical constraints, the field incubation was rather short (1 year). It is difficult to predict what similar, but longer incubations would have concluded; however, it is likely that greater charcoal effects would have been observed, especially in the laboratory incubation. However, the fact that fresh litter was used is helpful when considering the results observed here because its higher nutrient content undoubtedly promoted faster microbial attack.

The other major constraint of this study is that it was difficult to add realistic amounts of charcoal to the litter since current understanding of charcoal's abundance in the forest floor and mineral soil is limited (see Chapter 1). The amounts added here are

likely to be overestimates, but this loading rate was chosen to push the system.

Nonetheless, clear decomposition patterns were observed by adding charcoal to the litter types in this study, and this appears to be the first of such demonstrations for ponderosa pine ecosystems to date. Therefore, future research in this area is definitely warranted and should be able to shed greater light on the role of charcoal by using longer term incubations, both in the lab and the field.

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