



**School of Agriculture, Food and Rural Development**

# **Assessing the Use of Hydrogen Peroxide Oxidation to Quantify Black Carbon in Amazonian Soils**



**Greta C. Dargie**

**Student Number: 099059101**

**Agricultural and Environmental Science MSc.**

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## **Origin and Organisation of the Project**

The purpose of the expedition to Maranhão state Brazil was to carry out field and laboratory work for an MSc dissertation for the degree programme Agricultural and Environmental Science, School of Agriculture, Food and Rural Development, Newcastle University. The field and laboratory work in Brazil was carried out through the Universidade Estadual do Maranhão (UEMA). Communication and links between Newcastle University and UEMA were established through an acquaintance between Dr Elizabeth Stockdale, School of Agriculture, Food and Rural Development, Newcastle University and Dr Christoph Gehring, Laboratorios do Solo, UEMA. Through these communications permission was granted by Dr Christoph Gehring for the project to be carried out using the facilities and under the supervision of UEMA in cooperation with Newcastle University. The project that was carried out was selected because of its interest to myself, its global relevance and also because it was hoped that the results would be of interest and of use to other future projects carried out at UEMA.

## **Abstract**

Progress in the study of black carbon (BC) is being hindered by the lack of a low cost, effective method to quantify BC that can be carried out in most laboratories. Kurth *et al.* (2006) report the successful use of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) oxidation with weak nitric acid to quantify BC contents in temperate soils. The aim of this study was to assess the use of H<sub>2</sub>O<sub>2</sub> oxidation, as described by Kurth *et al.* (2006), as a low cost and effective method to quantify the BC content of Amazonian soils with varying fire histories from around Maranhão state, Brazil. Soils were amended with varying concentrations of charcoal (0-50 g kg<sup>-1</sup>) to test the methods ability to recover a range of BC concentrations in the soils. Soils that had not been amended with charcoal were also used to quantify the *in situ* BC concentrations of the soils. Following H<sub>2</sub>O<sub>2</sub> oxidation, loss on ignition was used to quantify the isolated BC. The results from LOI showed exceptionally high mass losses. These exceptionally high mass losses were mainly attributed to structural water loss from clay compounds, as opposed to mass losses that were mainly attributable to organic matter loss. A further problem was the selection of a suitable LOI correction factor that would take into account the higher C concentrations of BC compounds. Therefore the use of LOI was found not to be suitable when quantifying BC contents in soil. Despite this, regression analysis found a good relationship (Rsq. values ranged from 0.9697 to 0.9974), that was highly significant (p<0.001) between the concentration of charcoal added to the soil and the LOI mass loss (g LOI g<sup>-1</sup> dry soil), suggesting that the H<sub>2</sub>O<sub>2</sub> oxidation successfully recovered the charcoal added to the soil. A preliminary analysis of the oxidised soils using a LECO C autoanalyser suggests that the BC concentrations of the soils in this study ranged from 1.03 to 7.23 g kg<sup>-1</sup> dry soil. Further analysis with the autoanalyser will help confirm these figures and the success of H<sub>2</sub>O<sub>2</sub> oxidation.

## **1.0 Introduction**

### **1.1 The Role of Soil in the Carbon Cycle**

As the largest terrestrial store of carbon (C) (Garcia-Oliva and Masera, 2004, Schimel, 1995), storing an estimated 1580 Gt C (Schimel, 1995) soil plays a very important role in the carbon cycle. The way in which soil can either act as a sink or a source of carbon dioxide (CO<sub>2</sub>) has meant that the role of soil in the C cycle has received a lot of attention in recent years as the issue of rising levels of atmospheric greenhouse gases (GHG's), in particular CO<sub>2</sub>, becomes more prominent (Cresser *et al.*, 1991, Sleutel *et al.*, 2007). The basic process, through which C enters the soil, is through the absorption of CO<sub>2</sub> by plants and the incorporation of this C into the plant tissue (Brady and Weil, 2008). The majority of this C then reaches the soil through the shedding of plant litter (Brady and Weil, 2008) to become part of the soil organic matter (OM). Once incorporated into soil, the OM is then decomposed forming the main energy source for the soil microbial population. The rate at which decomposition takes place is dependent on the environmental conditions of the soil. The temperature, moisture content and the degree to which the soil is aerated all have an impact on the activity of the soil microbial biomass and therefore influence the rate at which decomposition of OM takes place (Brady and Weil, 2008). About half of the C is released as CO<sub>2</sub>, with the other half being transformed into microbial tissue (immobilisation) or transformed into more stable forms, often known as humus (Brady and Weil, 2008).

In the tropics OM accumulation in the soil is low as high temperatures and moist conditions cause high litter decomposition and mineralisation rates, resulting in low levels of OM accumulating in the soil (Rumpel *et al.*, 2006a). In Amazonia, therefore the accumulation of C in the soil is often low (table 1.1).

**Table 1.1 Total C contents for Amazonian soils for different soil types and depths.**

Location	Values Reported By Other Authors			
	Soil Type	Soil Depth	C Content (t ha <sup>-1</sup> )	Author and Year Published
Amazonia	-----	0-20cm	20-150 most in 60-80 range	Cerri <i>et al.</i> (2007)
Amazonia	ultisol	0-20cm	20.0±1.1	McGrath <i>et al.</i> (2001)
	oxisol	0-20cm	29.2±8.5	
Pará, Brazil	sandy	0-10cm	12.07±0.68	Silver <i>et al.</i> (2000)
	sandy	40-100cm	16.46±1.25	
	clayey	0-10cm	39.28±2.21	
	clayey	40-100cm	31.68±1.76	

Boxes filled with a dashed line (-----) indicate that the data was not available or presented in the paper.

The accessibility of the OM to the microbial population will also control the rate at which decomposition occurs. The physical location of OM in the soil influences the rate at which OM is decomposed. For example, if located within the interior of a soil aggregate OM will be more inaccessible to the microbial population than if located between aggregates (Brady and Weil, 2008). The chemical structure of the OM can also render OM inaccessible to some soil microbes and results in a slow rate of decomposition, even when environmental conditions favour decomposition (Brady and Weil, 2008). One group of C compounds, often referred to as black carbon, or sometimes elemental carbon or biochar, can be considered as chemically resistant to decomposition (Brady and Weil, 2008).

## **1.2 Black Carbon**

Charring of the aboveground biomass and soil organic matter (SOM) or the condensation of volatilised particles can lead to the formation of carbon compounds with high levels of recalcitrance (Fearnside, 2000, Lehmann *et al.*, 2006, Rumpel *et al.*, 2006a, Knicker, 2007), known as black carbon (BC). As a

product of fire BC is found in soils throughout the world. Table 1.2 provides an insight into the levels of and concentrations of BC in soils from around the world.

**Table 1.2 Soil black carbon contents or concentrations from around the world reported in the literature.**

	Values Reported By Other Authors			
Location	Soil Type	Soil Depth/Horizon	BC Content	Author and Year Published
Brazilian Amazon	<i>Oxisol (terra preta)</i>	0-30cm	25±10 g kg <sup>-1</sup>	Glaser <i>et al.</i> (2001)
		30-100cm	25±9 g kg <sup>-1</sup>	
Global Estimate	-----	-----	1-6% of total C	Gonzalez-Perez <i>et al.</i> (2004)
Luang Prabang province, Northern Laos.	Dystrocrepts, Alfisols, Inceptisols	A horizon	5.5-7.3% of total C	Rumpel <i>et al.</i> (2006a)
		B horizon	2.9-4.6% of total C	
Missoula Valley, Montana, U.S.A.	Udic haplustepts Typic eutrocryalfs Calcic argixerolls	60-120cm	2.91±0.32- 9.17±0.47 g kg <sup>-1</sup>	Kurth <i>et al.</i> (2006)
Florida, U.S.A.	Humic acids from peat	-----	0.63% of total C	Forbes <i>et al.</i> (2006)
Germany	Chernozenic soils	A horizon	2-45% of total C 0.4-7.6 g kg <sup>-1</sup>	Schmidt <i>et al.</i> (1999)

Information on soil type and depth is presented where known. A dashed line (-----) indicates that the information was not available or presented in the paper.

The aromatic, planar structure and relatively few polar functional groups give BC its high level of recalcitrance (Cornelisen, *et al.*, 2005). The high level of recalcitrance means that BC is not easily degraded and therefore could possibly be considered a long term C sink with turnover times in the soil potentially as long as 1000-2000 years (Glaser *et al.*, 2002, Kuzyakov *et al.*, 2009). BC is therefore one way in which C can be removed from the global C cycle long term and the deliberate formation of BC compounds has been proposed as one way of offsetting anthropogenic C emissions (Lehman *et al.*, 2006). The low degradation rate of BC compounds means that their formation can also be considered as a way of increasing SOM levels long term (Glaser *et al.*, 2002, Rumpel *et al.*, 2006a, De Neergard *et al.*, 2008). This is of particular importance in regions of the world where productive agriculture can be difficult to achieve because of low SOM levels, such as in the tropics.

### **1.3 Slash-and-Burn Agriculture**

Slash-and-burn agriculture is widely practised throughout the tropics. It is estimated that between 300 to 500 million people rely on slash-and-burn agriculture (Brady 1996) and that slash-and-burn systems make up ~30% of cultivated land worldwide (Brady, 1996, Metzger, 2002). Under a slash-and-burn agricultural system the aboveground vegetation is slashed to create a space for cultivation and then burned. The resulting ash from the burned vegetation provides nutrients for the succeeding crops and increases the soil pH in what are often nutrient-poor, acidic tropical soils (Kleinman *et al.*, 1996, Lessa *et al.*, 1996, Ketterings *et al.*, 2002). Usually after two to three years of cropping, soil fertility decreases to a point where cultivation is no longer viable and the plot is left for fallow and a new area of land is cleared (Brady, 1996).

The slash-and-burn system has proved to be a sustainable form of agriculture for hundreds or possibly thousands of years in the tropics (Brady, 1996). However, increasing population pressures, increasing food demand and land restrictions have led to an intensification of slash-and-burn systems and threaten their sustainability (Brady, 1996). Intensification has taken the form of reduced fallow periods and/or expansion into previously uncultivated forest (Brady, 1996). The habitat destruction and increased GHG emissions from the increased levels of deforestation and burning has led to the United Nations identifying slash-and-burn agriculture as a global environmental concern (Kotto-Same *et al.*, 1997).

In the Amazon basin slash-and-burn agriculture accounts for ~30-35% of total deforestation (Metzger, 2002). Deforestation occurs in all Amazonian countries. However, most deforestation has occurred and

continues to occur in Brazil (Wood, 2002). The deforestation in Brazil is not evenly distributed, with some regions experiencing much higher rates than others (Wood, 2002).

#### **1.4 Formation and Role of Black Carbon Under Slash-and-Burn Systems**

Under slash-and-burn agriculture large amounts of nutrients and organic carbon are released to the atmosphere during burning from the aboveground biomass and also from organic material already present in the soil (Lehmann, *et al.*, 2006). However, as has already been mentioned, the burning process can also lead to the formation of BC.

Only a small percentage of the C exposed to fire is converted to BC, however. Fearnside (2000) estimates that of the  $3.1 \times 10^6$  t of tropical forest biomass C exposed to burning,  $1.1 \times 10^9$  is emitted to the atmosphere and only  $49 \times 10^6$  t forms charcoal,  $26-31 \times 10^6$  t of which forms highly recalcitrant BC. Similarly Lehmann, *et al.* (2006) estimate that only around 3% of the biomass C is converted to BC following burning. It has been proposed that the loss of SOM during burning reduces the cation exchange capacity (CEC) of the soil under slash-and-burn agriculture and therefore results in even higher rates of nutrient leaching in tropical soils (Cerri *et al.*, 1991, Kleinmann *et al.*, 1996). However, some studies have found the CEC to increase post-burning (e.g. Hölscher *et al.*, 1997, Glaser *et al.*, 2002). It is believed that the high CEC of some tropical soils, e.g. *terra preta de Indios* soils, can be directly attributed to a high concentration of BC in the soil (Glaser *et al.*, 2001, Liang *et al.*, 2006). The high CEC of BC compounds is thought to be a result of large surface areas and the presence of carboxylic functional groups along the aromatic backbone of the compounds (Glaser *et al.*, 2001, Glaser *et al.*, 2002, Liang *et al.*, 2006) or indirectly through the adsorption of organic matter onto the surface of BC compounds (Liang *et al.*, 2006). Therefore the presence of BC in the soil could increase the nutrient retention of the soil and potentially reduce fertilizer requirements for soils under agricultural use in the tropics (Glaser *et al.*, 2002).

However, some studies have also shown that BC may be preferentially eroded from the soil (Rumpel *et al.*, 2006b, Rumpel *et al.*, 2008) and therefore the benefits of BC inputs into the soil may be short-lived. It is possible that the lack of functional groups to bind the BC with other compounds means that BC is easily eroded from the soil in comparison to mineral bound C compounds (Rumpel *et al.*, 2006b, Rumpel *et al.*, 2008). However, it has also been hypothesised that the more aromatic BC compounds are more hydrophobic, whilst BC compounds containing more carboxylic C groups are more soluble and therefore more likely to be leached from the soil (Knicker, 2007). Glaser *et al.* (2002) postulate that the

hydrophobicity of the more aromatic BC compounds may actually reduce leaching overall in the soil. They suggest that the hydrophobic nature of BC prevents water from entering the soil aggregate pores, improving soil aggregate stability and protecting other nutrients from being leached. They also suggest that the high porosity of some BC compounds may increase soil water retention. Research into BC has increased considerably in the last 30 years or so. However, knowledge of the processes by which BC is lost from environments is still lacking (Masiello, 2004).

It is clear that BC has the potential to play a beneficial role in tackling some of the environmental and social problems facing the world today, but it is also clear that in order to utilise any of these potential benefits, further research into BC and its role in the C cycle and effect on soil properties is essential. However, issues with the methodologies used to quantify black carbon are hampering, to a considerable extent, any further progress in the study of BC.

### **1.5 Methods to Measure Soil Organic Carbon- Loss on Ignition and Dry Combustion Carbon**

#### **Autoanalysers**

There are a variety of methods available for the determination of soil organic C in soils. All have their advantages and disadvantages. Accuracy, cost, ease and availability are all factors that help determine which method is adopted. The wet chemical oxidation method, acid dichromate oxidation is often used to determine the organic C content of soils. Acid dichromate oxidation works on the principle that organic matter in the soil will be oxidised by the dichromate. Organic C is then determined by back titration of the excess dichromate (Bird and Grocke, 1997, Chan *et al.*, 1995). However a limitation of acid dichromate oxidation is that more recalcitrant organic C has been found to resist oxidation and so a correction factor to account for the unoxidised organic C has to be applied (Santi *et al.*, 2006). In addition to the wet chemical oxidation methods, loss on ignition (LOI) and dry combustion using C autoanalysers are two other possible methods to measure organic C in soils.

