SOIL ORGANIC CARBON AND MOLECULAR CHARACTERISATION OF SOILS AND VEGETATION INPUTS ALONG A SAVANNAH-RAINFOREST BOUNDARY IN CENTRAL GUYANA, SOUTH AMERICA

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Declaration

I hereby certify that the work presented in this thesis is my original research work. Due reference is given to the literature and any research collaborations where appropriate. No part of this thesis has been submitted previously for a degree at this or any other university.

Jasmine Elizabeth Black

Abstract

Amazonian soils have been estimated to contain a globally substantial 66.9 Pg C within 1 m depth. Current uncertainty in model projections for future climate scenarios emphasises the need to better understand soil and vegetation carbon stocks which may become significant sources of CO_2 and CH_4. Contemporary data of bulk and molecular carbon stocks for full soil profiles and corresponding above ground inputs is needed to understand how these stocks may alter with climate change. The savannah-rainforest boundary is particularly sensitive to alteration in response to these local climatic changes and is thus a focal point of international research.

The study site in Central Guyana, which lies within the north eastern Amazon, encompasses pristine and relatively unexplored savannah-rainforest boundary, providing an advantageous location for assessing both soil and vegetation carbon. Soil profiles classified as gleysols (FAO) under rainforest have the greatest soil organic carbon (SOC) stocks of those studied, and are 43% greater than previously published data for tropical regions (up to 1 m depth). Further, estimations of the full soil profile SOC stocks show a 94% increase compared to previous 1 m depth data. Although not inclusive of the whole boundary region, these SOC stocks emphasise the significance of local responses to more extreme weather conditions induced by climate change.

Molecular surface SOC characteristics are site specific: likely influenced by local water table depth, mineralogy, vegetation inputs and microbial activity. However, measured environmental variables (pH and water content) show no relationship to molecular characteristics. Gleysols have the most degraded lignin and carbohydrates, indicating high inputs and a faster turnover than the bulk SOC. Drier savannah woodland plinthosols have the greatest amounts of lignin, tannin and carbohydrates, reflecting high inputs. Despite this, this soil has significantly lower SOC stocks than gleysols. If local weather patterns alter towards postulated longer and more intense dry seasons, rainforest die-back may occur. With savannah encroachment, the release of SOC stocks from the swamp forest and forest island gleysols is likely to occur. Phenol-rich soil organic matter may preside in developing areas of savannah woodland, but nevertheless a net decrease in SOC stocks is likely to result. The data collected here can be used to inform management policies and practices to help conserve and monitor the significant stocks of SOC in the swamp forests and forest island on these boundaries.

Dedication

I would like to dedicate this thesis to my late brother, Stephen Black. An incredibly charismatic character who achieved great success in too short a life. Thoughts of you have provided me with constant motivation and drive throughout, and have given me a perspective on life to enjoy every moment, believe that the impossible is possible and always strive to create positive change. Forever in my heart and thoughts.

I would also like to dedicate this thesis to the communities who protect the land from which my project has developed. To the people of the Northern Rupununi: during my time in your region, you showed such passion and commitment to the beautiful savannah and rainforest in which you live. Long may this continue and be respected.

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Chapter 1. **Introduction and literature Review**

1.1 Introduction

It has been recognised since the 1960's that anthropogenically-induced climate change has been occurring as a result of greenhouse gas (GHG) emissions, originating largely in the industrial era (Bindoff and Sebbari, 2013). At the global scale this climate change has been recognised as a warming of the atmosphere with numerous local expressions and amplifications. Recently, the IPCC published its Fifth Assessment Report on Climate Change, detailing that the average warming of land and ocean surfaces from 1880 to 2012 has been 0.85 ˚C (IPCC, 2014). This increase in temperature is extremely likely (95%) to be due to anthropogenic GHG emissions originating from fossil fuels and land use change, which have increased due to economic and population growth since the pre-industrial era (IPCC, 2014). As a consequence of global warming, extreme weather events appear to have become more frequent since 1950 (IPCC, 2014). Future predictions also suggest further warming of up to 2 ˚C by 2100, along with more frequent hot temperature extremes and changes in precipitation, depending upon the region (IPCC, 2013). Tropical regions are likely to see intensified weather patterns, however, uncertainty remains with regards to the spread and scale of these events due to high natural variation (IPCC, 2014).