The principle idea behind the LOI method is that if at a temperature of 200°C or above OM is oxidised from the soil (Santisteban *et al.*, 2004), then igniting the soil at temperatures above this should result in the complete oxidation of OM from the soil (Schumacher, 2002). The OM content of the soil should then be equal to the change in mass before and after ignition (Schumacher, 2002, Bianchi *et al.*, 2008, Cresser *et al.*, 1991). The C concentration of the soil can be calculated using standardised values for the C concentration of OM (Schumacher, 2002). LOI is a method which is widely used to measure organic C contents in soil because it is a simple and inexpensive method (Barillé-Boyer *et al.*, 2003, Christensen

and Malmras, 1982) and can therefore be applied in most laboratories. It also allows a large number of samples to be processed at once (De Vos *et al.*, 2005). However, numerous articles have debated its accuracy in measuring C contents (e.g. Leon *et al.*, 2009, Santisteban *et al.*, 2004). Whilst some recent studies still uphold loss on ignition as an adequate method to determine soil C concentrations (e.g. De Vos *et al.*, 2005), others have found that the method can be subject to considerable systematic errors (e.g. Santisteban *et al.*, 2004, Schumacher, 2002, Christensen and Malmras, 1982). Sources of error reported include the oxidation of carbonates in the mineral fraction (Santisteban *et al.*, 2004, Cresser *et al.*, 1991, Christensen and Malmras, 1982) or mass loss attributable to the volatilisation of compounds other than carbon, for example loss of structural water from clays (Santisteban *et al.*, 2004, Cresser *et al.*, 1991, Christensen and Malmras, 1982).

Growing in popularity is the use of dry combustion C autoanalysers to quantify C concentrations. Dry combustion autoanalyser instruments work on the principle that samples are heated to temperatures usually around 1300°C in a stream of pure oxygen and converted to simple gases, such as CO<sub>2</sub>, H<sub>2</sub>O, N<sub>2</sub>, SO<sub>2</sub> (Sleutel *et al.*, 2007). These gases are then separated by specific absorption columns and their amount is usually determined either by their thermal conductance (Pérez *et al.*, 2001, Sleutel *et al.*, 2007) or by infrared detectors (Kowalenko, 2001). Analysis with a dry combustion C autoanalyser has the advantage over other C determination methods in that it is simple and rapid (Kowalenko, 2001, Sleutel *et al.*, 2007, Wright and Bailey, 2001). It also avoids the use of hazardous chemicals, such as the use of potassium dichromate used in the acid dichromate oxidation method, which can be damaging to the environment and pose a risk to human health (Pérez *et al.*, 2001, Sleutel *et al.*, 2007, Wright and Bailey, 2001). Recovery rates of C from samples have been found to be as high as 98% (Wright and Bailey, 2001) and have been found to be highly correlated with wet chemical oxidation methods, but with the advantage that no correction factor needs to be applied to the results (Kowalenko, 2001, Sleutel *et al.*, 2007). In soils which contain carbonates, organic carbon may need to be determined separately, but in acidic soils the total C measured during dry combustion analysis can be assumed to be the organic carbon (Kowalenko, 2001, Santi *et al.*, 2006). Dry combustion autoanalysers also have the benefit that other elements, such as nitrogen or sulphur, can be measured simultaneously (Pérez *et al.*, 2001, Sleutel *et al.*, 2007). The downside to dry combustion C autoanalysers is that the initial purchasing cost and maintenance costs can be high and so dry combustion analysis may not be an option that is available to some laboratories (Santi *et al.*, 2006).

## **1.6 Measuring Black Carbon**

Measuring how much BC is present in the soil is complicated by the fact that BC does not have specifically defined characteristics; there is a BC continuum with varying levels of recalcitrance (Manning and Lopez-Capel, 2009). There is also no clear consensus on the methodology that should be adopted to measure the BC content of soils, with different methods resulting in either an overestimation or underestimation of the BC content or only part of the BC continuum being measured (De La Rosa Arranz *et al.*, 2008, Manning and Lopez-Capel, 2009). A problem that many of the methods face is distinguishing between BC and other highly recalcitrant C compounds that have similar chemical characteristics to BC but are not combustion derived, for example, C compounds such as coals, kerogens and melanoidens (De La Rosa Arranz *et al.*, 2008). Thermal oxidation methods have the potential problem of premature oxidation of BC compounds or the charring of more labile compounds during the oxidation process leading to an overestimation of the BC content (Hammes *et al.*, 2007, Manning and Lopez-Capel, 2009). Wet oxidation methods avoid the problem of charring, but have the problem of very light soot particles being lost during the rinsing of the sample and so have the potential to underestimate the total BC content (Cornelissen *et al.*, 2005).

The low removal rate of more recalcitrant organic C compounds has meant that wet chemical oxidation methods used to quantify soil organic C have been adapted to measure BC. Instead of the focus being on the C oxidised, the focus is on the C that survives oxidation, which can be determined through elemental analysis (Manning and Lopez-Capel, 2009). Acid dichromate oxidation has the benefit that it is one of the cheapest methods for quantifying BC and does not require specialist equipment and can therefore be carried out in most laboratories (Hammes *et al.*, 2007). However, the accuracy of acid dichromate oxidation is often questioned. It has been put forward that oxidation with acid dichromate could possibly result in a change in the chemical composition of the BC (Masiello, 2004) and that it fails to recover soluble forms of BC as these tend to be dissolved in the acid (Hammes *et al.*, 2007). However, the main problem with acid dichromate oxidation is that it is often found to overestimate the BC content of soils for the reason that it assumes that all C compounds that survive the oxidation process are BC (Hammes *et al.*, 2007).

One way to overcome the problem of determining whether the C left after acid dichromate oxidation is mineral derived or fire derived is through the use of  $^{13}\text{C}$ -NMR analysis (Hammes *et al.*, 2009).  $^{13}\text{C}$ -NMR provides a qualitative assessment of the chemical structure of compounds. It characterises the number and type of different C bonds in a C compound and so can be used to distinguish between BC and non-

fire derived mineral C (Masiello, 2004). However,  $^{13}\text{C}$ -NMR has the disadvantage that it is a very expensive and time-consuming method and so is less suitable for use on a large number of samples (Hammes *et al.*, 2007, Manning and Lopez-Capel, 2009). Therefore its use alongside acid dichromate oxidation detracts from any advantages that can be gained through the use of acid dichromate oxidation.

An alternative to acid dichromate oxidation is wet chemical oxidation with hydrogen peroxide. This is a method which has often been used to remove the OC fraction in soil mineralogical and textural studies (Mikuta *et al.*, 2005). However, many studies have found this method to have a low removal rate of OC, which has been attributed to its inability to oxidise more recalcitrant OC compounds (Mikuta *et al.*, 2005). Therefore some authors have used an adapted version of hydrogen peroxide oxidation to isolate the BC fraction of soil (e.g. Wu *et al.*, 1999, Kurth *et al.*, 2006, Jantalia *et al.*, 2007, Bélanger and Pinno, 2008). After hydrogen peroxide oxidation, the C remaining in the sample that failed to oxidise is assumed to be the BC fraction. The BC fraction can then be quantified via the loss on ignition method or through the use of a C autoanalyser (Kurth *et al.*, 2006). Although this is not as widely practised as the potassium dichromate oxidation method, Jantalia *et al.* (2007) found a good agreement between the proportions of OC oxidised using the hydrogen peroxide oxidation method and potassium dichromate oxidation method in their study of soils from the Brazilian cerrado. However, they concluded that both methods were not completely successful in providing accurate estimates of the BC content of the soils. However, Kurth *et al.* (2006) found the repeatability of their adapted version of hydrogen peroxide oxidation, using a weak nitric acid, to be reliable and less variable than acid dichromate oxidation. In comparison to other methods used to quantify BC, very few papers report the use of hydrogen peroxide oxidation and these are across a limited range of soils. Studies that have used the method to remove OC have found that, as with the potassium dichromate oxidation method, the assumption that the only C compounds remaining after oxidation are BC is not always valid (Mikuta *et al.*, 2005). Therefore the use of hydrogen peroxide oxidation to isolate BC fractions is a method that may benefit from further assessment.

Simpson and Hatcher (2004) contend that BC measurements reported by some studies will simply be an artefact of the method used rather than a true measurement of the BC content of the soil/ sediment. They also argue that many studies define BC as what is measurable with their chosen method. The large discrepancies between methods make it hard to compare the results of different studies looking at BC. Masiello (2004) calls for more methodological intercomparisons in studies so that progress can be made

towards amending the discrepancies that arise from the different methodologies. However, there is also a need to find a standardised, reliable method which can be readily carried out in most laboratories.

## **2.0 Aims**

The overall goal of this study was to increase the knowledge of BC in Amazonian soils, so that further steps can be made towards fully utilising any benefits that BC provides with regards to soil fertility and carbon sequestration. For this reason the main aim of this study was to assess the use of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) oxidation as a method to quantify BC in Amazonian soils. A further aim of this study was to quantify the BC contents of burned and unburned soils from around Maranhão State, Brazil.

The specific objectives of this study were therefore to:

- establish the use of hydrogen peroxide oxidation as a cheap and effective method to quantify BC in the laboratories of the Universidade Estadual do Maranhão, São Luís.
- amend soils with known concentrations of charcoal, to assess the ability of hydrogen peroxide oxidation to recover varying concentrations of BC.
- carry out hydrogen peroxide oxidation on soils of different textures and land use histories to assess whether the performance of hydrogen peroxide oxidation is consistent across a range of Amazonian soils.
- quantify the *in situ* BC concentrations in the soil from four sites around Maranhão state, through the use of hydrogen peroxide oxidation.

## **3.0 Materials and Methods**

### **3.1 Study Sites**

The project was carried out in the State of Maranhão in the northeast of Brazil (figure 3.1).



**Figure 3.1** Map of Brazil. The location of Maranhão state is indicated by a red circle. Original image sourced from <http://www.dholmes.com/master-list/brasil.html> (date accessed 2nd September, 2010).

The state of Maranhão is one of the poorest regions of Brazil and has the highest rural population density in Brazil's Legal Amazon (Porro, 2002, Porro, 2005). Of the original tropical moist forest biome, only small patches remain (Porro, 2002, WWF, 2002), whilst most of the region is anthropogenic secondary forest, mainly dominated by the babaçu palm or degraded pasture (Porro, 2002, WWF, 2002). Slash-and-burn agriculture, plantation agriculture and cattle ranching are the main land uses (WWF, 2002). Market incentives and government subsidies have seen an expansion in the area under cattle ranching and plantation agriculture (Porro, 2005). This, coupled with property right reforms favouring the agrarian elite, has meant less land is available for the poorer farmers who rely on slash-and-burn agriculture for their livelihoods (Porro, 2005). With less land available for cultivation, fallow periods have been reduced to three to four years. The resulting decrease in soil fertility and subsequent decline in crop yields means that those who were already vulnerable to poverty have become even more so (Porro, 2002).

Soil sampling was carried out at four sites from around two locations in Maranhão. All laboratory work was carried at the Universidade Estadual do Maranhão in the state capital city of São Luís.

### 3.1.1 Gurupi Biological Reserve

Gurupi Biological Reserve is one of the last Amazonian forest remains in Maranhão state, covering an area of 272,375ha. Under the Köppen climate classification, it is classified as class Aw, a tropical wet climate, with a pronounced dry season, with the driest month having a rainfall less than 50mm and the lowest average monthly temperature never falling below 18°C. As one of the forest refuges that survived the Pleistocene glaciations, Gurupi is an area that is very rich in biological resources, with new species of flora and fauna still being discovered to this day. In 1961 Gurupi became a Reserva Florestal (a forest reserve) and in 1984 deforestation in the reserve became completely prohibited. However, the enforcement of this law has been poor and so deforestation continues to this day and is a serious threat to this rare and valuable forest resource (*Amazônia Maranhense*, Anon., 1997)



**Figure 3.1.1** Satellite image of the Gurupi Biological Reserve showing the location of the two sampling sites (image sourced from Google Earth).

In the Gurupi Biological Reserve two areas (the location of which is shown in figure 3.1.1) were selected as sampling sites with contrasting burning histories:

- The first sampling site (Gurupi unburned) was inside the reserve boundaries (03.92192S, 046.76233W) at 219m a.s.l., in an area of the forest which had never been burned.
- The second sampling site (Gurupi burned) was just outside the reserve boundaries (04.00145S, 046.83564W) at 262m a.s.l., in fallow of around 5 years, which had been subjected to one burning.

### 3.1.2 Ilha Do Livramento

Ilha Do Livramento is an island located just off the coast, to the south west of the city of Alcântara, in the state of Maranhão, Brazil. The climate is characterised by a rainy season from January to June and a dry season from July until December and an annual accumulated rainfall between 2100-2400mm (INMET, 2008) with low average monthly rainfalls between 10-30mm occurring in November and high average rainfalls of 350-450mm occurring in April (INMET, 2008). Average annual temperatures are around 26.5°C (Alcântara Cyclone Space, 2010). As can be seen from figure 3.1.2 the island is mostly forested, with a large part of it being secondary forest; land use history of the island is not well known. The current population of the island stands at only two inhabitants.



**Figure 3.1.2** Satellite image of Ilha Livramento showing the location of the two sampling sites (image sourced from Google Earth).

On the Ilha Do Livramento two areas were selected as sampling sites with contrasting burning histories (the locations of which are shown in figure 3.1.2):

- The first sampling site (Livramento unburned) was located in an area of secondary forest to the north of the island ( $2^{\circ}25'3.20''S$   $44^{\circ}25'20.20''W$ ), 19 m a.s.l., which had never experienced burning.
- The second sample site (Livramento burned) was located in another area of secondary forest to the south of the island ( $2^{\circ}25'25.57''S$   $44^{\circ}25'31.45''W$ ), 1 m a.s.l., which had been subjected to one burning around 6 years ago.

### **3.2 Collection and Characterisation of Soil Samples**

#### **3.2.1 Soil Sampling**

At each site soil samples were taken from 0-5cm and 90-100 cm depths.

At each sampling site a pit was dug of at least 1 m in depth and soil sampled from three faces of the pit at a depth of 90-100 cm. Three replicate bulk density samples were taken using thin walled, steel sampling tubes 5 cm long and 6.1 cm in diameter. These tubes were pushed into the pit walls at the appropriate depth using a rubber hammer and a piece of wood to minimize soil compaction. The tubes were then excavated and the soil inside the tubes was placed in a sealed sampling bag. This process was repeated twice for each of the three faces of the pit, so that samples from two tubes formed one composite sample for bulk density.

Bulk soil samples were collected by excavating, through the use of a trowel, between 2-3 kg of soil from the 90-100 cm depth for the three corresponding faces of the pit. The soil was then placed inside a sealed plastic bag.

For the 0-5 cm layer soil was sampled in the surrounding area of the pit at three sites that were around 1m from the edge of the pit in directions corresponding to the three pit faces that would be sampled for the 90-100 cm layer. The 0-5 cm layer was not sampled within the pit due to soil compaction and contamination with soil from lower layers during the excavation of the pit. As the sampling tubes were 5cm in length, bulk density samples were collected by pushing the sampling tubes into the surface of the soil using the rubber hammer and block of wood, until the top of the sampling tube was level with the soil surface. The tubes were then excavated and the soil was placed inside a sealed plastic bag. Again this process was repeated twice for each of the three sites. Bulk samples were collected at the same location as the bulk density samples and were collected by excavating between 2-3kg of soil down to a depth of 5cm and placing the soil in a sealed plastic bag.

### 3.2.2 Determination of Soil Moisture Content and Bulk Density

Bulk density samples were taken back to the laboratory where they were weighed and then dried at 105°C in an oven to constant weight. Once dried, soil samples were weighed again.

The water content of soil measured in  $\text{g g}^{-1}$  dry soil was calculated by the following:

$$\text{WC} = ((m_s - m_e) / m_e)$$

where WC is the water content expressed as a percentage,  $m_s$  is the mass of the soil at the start and  $m_e$  is the mass of soil after drying.

The soil bulk density (BD), measured in  $\text{g cm}^{-3}$ , was calculated by the following equation:

BD= M/V

where M is the mass of the soil from the two bulk density samples once dried, measured in grams, and where V is the volume of the soil in the two sampling tubes combined, measured in cm<sup>-3</sup>. This way an average bulk density for the two replicate samples could be produced.

### 3.2.3 Soil Textural Analysis

Soil textural analysis was carried out on the three bulk density samples for each sample site and depth using the hydrometer method from Bouyoucos (1951).

### 3.2.4 Soil pH Analysis

Soil pH was determined in a 1:5 soil:0.01 M CaCl<sub>2</sub> suspension (as described by the NSW Government DECC, 2008).

### 3.2.5 Processing and Preparation of Bulk Soil Samples

The bulk soil samples (c. 3 kg) were dried in an oven at 45 °C to constant weight. The soil was then ground, using a mortar and pestle to pass through a 2 mm mesh sieve to remove roots and stones. The soil was then ground again to pass through a 250 µm mesh sieve. Soil particles which did not pass through the 250 µm mesh were ground a further 3 times. If after 3 times the soil particles still did not pass through the 250 µm mesh, these particles were then removed. Once this process was complete, the soil particles that did not pass through the 250 µm sieve were then returned to the ground sample. This was done to avoid exclusion of any particular soil fraction, such as sand particles. The three replicate bulk samples were then combined to form one composite sample (c. 6-9 kg) for each site.

## **3.3 Quantification of the BC Content in Burned and Unburned Soils with a Range of Charcoal Amendment Levels.**

### 3.3.1 Selection of Method for Determination of BC - Hydrogen Peroxide Oxidation

A wide ranging search of the literature on the topic of BC quantification methods was carried out in order to assess the advantages and disadvantages of a number of potential methods. Taken into consideration was the accuracy and repeatability of the method. As one of the objectives was to find a method which could easily be carried out in most laboratories at an affordable cost, methods which did not require the use of specialist equipment or involve costly analysis were given precedence.

The H<sub>2</sub>O<sub>2</sub> oxidation method, as used by Kurth *et al.* (2006) did not require the use of any specialist equipment and therefore could feasibly be carried out in the laboratories at the Universidade Estadual do Maranhão and within the financial budget of this project. More importantly, the H<sub>2</sub>O<sub>2</sub> oxidation method has been presented by Kurth *et al.* (2006) as a chemical oxidation method which, unlike some more commonly used chemical methods, can reliably quantify BC in temperate soils.

The H<sub>2</sub>O<sub>2</sub> oxidation with weak nitric acid method is based on the principle that during oxidation all form of C, including mineral C should be destroyed, with the exception of any BC present in the soil.

### 3.3.2 Identification of Added C Source and Levels

For soil amendment with known amounts of charcoal, charcoal was purchased from a supermarket in São Luís. Although this was commercial charcoal, it was derived from natural forest from the state of Pará next to Maranhão (figure 3.1). Therefore it is likely to be fairly representative of the type of charcoal which would be formed from forest burns across the state of Maranhão. Like the soil samples, the charcoal was ground and sieved using a 250µm sieve.

From each composite bulk soil sample, seven sub-samples of 150 g were taken and amended with the following concentrations of charcoal: 0, 0.05, 0.1, 0.5, 1.0, 2.0 and 5.0 % w/w. The levels of charcoal amendment were taken from the article by Kurth *et al.* (2006). This would allow the results obtained from their study to be easily compared to the results obtained from this study. As can be seen from table 1.2.1 the charcoal amendments used in this study are at the lower end of some of the concentrations reported for the following reason. When applying this method to soils from sites that had not experienced fire in recent history, it would be expected that if any BC were present it would be in very low levels. Therefore it would be important to know whether the method was capable of recovering very low levels of BC in soils.

### 3.3.3 Total C Content and Residual Water Content

From each charcoal amended sample 5 g of each soil sample was accurately weighed to four decimal places into a crucible and placed in an oven overnight at 105°C. Once dried, soil samples were placed in a desiccator and left to cool. Once cooled the soil samples were re-weighed.

The residual water content of the air-dried samples was calculated by the following:

$$WC_{res} = 100 * ((m_s - m_d) / m_s)$$

where  $WC_{res}$  is the residual water content measured in  $g\ g^{-1}$  of oven dried soil,  $m_s$  is the mass of the soil at the start and  $m_e$  is the mass of soil after drying at  $105^\circ C$ .

The samples were then placed in a furnace at  $850^\circ C$  for 30 minutes to determine carbon concentration via the loss on ignition (LOI) method (as described by Ball, 1964).

After ignition the crucibles containing the remaining soil were left to cool in a desiccator and then reweighed. The loss on ignition ( $g\ LOI\ g^{-1}$  dry soil) was calculated by the following:

$$LOI = (m_e - m_i) / m_e$$

where  $m_e$  is the mass of soil after drying at  $105^\circ C$  and  $m_i$  is the mass of the soil after ignition.

#### 3.3.4 Hydrogen Peroxide Oxidation

For the hydrogen peroxide oxidation the method used was based on the methods reported by Kurth *et al.* (2006). From each charcoal amended soil sample, 5 g of soil was weighed out into a 250 ml Erlenmeyer flask. 100 ml of 30%  $H_2O_2$  and 50 ml of 1 M  $HNO_3$  were then added to each Erlenmeyer flask. Flasks were then left at room temperature for an hour and swirled occasionally before being transferred to a hot plate and heated to  $70-100^\circ C$  for 14 hours. During this period samples were swirled occasionally. After 14 hours, if there was no more evidence of effervescence from the sample, then the digestion of the sample was assumed to be complete (Kurth *et al.*, 2006). If effervescence still occurred after 14 hours the sample was returned to the hot plate until no further effervescence was observed.

After oxidation was complete, the residue was then filtered out of the solution using Frama filter papers. The filter papers containing the soil residue were then dried in an oven at  $105^\circ C$  for 48 hours. They were then removed and placed in a desiccator to cool. Once cool, the filter papers containing the soil residue were removed one by one and weighed. The soil was then recovered from the filter paper, placed in a crucible and re-weighed before being placed in the furnace to determine loss on ignition as described above (Section 3.3.2).

Loss on ignition mass loss ( $g\ LOI\ g^{-1}$  dry soil) was then calculated using the following equation:

$$LOI = (m_e - m_i) / m_e$$

where  $m_e$  is the mass of soil residue after drying at  $105^\circ C$  and  $m_i$  is the mass of the soil residue after ignition.

For every charcoal amended soil three replicate samples were analysed in this way following this procedure.

A further three replicate digestions were carried out for each sample. However, instead of using LOI to measure BC concentrations, the soil residues from each digestion have been kept aside for C concentration analysis via a dry combustion C autoanalyser. Three replicate undigested soil samples from each charcoal amended soil have also been kept aside for total C analysis via a C autoanalyser. This will allow a second replicate data set to be produced which will allow an assessment to be made of the use of loss on ignition to measure total C and BC concentrations in soil.

In order to give an early indication of the performance of the loss on ignition method to accurately measure the BC and total C concentrations of the soils sampled, one replicate sample of digested soil residue and one replicate undigested soil sample from each 0% charcoal amended soil was sent to the Laboratorio de Ecofisiologia e Propagacao de Plantas (the Laboratory of Ecophysiology and Plant Propagation), Embrapa Amazonia Oriental in Belem, Brazil for BC and total C concentration analysis via dry combustion using a LECO CNS 2000 autoanalyser. In studies reviewing the performance of the Leco CNS 2000 dry combustion autoanalyser, the instrument has been found to perform well when measuring C contents of samples (Kowalenko, 2001, Matejovic, 1997).

### 3.3.5 Total C and BC Content

The total C content of the soil, expressed in  $\text{g kg}^{-1}$  and  $\text{t ha}^{-1}$  in each soil layer was calculated by the following equations:

$$\text{Total C (g kg}^{-1}\text{)} = C_{\text{conc}} * 10$$

$$\text{Total C (t ha}^{-1}\text{)} = C_{\text{conc}} * V * \text{BD}$$

where  $C_{\text{conc}}$  is the C concentration, measured by the autoanalyser, expressed as a fraction, BD is the bulk density and V is the volume in  $\text{m}^3$  over  $1 \text{ ha}^{-1}$  for the corresponding soil depth. For example if the sample depth is 5cm then the corresponding volume will be  $500 \text{ m}^3$  or if the sample depth is 10cm then the corresponding volume will be  $1000 \text{ m}^3$ .

The total BC content for each location, expressed in  $\text{g kg}^{-1}$  and  $\text{t ha}^{-1}$ , will be calculated by the following calculation:

$$\text{Total BC (g kg}^{-1}\text{)} = \text{BC}_{\text{conc}} * 10$$

$$\text{Total BC (t ha}^{-1}\text{)} = \text{BC}_{\text{conc}} * V * \text{BD}$$

where  $\text{BC}_{\text{conc}}$  is the BC concentration, measured by the autoanalyser, expressed as a fraction, BD is the bulk density and V is the volume in  $\text{m}^3$  over  $1 \text{ ha}^{-1}$  for the corresponding soil depth.

### **3.4 Data Collation and Statistical Analysis**

Statistical analysis was carried out using the statistical package Minitab 15. Regression analysis was used to look for a relationship between the concentration of charcoal added to the soil and the concentration of charcoal recovered following hydrogen peroxide oxidation. ANOVA General Linear Model's were used to look for significant differences in soil properties between sites and Tukey's test was used to see which sites were significantly different from one another. When looking to see if there were any significant differences between soil properties at the same site for the different soil depths, a student's 2 sample T-test was used. All data were tested for normality using the Anderson Darling test. Where data were found to be not of a normal distribution, the data were transformed until normality was reached. Where the data were not found to be of a normal distribution, it has been stated in the text along with the following data transformations that were made.

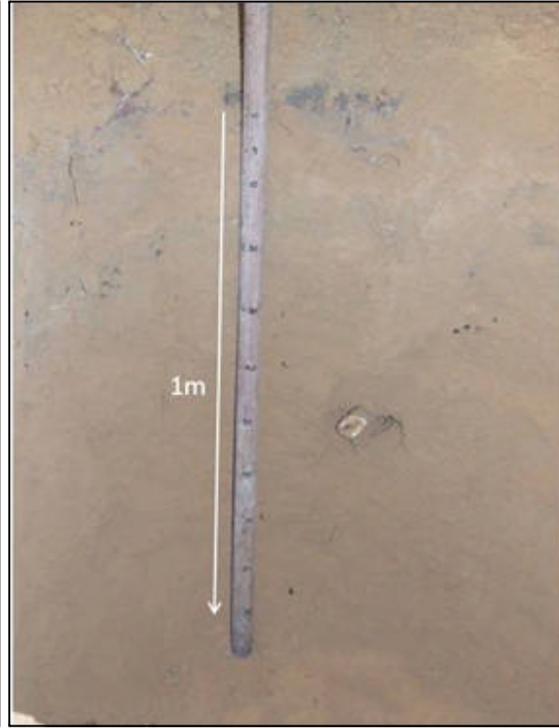
## **4.0 Results**

### **4.1 Soil Profiles**

As can be seen from figures 4.1 to 4.4 and table 4.1, there were clearly observable differences between the soil profiles sampled in Gurupi and the soil profiles sampled on Ilha do Livramento. There were also considerable differences between the soil properties of the two sites on Ilha do Livramento, with the Livramento burned site showing clear signs of anaerobic conditions, probably as a result of groundwater level fluctuations. As can be seen in figure 4.4, groundwater was observable in the bottom of the pit. At both of the unburned sites no charcoal was visible. At the Gurupi burned site charcoal was clearly visible in the soil until a depth of 30cm. Although in the surrounding area of the pit the trees showed visible signs of burning at the base of their trunks, no charcoal was visible in the soil profile at the Livramento burned site.



**Figure 4.1** Photo of the soil profile at the Gurupi unburned site.



**Figure 4.2** Photo of the soil profile at the Gurupi burned site.



**Figure 4.3** Photo of the soil profile at the Livramento unburned site.



**Figure 4.4** Photo of the soil profile at the Livramento burned site.

Table 4.1. Soil profile properties from the four sampling sites.

Soil Profile Properties				
Referred to in the text as:	Gurupi unburned (GU)	Gurupi burned (GB)	Livramento unburned (LU)	Livramento burned (LB)
Location	Gurupi	Gurupi	Ilha do Livramento	Ilha do Livramento
Vegetation Present	primary forest	secondary forest	secondary forest	secondary forest
Fire History	Unburned	Burned	Unburned	Burned
Horizon Development	Shallow litter layer. Shallow A horizon 0-10 cm, with diffuse boundary. Highly leached and weathered B horizon, 10-100 cm. Red colour indicating the presence of iron oxides.	Shallow litter layer. Shallow A horizon 0-2cm, with quite sharp boundary. Highly leached and weathered B horizon, 2-100 cm. Red colour indicating the presence of iron oxides.	Shallow A horizon, 0-5cm. Reddish-brown B horizon, 5-100 cm.	Grey/brown horizon 0-70 cm. Light grey, permanently anaerobic horizon, 30-100 cm. Reddish brown mottles present 30-100 cm.
Soil Texture	0-5 cm: sandy loam 90-100 cm: sandy clay	0-5 cm: silty clay 90-100 cm: clay	0-5 cm: sandy loam 90-100 cm: loam	0-5 cm sandy loam 90-100 cm sandy clay loam
Root Abundance	Fine roots concentrated in the 0-5 cm depth, but coarse roots present throughout the 1 m profile.	Fine roots concentrated in the 0-2 cm depth. Below this depth roots are infrequent.	Fine and coarse roots present throughout the 1 m profile, but with most concentrated in the top 30cm.	Fine roots concentrated in the 0-7 cm depth. Coarse roots present throughout the profile.