Guyana is a part of the wider Amazon region, located in the north east of South America. Guyana's seasons are influenced by the Inter-Tropical Convergence Zone (ITCZ) which moves over the country twice a year. When in its northern position during May – July, the subtropical area of high pressure weakens and heavy rainfall ensues creating the wet season. As it moves into a southern position over the Brazilian Amazon during November – January, the high pressure strengthens bringing the dry season. Within the Amazon region, annual temperature is predicted to rise up to 1.5 ˚C in the next 21 years, whilst annual precipitation may decrease by 10% in the same time period (IPCC, 2013). However precipitation changes are uncertain and may also show increases by 10% annually (IPCC, 2013). Such climatic change may cause disturbance to the length and intensity of local wet and dry seasons within Guyana. This tropical country may therefore experience effects from these local alterations similar to those seen during El Niño Southern Oscillation (ENSO) events: drought stress of trees, forest fires and resulting deforestation (Laurance and Williamson, 2001; Doughty et al., 2015). However, uncertainty in alterations to weather patterns still remains (IPCC, 2014).

Although historically deforestation rates in Guyana are low (Food and Agriculture Organisation of the United Nations, 2005), potentially, these climate alterations may lead to both environmental and social problems (such as economic and population growth) which could result in pressure upon the existing rainforest and savannah biomes (Terracarbon, 2009). Such problems may include deforestation due to drier, hotter climates as well as increased pressure on rainforest resources (Nobre et al., 1991; Nepstad et al., 2008). Deforestation may occur through climate or anthropogenically-induced fires, of which tropical fires have been shown to release a significant 2.6 Pg C yr^{-1} (van der Werf et al., 2003). In turn, this exposes forest types usually found further from the boundary, and creates greater vulnerability to fires through edge effects, including increases in surface temperatures and decreases in annual evapo-transpiration and precipitation (Nobre et al., 1991). If a tipping point is breached, savannah expansion into forested areas may result (Hoffmann et al., 2002; Malhi et al., 2008).

As a result of an awareness of global climate change effects, the Kyoto Protocol was ratified in 1997 with the aim of all participating countries committing to a reduction in GHG emissions as part of the United Nations Framework Convention on Climate Change (UNFCCC). An extension beyond the 2008 – 2012 agreement under the name the 'Bali Roadmap' was created following recognition of the need to protect rainforest nations through financial incentives or voluntarily (Santilli et al., 2005). The significance of Guyana's rainforests, which cover 85% of its land area (Palo, 1994), was acknowledged and a response developed for their protection through the Low Carbon Development Strategy (LCDS) and Reducing Emissions from Deforestation and Forest Degradation (REDD+) mechanism in 2009 (GRIF, 2014; Office of Climate Change, 2015).

In order to aid in climate change mitigation through GHG emission reduction and the protection of ecologically valuable biomes within Guyana, the existing carbon stocks of these biomes needs to be accurately assessed. This will provide a baseline understanding of how carbon emissions from climate change may alter these stocks. Soil carbon stores are twice that of biomass or the atmosphere (Batjes, 1996) and play a vital role in ecosystem processes, feedback mechanisms and the speed of climate change (Raich and Potter, 1995; Trumbore et al., 1996). Therefore, having a clear picture of current SOC stocks is critical for understanding and interpreting their future

changes. The Amazon region is estimated to hold a globally significant amount of SOC across a variety of biomes, sub-environments and soil types: a total of 66.9 Pg C within 1 m of the surface (Batjes and Dijkshoorn, 1999). This region is greatly important in terms of potential SOC loss through CO_2 emissions with climate change as well as future land use changes, which may have significant negative impacts upon the quality of these vulnerable soils (Sombroek et al., 1993). Therefore, it is of particular importance to assess the SOC stocks within Guyana. The significant 85% rainforest land cover in Guyana (Palo, 1994) is bordered by an area of savannah, known as the Rupununi (see Chapter 3: Figure 3.1). This region of rainforest and savannah has two dry-wet seasons and one dry-wet season, respectively (Bovolo et al., 2012). These seasons are likely to affect SOC stocks and molecular characteristics: soils will become anaerobic during flooding causing a decrease in microbial decomposition; however this may reverse to intensive degradation during dry seasons, as microbes utilise moisture remaining from the wet season (Fenner and Freeman, 2011). Different vegetation types are also likely to affect SOC stocks, through variations in leaf and wood litter chemistry such as the amount and type of lignin, tannin and carbohydrates present (Carr et al., 2013).