#### 4.2 Bulk Density

Bulk density ranged from 0.58 to 1.71 g cm<sup>-3</sup> in the sites sampled. In site variability was low; however the Gurupi burned site had a noticeably higher in site variability in the 0-5 cm than any other site. With the exception of Gurupi unburned, all sites had significantly lower bulk densities in the top 0-5 cm than in the 90-100 cm soil depth (table 4.2). The bulk density of the 0-5 cm soil depth was significantly different between the four sample sites ( $p < 0.05$ ; table 4.2). The Gurupi burned site had a significantly lower bulk density in the top 5 cm than the Gurupi unburned site. The Gurupi burned site had a

significantly lower bulk density at 90-100 cm soil depth than the other three sites and the Livramento burned site had a significantly higher bulk density than the other three sites (table 4.2).

**Table 4.2 Mean bulk densities for the four different sample sites and different soil depths.**

Soil Bulk Density ( $\text{g cm}^{-3} \pm \text{SD}$ )		
Site	Soil Layer	
	0-5cm	90-100cm
GU	1.26 $\pm$ 0.11a	1.40 $\pm$ 0.04A
GB	0.73 $\pm$ 0.14*b	1.13 $\pm$ 0.04*B
LU	1.08 $\pm$ 0.14*ab	1.37 $\pm$ 0.01*A
LB	1.09 $\pm$ 0.13*ab	1.67 $\pm$ 0.06*C

\* indicates that there was a significant difference ( $p < 0.05$ , 2 sample t-test) in bulk densities between the 0-5cm and 90-100cm soil depths in the same soil profile. Different lower case letters indicate significantly different ( $p < 0.05$ , Tukey's test) bulk densities in the 0-5cm soil depth at the different sites. Different uppercase letters indicate significantly different ( $p < 0.05$ , Tukey's test) bulk densities in the 90-100cm soil depth between the different sites.

#### **4.3 Water Content at Sampling and Residual Water Content in Air Dry Soils**

Water contents ranged from 0.0069 to 0.0672  $\text{g H}_2\text{O g}^{-1}$  air dried soil ( $45^\circ\text{C}$ ) in the sites sampled. In site variability was low for the 90-100cm depth samples at all sites. For the 0-5cm depth samples in site variability was a lot higher, particularly for the Livramento burned site. In general water contents were higher in the 0-5 cm depth, than in the 90-100 cm depth, however this difference was only significant for the Gurupi burned and Livramento unburned sites. At the Gurupi unburned site, the water content was slightly lower in the 0-5 cm depth, but this was not significant. Water contents were found to be significantly different in the top 0-5 cm ( $p = 0.001$ ) and in the 90-100 cm depth ( $p < 0.001$ ) between the four sites. In the 0-5 cm depth the Gurupi unburned site had a significantly lower water content than the other three sites, whilst the Gurupi burned site had a significantly higher water content than the Gurupi unburned and Livramento burned sites, but not the Livramento unburned site (table 4.3.). A similar pattern was observed in the 90-100 cm depth, with the Gurupi unburned site having a significantly lower water content than the other three sites and the Gurupi burned site having a significantly higher water content than the other three sites (table 4.3).

**Table 4.3 Real water contents of the soil at sampling for the four different sites and different depths.**

Soil Water Contents At Sampling				
Site	0-5 cm		90-100 cm	
	Real Water Content (g H <sub>2</sub> O g <sup>-1</sup> dry soil)	Water Content (g H <sub>2</sub> O g <sup>-1</sup> air dried (45°C) soil ±SD)	Real Water Content (g H <sub>2</sub> O g <sup>-1</sup> dry soil)	Water Content (g H <sub>2</sub> O g <sup>-1</sup> air dried (45°C) soil ±SD)
GU	0.14	0.13±0.02A	0.16	0.15±0.00a
GB	0.44	0.39±0.05*B	0.32	0.30±0.00*b
LU	0.41	0.35±0.04*BC	0.36	0.28±0.01*c
LB	0.29	0.27±0.07C	0.22	0.19±0.00d

Real water contents were calculated from the mean water content of soil dried at 45°C and the mean residual water content of soil dried at 105°C for corresponding samples. For this reason no standard deviations are presented and no statistical analysis were carried out. As residual water contents made a reasonably small contribution towards the real water content, the mean water contents of the soil dried at 45°C are presented along with the results of any statistical analysis carried out on these data. \* indicates that there was a significant difference ( $p < 0.05$ , 2 sample t-test) in water contents between the 0-5 cm and 90-100 cm soil depths in the same soil profile. Different uppercase letters indicate significantly different ( $p < 0.05$ , Tukey's test) water contents in the 0-5 cm soil depth at the different sites. Different lowercase letters indicate significantly different ( $p < 0.05$ , Tukey's test) water contents in the 90-100 cm soil depth between the different sites.

#### **4.4 Soil Textural Analysis**

There was a lot of variation in the textural properties between the four sites; coarse sand contents ranged from 0 to 21 %; fine sand contents ranged from 2 to 72 %; silt contents ranged from 4 to 50 % and clay contents ranged from 13 to 62 %. In site variation was generally low, although where a granular component of the soil was low, variability was often high. With the exception of the Gurupi burned site, the fine sand fraction was the dominant fraction at all sites in both the 0-5 cm and 90-100 cm depths. At the Gurupi burned site, however, the sand content of the soil was exceptionally low in comparison to the other three sites, with clay and silt contents being far more dominant. At all sites the sand content was found to be higher in the surface 0-5 cm depth than in the 90-100 cm depth. The opposite pattern was observable for the clay contents (table 4.4).

**Table 4.4 Results from a textural particle size analysis showing the granular composition of the four sites and different depths.**

Site/Soil Depth	Average Granular Composition (% $\pm$ SD)			
	Sand: Coarse (2-0.2mm)	Sand: Fine (0.2-0.05 mm)	Silt	Clay
GU 0-5 cm	7.33 $\pm$ 0.58	71.33 $\pm$ 0.58	5.67 $\pm$ 0.58	15.67 $\pm$ 0.58
GU 90-100 cm	4.00 $\pm$ 0	44.33 $\pm$ 0.58	16.33 $\pm$ 0.58	35.33 $\pm$ 0.58
GB 0-5 cm	2.33 $\pm$ 0.58	4.33 $\pm$ 0.58	49.33 $\pm$ 0.58	44.00 $\pm$ 1.00
GB 90-100 cm	0 $\pm$ 0	2.00 $\pm$ 0	37.33 $\pm$ 1.15	60.67 $\pm$ 1.15
LU 0-5 cm	11.00 $\pm$ 1.00	51.67 $\pm$ 1.53	19.67 $\pm$ 0.58	17.67 $\pm$ 0.58
LU 90-100 cm	7.00 $\pm$ 1.00	43.00 $\pm$ 1.00	29.33 $\pm$ 0.58	20.67 $\pm$ 0.58
LB 0-5 cm	20.67 $\pm$ 0.58	61.33 $\pm$ 1.15	4.33 $\pm$ 0.58	13.67 $\pm$ 0.58
LB 90-100 cm	16.67 $\pm$ 0.58	52.00 $\pm$ 1.00	10.00 $\pm$ 1.00	21.33 $\pm$ 0.58

#### **4.5 Soil pH**

The soil pH of the samples ranged from 3.89 to 5.48. In site variability was low (table 4.5). With the exception of the Gurupi unburned site, soil pH was found to be significantly higher in the 0-5 cm depth than in the 90-100 cm depth (table 4.5). For the 0-5 cm depth the soil pH was found to be significantly different ( $p < 0.05$ ) for the different sites. In general the Livramento sites had a higher pH, with both sites having a significantly higher pH than the Gurupi unburned site. Only the Livramento unburned site had a significantly higher pH than both of the Gurupi sites. Between the two Gurupi sites and between the two Livramento sites no significant difference in soil pH was found for the 0-5 cm depth (table 4.5). In the 90-100 cm depth the soil pH was also found to be significantly different ( $p < 0.001$ ) for the different sites. The two Livramento sites both had a significantly higher pH than both of the Gurupi sites (table 4.5). Again, no significant difference was found between the two Gurupi sites and between the two Livramento sites (table 4.5).

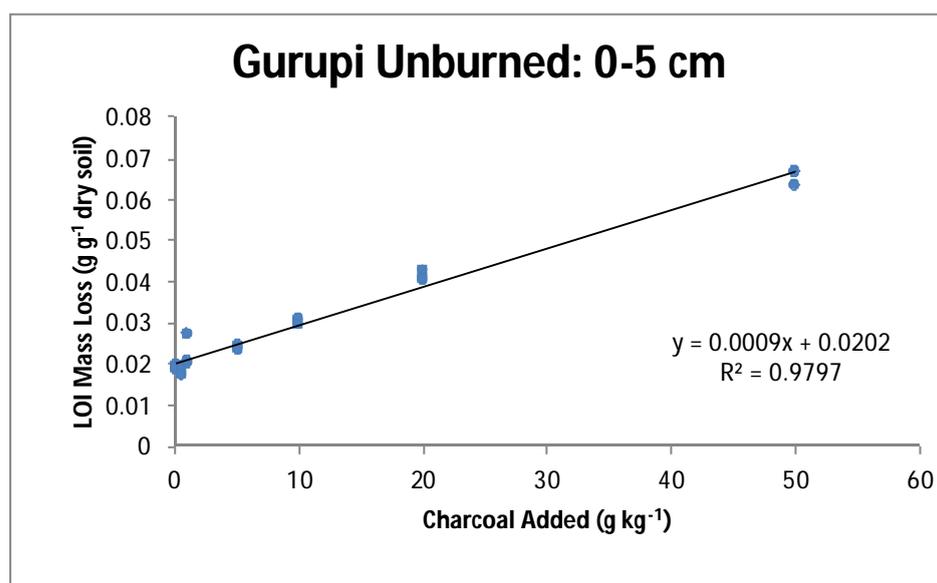
**Table 4.5 Soil pH results for the four sampling sites and different depths.**

Site	Soil pH ( $\pm$ SD)	
	0-5 cm	90-100 cm
GU	4.24 $\pm$ 0.27A	3.95 $\pm$ 0.07a
GB	4.70 $\pm$ 0.42*AB	3.87 $\pm$ 0.02*a
LU	5.42 $\pm$ 0.06** C	4.48 $\pm$ 0.04**b
LB	5.16 $\pm$ 0.10** BC	4.48 $\pm$ 0.08**b

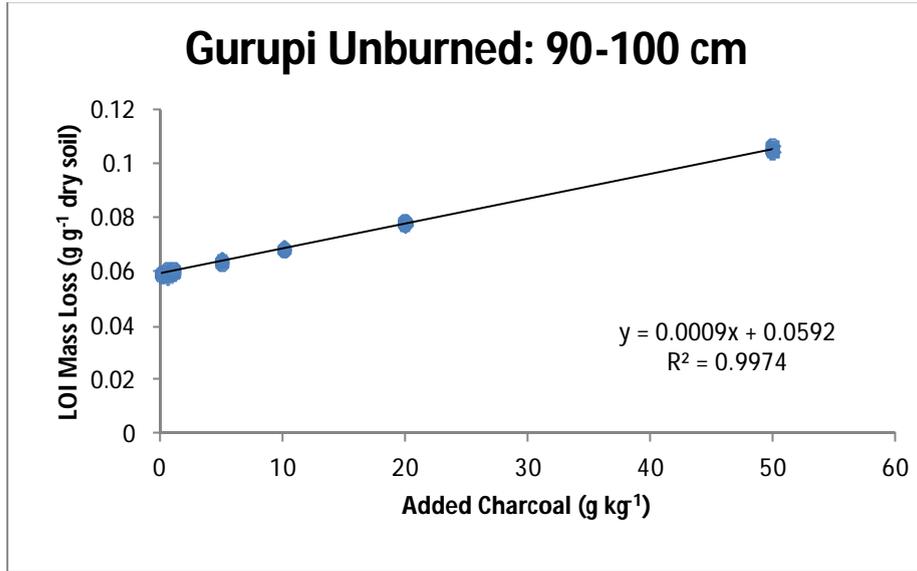
\* indicates that there was a significant difference ( $p < 0.05$ , 2 sample t-test) in soil pH between the 0-5cm and 90-100cm soil depths in the same soil profile. \*\* indicates that there was a highly significant difference ( $p < 0.001$ , 2 sample t-test) in soil pH between the 0-5cm and 90-100cm soil depths in the same soil profile. Different uppercase letters indicate significantly different ( $p < 0.05$ , Tukey's test) soil pH levels in the 0-5cm soil depth at the different sites. Different lowercase letters indicate significantly different ( $p < 0.05$ , Tukey's test) soil pH levels in the 90-100cm soil depth between the different sites.

#### **4.6 BC Recovery From Hydrogen Peroxide Oxidation**

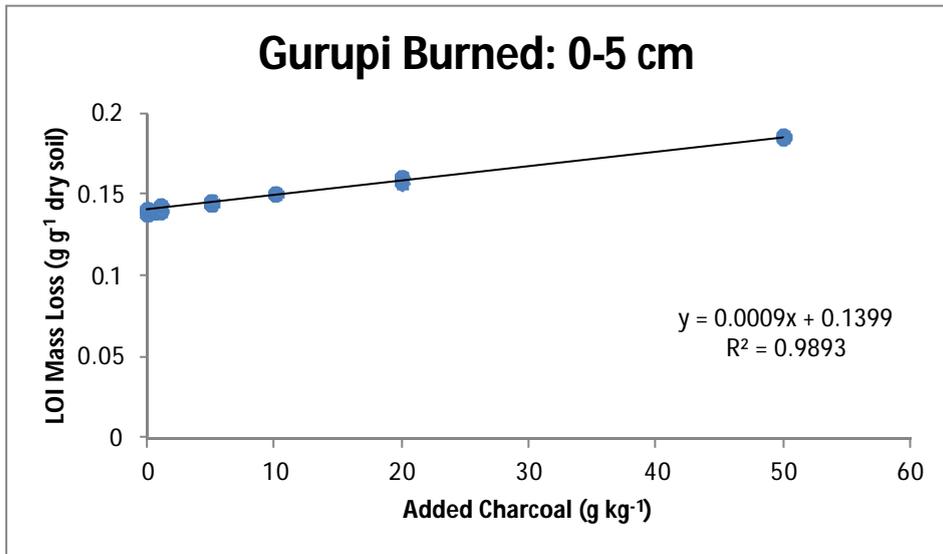
For all sites and soil depths the goodness of fit of the regression equations for the relationships between charcoal added and LOI mass loss for the digested soils (shown in graphs 4.6.1 to 4.6.8) was very high and all relationships were found to be highly significant ( $p < 0.001$ ).



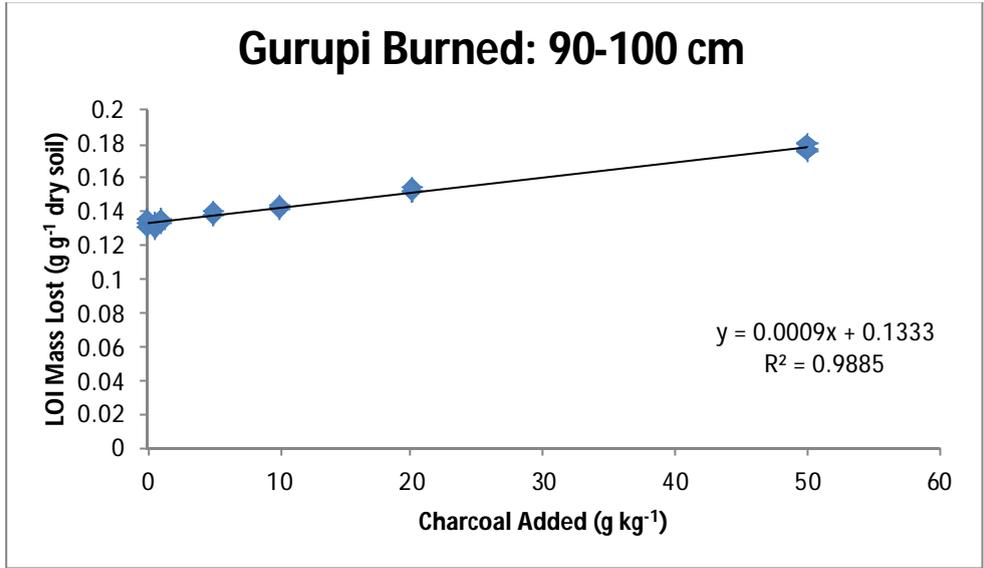
**Graph 4.6.1 LOI mass loss plotted against charcoal amendments for the Gurupi unburned site in the 0-5 cm depth. The regression line and equation are shown on the graph.**



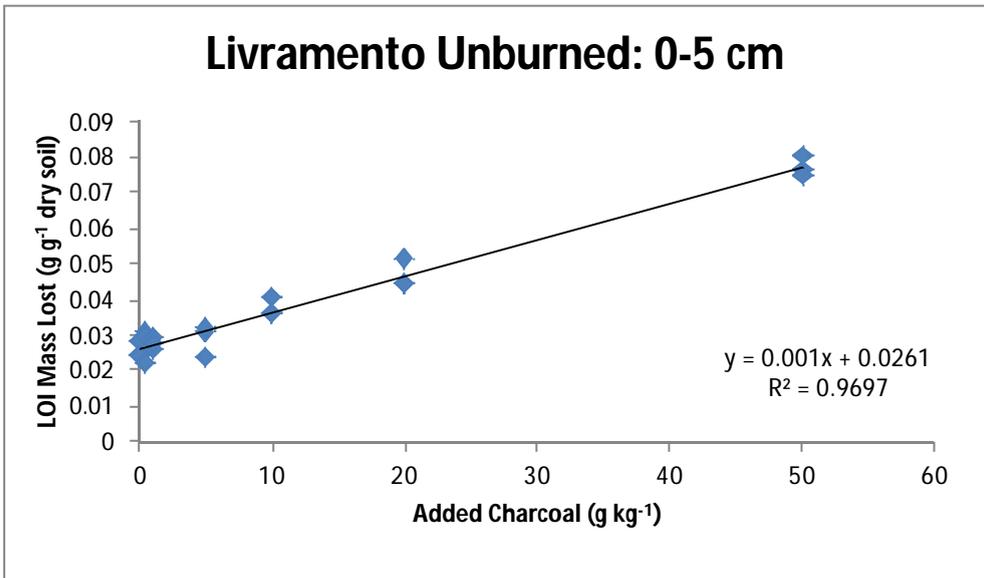
Graph 4.6.2 LOI mass loss plotted against charcoal amendments for the Gurupi unburned site in the 90-100 cm depth. The regression line and equation are shown on the graph.



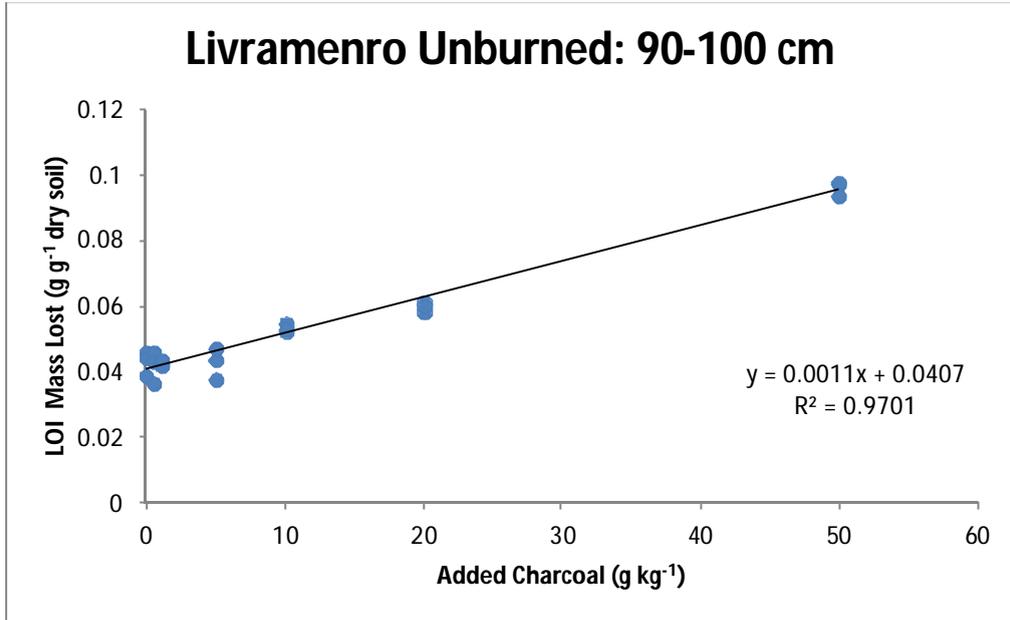
Graph 4.6.3 LOI mass loss plotted against charcoal amendments for the Gurupi burned site in the 0-5 cm depth. The regression line and equation are shown on the graph.



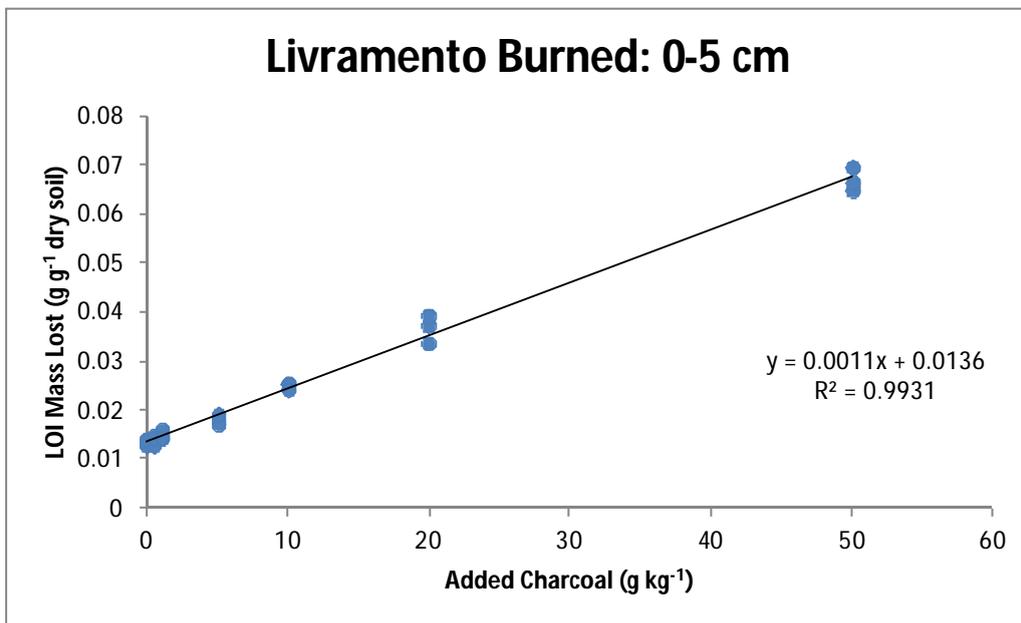
Graph 4.6.4 LOI mass loss plotted against charcoal amendments for the Gurupi burned site in the 90-100 cm depth. The regression line and equation are shown on the graph.



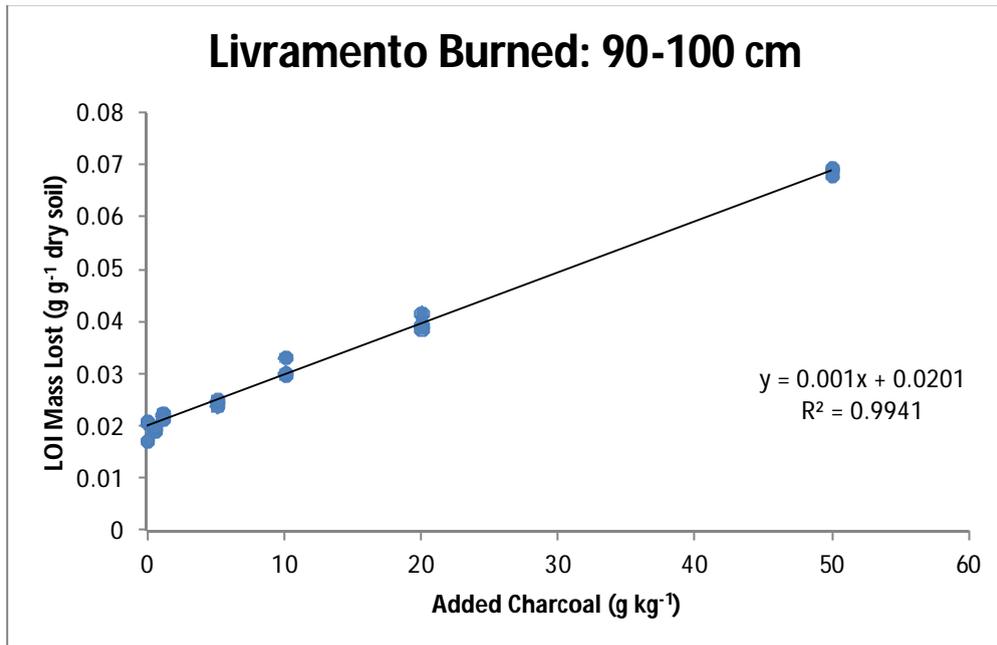
Graph 4.6.5 LOI mass loss plotted against charcoal amendments for the Livramento unburned site in the 0-5 cm depth. The regression line and equation are shown on the graph.



Graph 4.6.6 LOI mass loss plotted against charcoal amendments for the Livramento unburned site in the 90-100 cm depth. The regression line and equation are shown on the graph.



Graph 4.6.7 LOI mass loss plotted against charcoal amendments for the Livramento burned site in the 0-5 cm depth. The regression line and equation are shown on the graph.



**Graph 4.6.8 LOI mass loss plotted against charcoal amendments for the Livramento burned site in the 90-100 cm depth. The regression line and equation are shown on the graph.**

In order to establish whether a highly significant relationship between the concentration of charcoal added to and the LOI mass loss from the soil was consistent across the full range of BC concentrations, further regression analysis was carried out for the lower charcoal concentrations (ranging from 0-5 g kg<sup>-1</sup>) exclusively and the higher charcoal concentrations (ranging from 10-50 g kg<sup>-1</sup>) exclusively.

The results from the regression analysis of the higher concentrations of charcoal showed a strong relationship between the concentration of charcoal added and the concentration of charcoal recovered after digestion. Across all sample sites and soil depths the Rsq. values were high and all relationships were found to be highly significant (table 4.6). The Rsq. values for the lower charcoal concentrations were in general not as high as for the higher charcoal concentrations and for the Livramento unburned site no significant relationship was found between the concentration of charcoal added and the concentration of charcoal measured for both the 0-5cm and 90-100cm soil depths (table 4.6).

**Table 4.6. Rsq. values for the regression equations of the relationship between charcoal concentration added and LOI mass loss for low and high concentrations of charcoal amendment.**

Rsq. Values From Regression Analysis Of Charcoal Concentration Added and LOI Mass Loss For Low and High Charcoal Concentration Amendments				
Site	0-5 cm		90-100 cm	
	0-5 g kg <sup>-1</sup>	10-50 g kg <sup>-1</sup>	0-5 g kg <sup>-1</sup>	10-50 g kg <sup>-1</sup>
GU	0.4057*	0.9899**	0.8664**	0.9969**
GB	0.6956**	0.9898**	0.7039**	0.9923**
LU	0.0865	0.9758**	0.0018	0.9855**
LB	0.8452**	0.989**	0.7234**	0.9946**

\* indicates a significant relationship ( $p=0.05$ ) and \*\* indicates a highly significant relationship ( $p=0.001$ ) between the concentration of charcoal added and the LOI mass loss after digestion.

#### **4.7 LOI Mass Loss**

The mass of soil lost during LOI from the digested soils ranged from 0.0128 to 0.1412 g LOI g<sup>-1</sup> dry soil. In site variability in mass loss was low. LOI mass loss was found to be significantly higher for the soils from the 90-100 cm depth than for the soils from the 0-5 cm depth at all sites (table 4.7.1). For both the 0-5 cm and 90-100 cm depths, sites were found to have significantly different mass losses ( $p<0.001$ ) during LOI. The Gurupi unburned site had a significantly higher mass loss than the other three sites in both the 0-5 cm and 90-100 cm depths, whilst the Livramento burned site had significantly lower mass losses than the other three sites in both the 0-5 cm and 90-100 cm depths (table 4.7.1).

**Table 4.7.1 LOI mass losses for digested soils for the four different sites and different depths.**

Site	Digested Soil LOI Mass Loss (g LOI g <sup>-1</sup> dry soil)	
	0-5cm	90-100cm
GU	0.0194±0.0006A**	0.0591±0.0005a**
GB	0.1396±0.0018B*	0.1332±0.0019b*
LU	0.0255±0.0024C*	0.0430±0.0036c*
LB	0.0134±0.0005D*	0.0197±0.0021d*

\* indicates that there was a significant difference ( $p<0.05$ , 2 sample t-test) and \*\* indicates that there was a highly significant difference ( $p<0.001$ , 2 sample t-test) in mass loss between the 0-5 cm and 90-100 cm depths within the same soil profile. Different uppercase letters indicate a significant difference ( $p<0.05$ , Tukey's Test) in mass loss between sites in the 0-5 cm depth. Different lowercase letters indicate that there was a significant difference ( $p<0.05$ , Tukey's Test) in mass loss between sites in the 90-100 cm depth.