Lignin and tannin are the second and fourth most abundant biopolymers in terrestrial biomass, and thus contribute significantly to soil organic matter (SOM) (Crawford, 1981; Hernes and Hedges, 2000). Due to similarities in the structure of lignin and tannin monomers, it has been previously difficult to distinguish between the two biopolymers within biomass and soil samples. However, due to the introduction of a relatively new technique employing ^{13}C-labelled tetramethylammonium hydroxide (TMAH) as a derivatising agent, it is now possible to distinguish these biopolymers in order to accurately assess SOM degradation (Filley et al., 1999).

Within the savannah and rainforest biomes of central Guyana, a variety of sub-environments have been reported, such as savannah grassland, woodland, scrubland, forest islands, Mora (naturally occurring tree species) dominated forests and Kokret palm forests (Eden, 1964). Equally, soil types have been found to vary across these sub-environments e.g. gleysols, regosols and ferralsols (Suggett, 1964). This variety of biomass input (including grasslands, scrubs and trees) into soils and soil type may imply

profound variation in soil lignin, tannin and carbohydrate signatures as well as total SOC stocks across the savannah-rainforest boundary.

SOC estimates and molecular SOM characteristics of this region of the Amazon have not been previously published, and thus it is even more important that this analysis should be undertaken. The savannah-rainforest boundary is more susceptible to fire hazards from the savannah than deeper rainforest, but may expose these deeper forest areas after fire and drought induced die-back. The area of boundary studied in central Guyana is relatively pristine and thus provides a rare opportunity to assess SOC, and advance understanding of how alterations may be expressed with local weather extremes (Uhl and Kauffman, 1990; Nepstad et al., 2004). Although beyond the remit of this study, the knowledge acquired here can be transferred to crucial climate change mitigation and management strategies: such as comprehensive SOM monitoring over time (Batjes and Sombroek, 1997), and effective protection of areas with high SOC stocks (Malhi et al., 2008; Walker et al., 2009).

1.2 Literature Review

1.2.1 Climate change in the Amazon

The Amazon is renowned for its ecosystem services as a biodiversity hotspot, providing species with many major medicinal uses, and a vast expanse of vegetation that provides a global sink of CO_2 and a source of O_2 (Malhi and Grace, 2000; Dirzo and Raven, 2003). Highly weathered and deep soils also provide a significant sink of CO_2 here (Batjes and Dijkshoorn, 1999). As stated above (Section 1.1), the Amazon region is likely to undergo warming and precipitation changes within the current century and beyond (IPCC, 2013). Alongside this, it is also suffering from anthropogenic deforestation as a result of population and resource pressure (Nepstad et al., 2008). This stems from the demand for pastoral agriculture and timber resources (Binswanger, 1991; Inman, 1993; Soares-Filho et al., 2006).

Although fires are a natural part of the savannah ecosystem, anthropogenic fires which actively cut into the rainforest expose areas of forest that are less drought tolerant and susceptible to further die-back (Nepstad et al., 1999; Cochrane and Laurance, 2002). Intense El Niño Southern Oscillation (ENSO) events have also been known to affect otherwise stable Amazonian rainforests (Meggers, 1994). Climate-induced changes towards warmer temperatures and lower precipitation rates can also lead to the exposure of deeper forest areas after drought affected die-back (Nepstad et al., 2004). Tree mortality was found to have increased by ~50% following the 1997 El Niño event in central Amazonia (Williamson et al., 2000). The large carbon stock held within the soils beneath the Amazon rainforests may be released with such die-back and conversion to savannahs, exacerbating positive feedback effects (Batjes and Dijkshoorn, 1999). Thus, assessing SOC stocks of forest edges vulnerable to the above impacts is vital to understanding how much carbon may be released with climate change. No published data exists for SOC stocks on the savannah-rainforest boundary in Guyana, and so this study addresses the SOC characteristics of this exposed forest edge. However previous work in central Guyana highlighting soils as strongly acidic and nutrient poor has been conducted by the Tropenbos Project (van Kekem et al., 1997). Previous studies have also been conducted on soil properties close to the study region (Sugget and Braun, 1964; GLSC, 2005), which are described in Section 3.2.4.