LOI mass losses for the undigested soils ranged from 0.0264 to 0.2028 g LOI g<sup>-1</sup> dry soil. In site variability was again low. With the exception of the Gurupi unburned site, mass losses during LOI for the undigested soils, were found to be significantly higher for the 0-5 cm soil depth than for the 90-100 cm soil depth (table 4.7.2). For the Gurupi unburned site mass loss during LOI was significantly lower for the 0-5 cm depth (table 4.7.2). Between sites, the mass loss for both the 0-5 cm and 90-100 cm depths was found to be significantly different ( $p < 0.001$ ). The Gurupi burned site was found to have significantly higher mass losses than the other three sites in both the 0-5 cm and 90-100 cm soil depth (table 4.7.2). The Gurupi unburned site and the Livramento burned site were found to have significantly lower mass losses in the 0-5 cm soil depth than the other two sites. In the 90-100 cm soil depth, the Livramento burned site was found to have significantly lower mass losses than the other three sites.

**Table 4.7.2. LOI mass losses for undigested soils for the four different sites and different depths.**

Site	Undigested Soil LOI Mass Loss (g LOI g <sup>-1</sup> dry soil)	
	0-5 cm	90-100 cm
GU	0.0608±0.0003A**	0.0643±0.0002a**
GB	0.2016±0.0026B**	0.1359±0.0020b**
LU	0.0916±0.0029C**	0.0509±0.0043c**
LB	0.0662±0.0016A**	0.0276±0.0010d**

\* indicates that there was a significant difference ( $p < 0.05$ , 2 sample t-test) and \*\* indicates that there was a highly significant difference ( $p < 0.001$ , 2 sample t-test) in mass loss between the 0-5 cm and 90-100 cm depths within the same soil profile. Different uppercase letters indicate a significant difference ( $p < 0.05$ , Tukey's Test) in mass loss between sites in the 0-5 cm depth. Different lowercase letters indicate that there was a significant difference ( $p < 0.05$ , Tukey's Test) in mass loss between sites in the 90-100 cm depth.

#### **4.8 Hydrogen Peroxide Oxidation of Pure Charcoal**

As can be seen from table 4.8, the pure charcoal samples did experience some degree of oxidation during the digestion process. The degree to which a sample experienced oxidation was highly variable (table 4.8). The variability in LOI mass loss for pure charcoal samples was low. During LOI almost all the sample was oxidised.

**Table 4.8 The mass difference before and after digestion of the ten pure charcoal samples and the LOI mass loss of four pure charcoal samples which LOI was carried out on.**

Charcoal Digestion and LOI Results	
Mass Difference Before and After Digestion (% $\pm$ SD)	-5.58 $\pm$ 2.66
LOI Mass Loss (g LOI g <sup>-1</sup> dry charcoal $\pm$ SD)	0.95 $\pm$ 0.06

#### **4.9 Autoanalyser C and BC Concentrations**

The autoanalyser results show total C concentrations to be considerably higher in the 0-5 cm depth than the 90-100 cm depth across all sites (table 4.9). In the 0-5 cm and 90-100 cm depths the Gurupi burned site had the highest total C concentrations of all the sites, whilst the Gurupi unburned site had the lowest total C concentrations (table 4.9). When total C contents were calculated using the bulk density values for each site and depth, the Gurupi burned site had the largest total C content in the 0-5 cm depth, whilst the Gurupi unburned site had the lowest C content. In the 90-100 cm depth, the Livramento unburned site had a slightly higher C content than the Gurupi burned site. The Gurupi unburned site had a considerably lower C content than the other three sites at this depth.

**Table 4.9 Total C and BC concentrations and contents for the four different sites and different depths as measured and calculated using the autoanalyser C concentrations.**

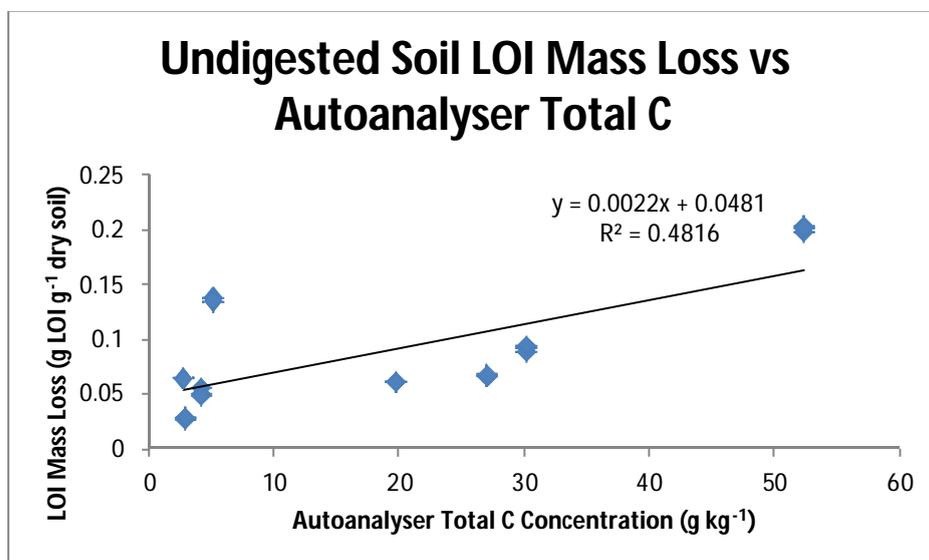
Autoanalyser Total C and Black Concentrations (g kg <sup>-1</sup> ) and Contents (t ha <sup>-1</sup> )										
	0-5 cm					90-100 cm				
	Total C		BC			Total C		BC		
Site	(g kg <sup>-1</sup> )	(t ha <sup>-1</sup> )	(g kg <sup>-1</sup> )	(t ha <sup>-1</sup> )	(% of total C)	(g kg <sup>-1</sup> )	(t ha <sup>-1</sup> )	(g kg <sup>-1</sup> )	(t ha <sup>-1</sup> )	(% of total C)
GU	19.70	12.42	2.73	1.72	13.84	2.73	1.91	1.03	1.44	37.82
GB	52.37	19.16	3.41	1.25	6.52	5.02	2.85	1.44	1.63	28.62
LU	30.08	16.29	3.01	1.63	10.01	4.18	2.86	2.01	2.74	47.98
LB	27.06	14.68	7.23	3.92	26.71	2.95	2.47	1.90	3.18	64.40

**Standard deviations are not presented because values presented are from only one repetition.**

The BC concentrations as measured by the autoanalyser were also much higher in the soil from the 0-5 cm depth than from the 90-100 cm depth. Of all the sites the Livramento burned site had the highest BC concentration in the 0-5 cm depth, but the lowest BC concentration in the 90-100 cm depth. The lowest BC concentration in the 0-5 cm depth was found at the Gurupi unburned site. The highest BC concentration for the 90-100 cm depth was found at the Livramento unburned site. For both the 0-5 cm and 90-100 cm depths, the Livramento burned site had the highest BC contents. The Gurupi burned site had the lowest BC contents in the 0-5cm depth, whilst the Gurupi unburned site had the lowest BC content in the 90-100 cm depth.

#### **4.10 Autoanalyser and LOI Relationships**

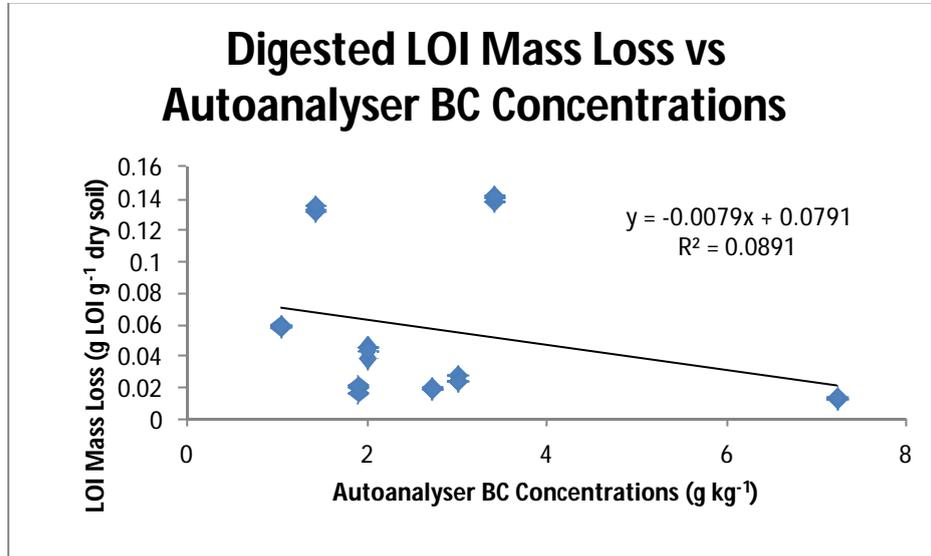
When LOI mass losses for the undigested, non-amended soils were plotted against the corresponding total C concentrations obtained from the autoanalyser, it could be seen that as the total C concentration of the soil increased, the LOI mass loss increased (graph 4.10.1). Regression analysis showed that although a lot of the variance in LOI mass loss was not explained by the total C content ( $R^2 = 0.48$ ), the relationship between the two variables was highly significant ( $p < 0.001$ ).



**Graph 4.10.1 LOI mass loss plotted against the corresponding autoanalyser total C concentrations for the undigested and non-amended soils. The regression line and equation are shown on the graph.**

When LOI mass losses for the digested, non-amended soils were plotted against the corresponding BC concentrations obtained from the autoanalyser, no clear relationship could be observed (graph 4.10.2).

BC concentrations did not explain a lot of the variance in LOI mass losses ( $R^2=0.09$ ) and no significant relationship was found between the two variables ( $p=0.157$ ).



Graph 4.10.2 LOI mass loss plotted against the corresponding autoanalyser total C concentrations for the digested and non-amended soils. The regression line and equation are shown on the graph.

## 5.0 Discussion

### 5.1 Hydrogen Peroxide- Measuring BC

In the study by Kurth *et al.* (2006), they state in their methodology that samples were heated to 100°C for 16 hours during H<sub>2</sub>O<sub>2</sub> oxidation. In this present study, it was found that lower temperatures were usually required, as in the initial stages of the oxidation the reaction between the reagents and organic matter was particularly vigorous, with some samples even having to be removed from the hot plate for a while. Even at a lower temperature and having been removed from the hot plate, samples often boiled over. It was observable that when this happened some of the light fraction of the soil was lost from the flask. It is likely that the majority of the soil that was lost from the flasks was labile organic matter, which should have been oxidised anyway. However, it is also possible, that smaller, less dense BC particles are also lost in this way, as their hydrophobic nature will mean that they have a tendency to accumulate at the air-water interface (Cornellissen *et al.*, 2005). However, the significant relationship between charcoal added to the soil and LOI mass loss for the digested soil and the low variation in results between replicate samples that will be discussed below, suggests that if part of the BC present in the soil

is lost in this way, it is not a considerable amount. Other studies which have used H<sub>2</sub>O<sub>2</sub> oxidation to remove the OM fraction, report heating of the sample to below 100°C (e.g. Wu *et al.*, 1999 and authors cited within Mikuta *et al.*, 2005). Therefore, if oxidation of organic matter is not impeded, the use of lower temperatures may be preferable, at least initially when the reaction is most vigorous, to avoid any potential losses of BC.

The significant relationship found between the concentration of charcoal added to the soil and the LOI mass loss for the digested soils (as charcoal concentrations increased LOI mass loss increased) indicates that the H<sub>2</sub>O<sub>2</sub> oxidation method was successfully able to recover the BC added to these soils. The high R.sq. values indicate that the method also provides consistent results. The slope values, which are reasonably close to zero and do not vary considerably between the sites, also indicate that the method was successful and consistent across all the sites. However, when the relationship between the concentration of charcoal added and the LOI mass loss for the digested soils was looked at separately for the low and high levels of charcoal concentration it could be seen that the goodness of fit between the regression equation and the measured values was not as strong for the lower charcoal concentrations as it was for the higher concentrations and for the Livramento unburned site, for both the 0-5cm soil depth and the 90-100cm soil depth, the relationship was found to be insignificant for the lower concentrations of charcoal. This suggests that the method performs best for higher concentrations of BC in the soil and is less accurate for quantifying lower levels of BC in the soil. These findings contrast with those of Kurth *et al.* (2006) who found the method to perform well across all BC concentration levels including the lower concentrations. Kurth *et al.* (2006) carried out the H<sub>2</sub>O<sub>2</sub> oxidation on soils described as being loams and silty clay loams. The soils in this study covered a range of textures. It could therefore be that the ability of the hydrogen peroxide oxidation method to accurately quantify low levels of BC in soils is not consistent across all soil types. However, at the Livramento unburned site, where no significant relationship was found between the lower levels of charcoal concentrations added and LOI mass losses, the soil was also found to have a loamy texture. Therefore, factors other than texture may also influence the method's ability to recover varying levels of BC. Kurth *et al.* (2006) use a C autoanalyser to measure the BC. It therefore may be the case that errors attributed to LOI may be responsible for the poor relationship between charcoal added and BC recovered at the low charcoal amendment levels.

## **5.2 Amendment With Charcoal**

Many methodological studies (e.g. De La Rosa Arranz *et al.*, 2008, Hammes *et al.*, 2007) into the quantification of BC use a variety of charred or pyrolysed materials ranging from charcoal to soot in

order to assess how well their selected method performs across the varying levels of recalcitrance of the BC continuum, with charcoal usually being used to represent the less recalcitrant end of the BC spectrum (Hammes *et al.*, 2007). Therefore the use of charcoal in this study to represent BC is one which is accepted in the literature and is common practice. The charcoal purchased for use in this study has the benefit of being derived from reasonably local, natural conditions. One disadvantage of this however is that the conditions of the fire event, such as temperature and oxygen availability are unknown. Many studies (e.g. Hammes *et al.*, 2007) use charred material created in laboratories so that burn conditions can be known. However, in reality when quantifying BC in soils, the burn conditions are rarely known, especially when quantifying BC that has resided in the soil for a long period of time.

The assumption with the hydrogen peroxide with weak nitric acid oxidation is that the C which survives the oxidation is BC and the C oxidised is the labile C fraction. The difference in mass before and after digestion for the pure charcoal samples is of interest, because it either challenges the assumption that the amendment of soils with X% of charcoal is equitable to the amendment of soils with X% of BC or the assumption that all BC survives the digestion process. Probably neither of these assumptions is entirely correct. Within its structure charcoal is known to contain non-charred material derived from the original plant material (Hammes *et al.*, 2007, Han *et al.*, 2007, Knicker *et al.*, 2007, Masiello, 2004). Equally and in keeping with the idea of the BC continuum, charcoal will contain material with varying degrees of charring, with some material having undergone considerable chemical changes during burning, whilst other material will still retain a considerable part of its original plant derived chemical structure (Knicker *et al.*, 2007, Masiello, 2004) and therefore may not survive the H<sub>2</sub>O<sub>2</sub> oxidation. If the first assumption is proven incorrect, then this requires a correction to be made to the recovered BC values from the digestion in order to avoid the underestimation of *in situ* BC. If the proportion of charcoal oxidized is constant or only slightly variable across all samples, then a high goodness of fit for the relationship between the concentration of charcoal added and the concentration of BC recovered would still be achievable and if BC were already present in the soils any loss of charcoal during digestion could go undetected, unless pure charcoal samples are included in the digestions. If the second assumption is shown to be incorrect, that some charred material is oxidized during H<sub>2</sub>O<sub>2</sub> oxidation, then the hydrogen peroxide oxidation is subject to the same problem that many of the BC quantification methods face - that only part of the BC continuum is measured.

It is also possible that variations in the proportion of charcoal oxidized may be contributing to the poorer relationship between charcoal added and BC recovered at the low charcoal amendment levels.

### **5.3 BC and Total C Quantities As Estimated By The C Autoanalyser**

As the autoanalyser BC and total C values are derived from only one repetition, no conclusive comments can be made on the true total C and BC contents of these soils until the results are obtained from the other samples awaiting analysis via an autoanalyser. However, the autoanalyser values do give an indication of the true total C and BC contents of the soil.

The results showed all sites to contain BC in both the 0-5 cm depths and the 90-100 cm depths. It would be expected that the burned sites would contain higher concentrations of BC in the soil than the non-burned sites. At the Livramento sites it can be seen that the burned site does contain noticeably higher levels of BC than the unburned site in the 0-5 cm depth. At the Gurupi sites however, the difference between the BC concentrations at the two sites is not that considerable. This does however fit with the findings of other studies, such as Zachrisson *et al.* (1996) and Knicker *et al.* (2006), where no observable relationship between fire history and BC contents in the soil was found. Kurth *et al.* (2006) also found no difference between the BC content of soils that had experienced regular burning and that of soils that had not experienced burning in over 100 years. The explanation put forward by Knicker *et al.* (2006) and considered by Kurth *et al.* (2006) to explain these findings is that any BC present in the soil is consumed by subsequent fires. Therefore a site with a more frequent occurrence of fire or a more recent fire history than another site may not actually have a higher BC content, as the accumulation of BC is inhibited. This may be particularly true in cases where the fire is particularly intense resulting in a higher consumption rate of the BC already present in the soil and a reduction in the production rate of charred material, as the proportion of organic matter that is volatilised increases (Knicker *et al.*, 2006).

When looking at BC as a percentage of total C it was observed that the BC fraction of the C pool is much higher in the 90-100 cm soil depth than in the 0-5 cm depth at all sites. As table 4.8 shows, this can be attributed to a lower labile C content, rather than a higher overall BC concentration at the 90-100 cm depth. Despite this, the BC concentrations in the 90-100 cm depth are not substantially lower than the BC concentrations in the 0-5 cm depth. The rate and the mechanisms which result in BC being lost from the soil are still under debate. However, if the values from the autoanalyser are correct then it is likely that BC is subjected to translocation within these soils or alternatively the BC has arrived at this depth from an earlier depositional event. However, without knowing the age of the BC found at the 90-100 cm depth it would be hard to estimate the rate at which this BC had been transported to this depth or if it is derived from an earlier depositional event. When considering the two Livramento sites, if translocation of the most recently formed BC at the burned site had already occurred, it would be expected that the

pattern of a higher BC concentration in the 0-5 cm depth at the burned site would also be reflected in the 90-100 cm depth. However, the BC concentration at this depth is actually higher in the unburned site, suggesting that the most recently produced BC has not been translocated to this depth.

Alternatively, it might be the case that at the Livramento burned site, the influence of ground water may result in a higher erosion rate of BC, inhibiting the BC enrichment of soil at this depth.

The overall BC content of the soils ( $\text{t ha}^{-1}$ ) was generally higher in the 90-100 cm depth (the exception being the Gurupi unburned site). This is a result of the higher bulk densities in the 90-100 cm depth than in the 0-5 cm depth. In the case of the Gurupi sites, the higher bulk density of the Gurupi unburned site led to an overall higher BC content than at the burned site in the 0-5 cm depth.

Referring back to table 1.2 in section 1.2 it can be seen that the BC concentrations of this study fall into the range of those presented by Schmidt *et al.* (1999). The BC as a percentage of total C found in this study is generally much higher than those reported by other authors and much higher than the global estimates produced by González-Pérez *et al.* (2004). However, in general, it is hard to make comparisons for the BC quantities of this study with the BC quantities of other studies. This is because of the way in which BC quantities are reported, as an absolute BC concentration ( $\text{g kg}^{-1}$ ) or as a relative quantity (% of total C) (an issue also raised by Forbes *et al.* (2006)). A further problem is that in each study the methods used to quantify BC vary and consequently the definition of what constitutes BC varies. This problem is one which has been raised many times by numerous authors (e.g. Masiello, 2004, De La Rosa Arranz *et al.*, 2008, Hammes *et al.*, 2007, Forbes *et al.*, 2006).

The sampling depth of 0-5 cm used in this study makes comparisons with BC contents reported by other studies harder, as most other studies have sampled to a depth of 0-10 cm or 0-20 cm. Therefore the BC contents of this study have to be extrapolated over a greater depth than was sampled, which has to make the assumption that the BC contents in the 0-5 cm depth are similar to the BC contents of the 5-10 cm or 5-20 cm depth, which may not be valid. However, when extrapolated to the depths of those used by the studies in table 1.1 section 1.1 then the total C contents of the soils in this study as measured by autoanalyser fall within the range of total C contents as reported by Cerri *et al.* (2007) for the 0-10 cm depth Amazon regions. When the 90-100 cm total C contents of the soil are extrapolated over the depth reported by Silver *et al.* (2000) it can be seen that the total C contents of this study are at the lower end of ranges reported in their study.

It has been proposed that the presence of BC in soils increases the overall C content of a soil not just through its high C concentrations and low degradation rates, but also through the adsorption of SOM onto the surfaces of the BC particles (Liang *et al.*, 2006, Glaser *et al.*, 2002). When looking at the Gurupi sites it can be seen that the higher total C concentrations are found at the Gurupi burned site where the higher BC concentrations also occur. However, at the Livramento burned site total C concentrations are lower than at the Livramento unburned site, despite BC concentrations being much higher. It may also have been expected that the anaerobic conditions at the Livramento burned site would inhibit OM decomposition resulting in a higher total C concentration. However, as was shown from the soil textural analysis, the Livramento burned site had a higher sand content than at the Livramento unburned site. Therefore the stabilisation of SOM that occurs at this site is probably lower than that which occurs at the Livramento unburned site as a result of the higher sand content (Silver *et al.*, 2000).

#### **5.4 Overestimation of C by LOI**

The large mass losses that occurred during LOI on both the digested soils and undigested soils, led to the suspicion that a considerable part of the mass loss was not attributable to C oxidation, especially since tropical soils are often very low in C content and should be even more so after digestion with  $H_2O_2$ . The results from the autoanalyser helped to confirm this suspicion, by showing the soils to contain low total C and BC concentrations. The significant positive relationship found between LOI mass loss and the total C concentration measured by the autoanalyser shows, however, that the oxidation of C does influence the mass loss during LOI. However, as this relationship did not explain a lot of the variance in mass loss between the four sites and different depths, this suggests that factors other than carbon are also influencing the mass loss during LOI. The fact that no significant relationship was found between the mass loss during LOI and the BC concentration as measured by the autoanalyser also support this idea by implying that when C concentrations are low, the signal from the other factors becomes more prominent, obscuring any patterns in mass loss that may be attributable to C loss.

If the difference between LOI mass loss ( $g\ H_2O\ g^{-1}$  dry soil) and C or BC concentrations ( $g\ C/BC\ g^{-1}$  dry soil) measured by the autoanalyser is taken to represent mass loss attributable to sources other than C, then it can be seen that the Gurupi burned site has the largest mass loss not attributable to C and that Livramento burned has the least mass loss not attributable to C. The soil textural analysis showed the soil at the Gurupi burned site to have the highest clay content out of the four sites, whilst the soil at the Livramento burned site was shown to have the lowest clay content. Numerous studies have shown the clay content of soils to have a strong impact on the mass loss of soil during LOI (Sun *et al.*, 2009, Sleutel

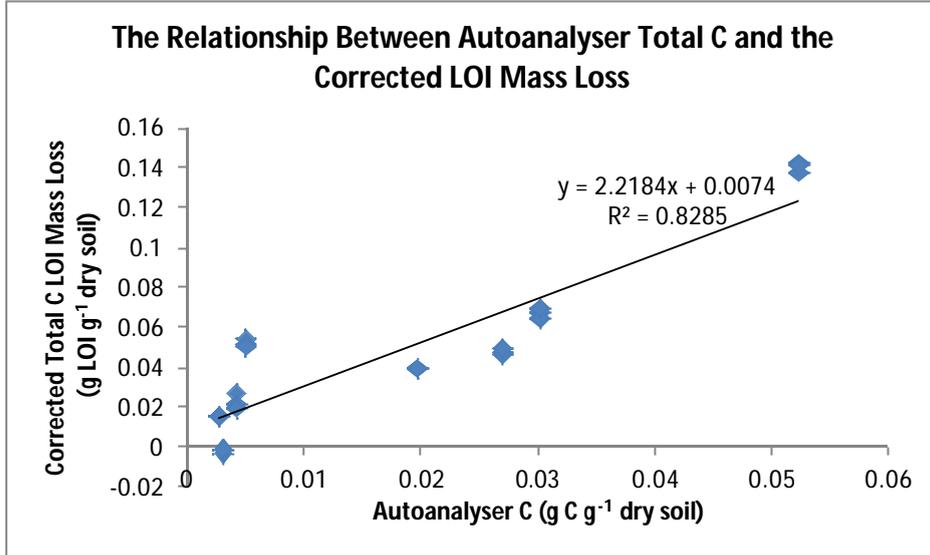
*et al.*, 2007, De Vos *et al.*, 2005, Santisteban *et al.*, 2004, Barillé-Boyer *et al.*, 2003). Clay minerals contain water within their crystal lattices, this is termed structural water. When heated to a high temperature, the crystal structure of clay minerals can breakdown, releasing this structural water and thus leading to a mass loss (Barillé-Boyer *et al.*, 2003). Therefore, the fact that the largest LOI mass losses occur for the soils which have the highest clay content and vice versa, suggest that the loss of structural water from clay minerals is largely responsible for the unexpectedly high LOI values. Other factors which could possibly contribute to mass loss during LOI include the loss of non-organic C (Barillé-Boyer *et al.*, 2003) and also the dehydration of metal oxides (Christensen and Malmräs, 1982, Lechler and Desilets, 1987, Santisteban, 2004). A high soil pH can be used as an indicator of the presence of carbonates in the soil and vice versa (Rumpel *et al.*, 2006a, Santi *et al.*, 2006). As the soils in this study were all found to have a pH below 6 the presence of carbonates in these soils seems unlikely. Therefore the volatilisation of carbonates can probably be ruled out as a significant source of error during LOI in this study. Dehydration of metal oxides is however a possibility in these soils. The reddish orange colour of the Gurupi soils is an indication of the presence of iron oxides (Schaefer *et al.*, 2008), as is the presence of mottles in the Livramento burned site (Ashman and Puri, 2002).

### **5.5 Correcting For Structural Water Loss From Clays.**

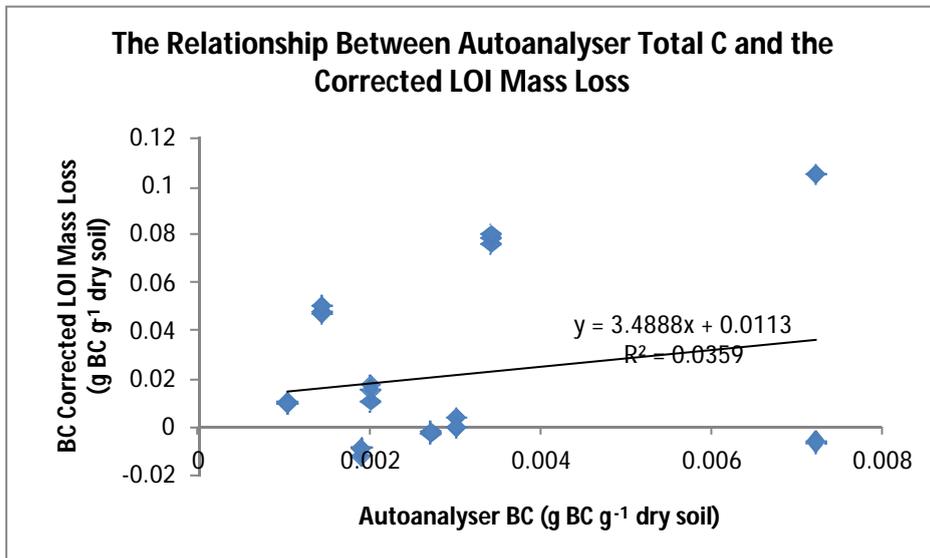
As kaolinite is the most dominant clay mineral in Brazilian soils (Schaefer *et al.*, 2008) it is therefore likely to be the most common clay mineral in the soils in this study. Structural water loss from the clay mineral kaolinite is also thought to occur around 350-600°C (Sleutel *et al.*, 2007). As LOI was carried out at 850°C it might be reasonable to assume that almost 100% of the clay structural water in the soil was volatilised during LOI. It would therefore be possible to calculate the theoretical potential structural water loss from the soil if the clay structural water content of the soil was known.

Using the data from the soil textural analysis to calculate the soil clay content and using a value of 13.96% of structural water for kaolinite (taken from a paper by Sun *et al.*, 2009) the theoretical structural water loss was calculated for each soil. These theoretical values were then be used to calculate the LOI mass loss attributable to the loss of C, by subtracting the theoretical structural water loss from the total LOI mass loss. In theory the C mass loss ( $\text{g C g}^{-1}$  dry soil) should be close to the C concentration ( $\text{g C g}^{-1}$  dry soil) measured by the autoanalyser. Slight divergences in the values would be expected, as the theoretical structural water loss did not include losses from iron oxides, and the assumption that the clay mineralogy of the soil is composed entirely of kaolinite is also likely to result in some degree of error. However, as can be seen from graphs 5.5.1 and 5.5.2, this led in some cases to a

negative C mass loss, particularly for the digested samples, showing that the theoretical structural water loss was too high.

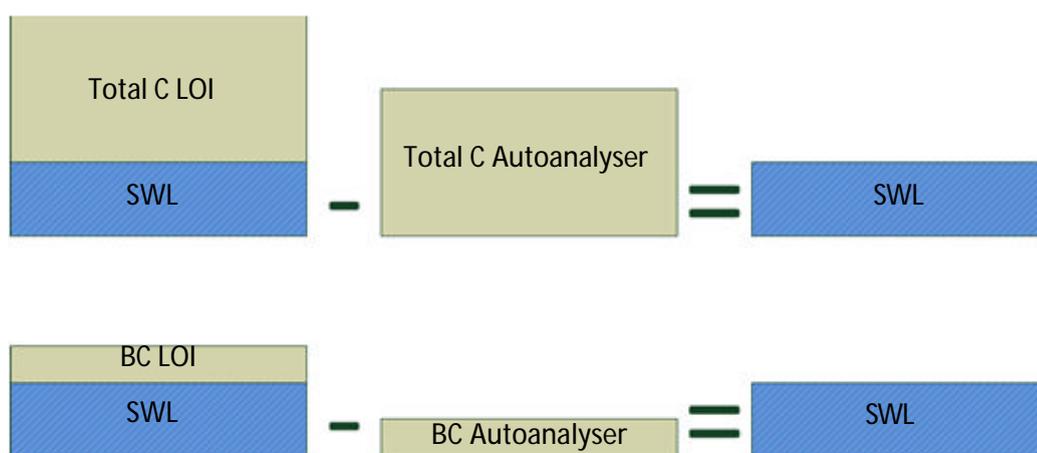


Graph 5.5.1. The relationship between total C content as measured by the autoanalyser and total C LOI mass loss after correction for the theoretical structural water loss.

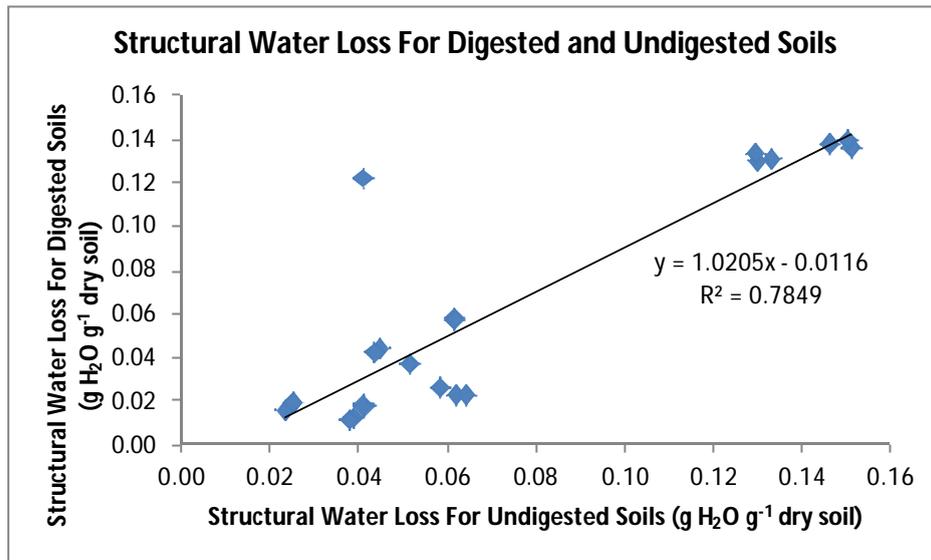


Graph 5.5.2. The relationship between BC content as measured by the autoanalyser and BC LOI mass loss after correction for the theoretical structural water loss.

The degree to which structural water loss took place should in theory be the same for the corresponding digested and undigested soils. Therefore the equation shown in figure 5.5.1 should be true. However, as graph 5.5.3 shows structural water loss for the undigested soils was higher than the structural water loss for the digested soils. This could be explained by a variety of reasons. Firstly, some studies have reported the structural alteration of clay minerals and the dissolution of metal oxides during oxidation with  $H_2O_2$  (Mikuta *et al.*, 2005). If this is the case, it may be that some of the structural water loss or other volatile components may be lost during the digestion process, which would then result in a lower proportion being lost during LOI. Alternatively it could be the result of differences in the drying procedure for the digested and undigested soils before LOI was carried out. As samples were wet from the digestion, digested samples were dried at 105°C for 48 hours in filter papers before LOI, whilst undigested samples were dried for only 24 hours (as these were already at an air dried stage) at 105°C in ceramic crucibles. It has been found that dehydration of some metal oxides can occur at temperatures as low as 105°C (Sleutel *et al.*, 2007). The better aeration facilitated by the filter papers compared to the ceramic crucibles and the longer drying period for the digested samples may have meant that if any structural water loss was occurring at this stage, it may have been higher for the undigested samples.



**Figure 5.5.1 Theoretical structural water equation. In theory structural water loss (SWL), measured in  $g H_2O g^{-1}$  dry soil, should be able to be calculated by the above equations and should be the same for undigested and digested soils.**



**Graph 5.5.3. The relationship between structural water loss for corresponding digested and undigested soils. Structural water loss was taken to be the difference between LOI mass loss and the C concentration measured by the autoanalyser for the digested and undigested soils.**

In theory it would be possible to calculate the labile C in the soil by subtracting the LOI totalC from the LOI BC. However, if the degree of structural water loss is not the same in the digested and undigested samples then this would not be possible.

### **5.6 The Use of a Lower Ignition Temperature**

Santisteban *et al.* (2004) state that OM begins to ignite at temperatures around 200°C and is completely ignited at around 550°C. Although some carbonates begin to volatilise at temperatures below 550°C, the majority of carbonates are not volatilised until higher temperatures of 700°C plus are reached (Santisteban *et al.*, 2004). Structural water loss from kaolinite, a common clay mineral in the tropics (Schaefer *et al.*, 2008) and therefore a potentially a common source of error in LOI measurements, is also thought to begin around temperatures of 500-600°C (Sun *et al.*, 2009). For this reason Christensen and Malmros (1982) suggest the use of lower ignition temperatures to avoid the volatilisation of materials other than OM. However, the oxidation of BC compounds occurs at much higher temperatures than more labile organic C compounds. For example, thermal oxidation techniques, such as DSC analysis are based on the assumption that BC oxidation does not occur until temperatures of around 500°C plus have been reached (De La Rosa Arranz *et al.*, 2008, Leifield, 2007). Therefore, since LOI was being used to quantify the BC concentration of the digestion residues and given the high levels of recalcitrance of BC compounds, it is likely that LOI using temperatures much lower than 850°C would fail to oxidise all

the BC present in the soil. Therefore, it is likely that the problem of C content overestimation is unavoidable when using LOI to quantify BC.

### **5.7 LOI Correction Factors and the Suitability of LOI for BC Quantification**

As OM is not composed entirely of C, mass losses attributable to the ignition of OM during LOI are not directly equitable to mass losses of C. Therefore a correction needs to be made to the LOI values to obtain the C content of soils. Traditionally a correction factor of 1.724 has been used (i.e.  $g\ C = g\ OM / 1.724$ ) which assumes that OM contains 58% organic C (Schumacher, 2002). However, the use of this correction factor is controversial, as OM has been shown to contain varying concentrations of organic C (Schumacher, 2002) and some authors even question the work on which this correction factor was based (Pribyl, 2010). It also does not take into account all the factors discussed above that may also impact on LOI mass loss and lead to an overestimation of C. Therefore many authors have used their own, alternative correction factors (e.g. Sleutal *et al.*, 2007, Cresser *et al.*, 2007, De Vos *et al.*, 2005, Christensen and Malmros, 1982), many of which will be specific to a particular site or environment. Consequently selecting or calculating an appropriate correction factor can be difficult. This is further complicated when using LOI to quantify the BC concentration of a soil. Charred organic materials have a higher C concentration than non-charred organic materials (Hammes *et al.*, 2007, Masiello *et al.*, 2004, Hedges *et al.*, 2000). The concentration of C will depend on the degree of charring and the original C concentration of the OM that the char is derived from (Hedges *et al.*, 2000). Fearnside *et al.*, (2001) use an average C concentration of 74.8% in char derived from primary forest in the Manuas region of Brazil based on C concentrations of manufactured charcoal in that area, whilst Lehman *et al.*, 2002 report the charcoal used in their study to have a C concentration of 70.8%. Therefore the traditional correction factor or any other correction factor based on C concentrations for non-charred OM is not likely to be suitable for the quantification of BC. However, C concentrations of charcoal could be used to calculate a new correction factor which could be applied when quantifying isolated BC through the use of LOI. In soils where BC had not been isolated two correction factors would need to be applied: one for the more concentrated, recalcitrant C and one for the less concentrated, more labile C. This, however, would first require the proportion of BC and the proportion of labile C in the soil to be known already.

It has been found that the degree of error associated with LOI is higher for soils with low a OM content, than for soils with a high OM content (Sun *et al.*, 2009, Santisteban *et al.*, 2004). Studies have reported particular problems with errors induced by structural water loss from clays in soils where the SOM has been below 6% (Sun *et al.*, 2009). The results from the C autoanalyser showed the soils in this study to

have below 6% OM, which is in the range of concentrations reported by other studies on similar soils (e.g. McGrath *et al.*, 2001, Silver *et al.*, 2000). The results from the C autoanalyser also showed all the digested soils to have *in situ* BC concentrations below 1%. Therefore it may be the case that, as a result of the errors associated with LOI when OM levels are low and all the problems of applying a suitable correction factor, LOI is not a suitable method to quantify organic C in tropical soils and even less suited for measuring BC in soils.

## **6.0 Conclusions**

The main aim of this study was to assess the use of H<sub>2</sub>O<sub>2</sub> oxidation as a method to quantify BC in Amazonian soils. In accordance with this H<sub>2</sub>O<sub>2</sub> oxidation followed by LOI was carried out at the laboratories of the Universidade Estadual do Maranhão, São Luís to quantify the BC contents of soils from around Maranhão state. LOI mass losses were found to be very high across all samples. It was expected that these soils would be very low in soil C. Therefore it seemed highly unlikely that this mass loss was attributable to C oxidisation. The preliminary C autoanalyser results helped to confirm that the mass loss was largely not attributable to C losses. The fact that the greatest mass losses corresponded with the highest clay contents, and vice versa, suggested that the excessive mass loss could largely be attributed to the loss of structural water from clays. Attempts to correct for structural water loss using the data available were unsuccessful. A further problem with LOI was the selection of a suitable correction factor to convert g LOI g<sup>-1</sup> dry soil into g C g<sup>-1</sup> dry soil. As BC has higher C concentrations than more labile C compounds, correction factors calculated using C concentrations of labile OM are not likely to be suitable when quantifying BC. The results from this study suggest that LOI is not a suitable method to quantify BC following H<sub>2</sub>O<sub>2</sub> oxidation and that the use of a dry combustion C autoanalyser would be preferable. For laboratories, such as the laboratories of the Universidade Estadual do Maranhão, São Luís, which do not own a C autoanalyser, this takes away from the economical advantage and the minimal equipment requirements of H<sub>2</sub>O<sub>2</sub> oxidation.

Despite the large LOI mass losses, the good relationship found between the concentrations of charcoal added to the soil and the LOI mass losses for the digested soils suggests that the method successfully recovers BC from soils. In general, however, this relationship was not as strong for the lower levels of charcoal concentrations, as it was for the higher concentrations. Contrary to the findings of Kurth *et al.* (2006), this could imply that the method is not so successful at recovering very low levels of BC in soils.

Alternatively it could be the result of a variation in the extent to which less recalcitrant charcoal is oxidised between samples. Another possibility is that the poorer relationship can be attributed to errors associated with LOI. The impending results from the C autoanalyser should help to ascertain whether the use of LOI is in part responsible.

The fact that the relationship between the charcoal concentrations added to the soil and the LOI mass losses for the digested soils was strong across all the sites and sampling depths suggests that the method works well and is consistent over a range of Amazonian soils that vary in texture and environmental conditions such as local hydrology.

As only one replicate analysis was carried out for each sample, no conclusive comments can be made on the *in situ* BC contents of the soils in this study. However the initial autoanalyser results suggest that the relationship between fire history and BC concentrations in the soil is not an obvious one, in that sites that had experienced burning recently did not necessarily have considerably higher BC concentrations than the sites which had no known fire history. More replicate analyses from the C autoanalyser would help determine whether the differences (or lack of differences) between the BC concentrations of the different sites were significant or not.

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## **9.0 Appendix**

### **9.1 Project Budget and Costing**

**Table 9.1.1 Sources of project funding. Sources of funding and the amount are shown.**

<b>Sources Of Project Funding</b>	
<b>Source</b>	<b>Amount (£)</b>
Newcastle University Expeditions Committee	1720
Tropical Agriculture Association Award Fund	1000
School of Agricultural and Environmental Science	362
<b>Total</b>	<b>3082</b>

**Table 9.1.2 Proposed project costs. Items and there expected costs are shown.**

<b>Proposed Project Costs</b>	
<b>Item</b>	<b>Estimated Cost (£)</b>
Return flights from the U.K. to Brazil	800
Return flights from Sao Paulo to São Luis	200
Accommodation	500
Food	300
Travel Insurance	110
Daily transport to field	100
Acid dichromate analysis	700
Routine soil preparation and characterisation	450

**Table 9.1.3 Actual project costs. Items and their cost are shown.**

<b>Actual Project Costs</b>	
<b>Item</b>	<b>Cost (£)</b>
Flights to Brazil	848.40
Flights to Sao Luis	238.80
Change of Flights	107.23
Transport to and from airports	60
Transport to Field	150
Transport to the laboratories	150
Accommodation	600
Accommodation in field	36.23
Food	500
Insurance	free
Vaccinations	free
Chemicals	250
equipment costs	150
Assistance in lab and field	30
<b>Total</b>	<b>3120.66</b>

## 9.2 Project Contacts

**Table 9.2 Project contacts. The main project contacts are shown along with their contact details and role in the project.**

Project Contacts in the U.K and Brazil			
Name	Institution	Contact Details	Role
Dr Elizabeth Stockdale	School of AFRD, Newcastle University, U.K.	Tel. 0191 222 6915 Email <a href="mailto:e.a.stockdale@ncl.ac.uk">e.a.stockdale@ncl.ac.uk</a>	Project Supervisor- Provided advice and feedback throughout project.
Dr Guillaume Rousseau	Laboratorios do Solo, UEMA, Brazil.	Universidade Estadual do Maranhão (UEMA) Pós- Graduação em Agroecologia Cidade Universitária Paulo VI S/N Tirirical São Luis-MA 65055- 970 Brasil Tél: 55 98 3231 0476  E- mail: <a href="mailto:guilirous@yahoo.ca">guilirous@yahoo.ca</a>	Host country supervisor - Provided advice and assistance throughout the project.
Dr Christoph Gehring	Laboratorios do Solo, UEMA.	Pesq. Visitante Mestrado em Agroecologia da UEMA, Laboratório de Solos CP 3004 São Luis, MA, Brasil Tel: (098)3232-1003, Fax: (098)3231-1067 e-mail: <a href="mailto:cgehring@uema.br">cgehring@uema.br</a>	Initial contact in host country. Allowed project to be carried out through the facilities of UEMA.