1.2.2 Land use and climate change within Guyana

As previously mentioned, (Section 1.1), Guyana currently has initiatives in place to protect its valuable rainforest resources, although historically deforestation for timber resources has been comparatively lower than in other tropical regions (ter Steege et al., 2002). In recent years rainforest protection policies such as the Forests Act 2009 (Guyana, 2009) and Protected Areas legislation in 2011 (Guyana, 2011) have been established.

Along the savannah-rainforest boundary within the Northern Rupununi, some areas of rainforest are currently utilised by the indigenous population for farming where soils are suitable (personal communication with indigenous community of Wowetta, 31st January 2012). However, due to the occurrence of laterite and nutrient-poor, unsuitable soils, there are areas where this is not possible and the rainforest remains unused by humans (personal communication with indigenous farmers of Wowetta, 31st January 2012 and field observations: see Chapter 3). This study focuses on these pristine areas in order to gain fundamental data on the existing SOC characteristics.

Previous work in this region has focused on characterising the climate system and its variability (Bovolo et al., 2012), where precipitation has been highlighted as the main regional climatic factor. The study also emphasises the sensitivity of the savannah-rainforest boundary to climatic changes; and thus the need for further investigation of this interface as an early indicator in establishing climate change effects (Bovolo et al., 2012). The movement of dissolved organic matter (DOM) from plants and soils into river waters has also recently been studied (Pereira et al., 2014). The latter study highlights that greater amounts of DOM are mobilised from plant litter and surface soils than deeper in the soil profile during rainstorm events, which may suggest a substantial portion of OM is lost before entering the soil. This further emphasises the need to characterise SOM to create a fuller picture of the carbon cycle in this region.

Strong ENSO events have been shown to be the main cause of fires in established forests, particularly when in conjunction with human interference, whilst forests on sandy soils in the Guianas, such as those at the savannah boundaries, are most likely to burn (Hammond and ter Steege, 1998). Charcoal records from the Holocene period also

provide evidence of fires within established forests in central Guyana (Hammond and ter Steege, 1998). This indicates that past climates have been dry enough to dramatically affect this area. With the predicted changes in climate within the current century and beyond (which may include stronger and more frequent ENSO events) it is possible that human induced savannah fires may spread into deeper forest areas (Laurance and Williamson, 2001), potentially causing large CO_2 release from biomass and soils (van der Werf et al., 2003). As climate and land use change occurs in Guyana, it is imperative to understand the possible effects this has on SOC stocks. As detailed in Section 1.2.10, Objective 1 focuses on identifying the different sub-environments and soil types on the savannah-rainforest boundary. This is in order to characterise a variety of SOC stocks at this important and vulnerable ecosystem interface.

1.2.3 Soil Organic Carbon (SOC)

The global carbon cycle includes three major pools, in which carbon is continuously cycled. These three major pools consist of an atmospheric pool (760 Gt), an oceanic pool (38000 Gt) and a terrestrial pool (8060 Gt), (Lal, 2004). Included in the terrestrial pool are the biotic (560 Gt) and soil (2500 Gt) pools, of which the soil pool is 4.5 times the biotic and 3.3 times the atmospheric pool. The soil pool is divided into SOC and soil inorganic carbon (SIC), of which the SOC accounts for 1550 Gt and the SIC 950 Gt (Lal, 2004).

The transformation of carbon from the atmosphere into the soil occurs through conversion of CO_2 into terrestrial biomass after which it can be released back to the atmosphere through respiration, or sequestered as soil organic matter (SOM) via physical and biochemical stabilisation mechanisms (Kay, 1999; Lorenz et al., 2007). This soil carbon stock is thus determined by the balance of inputs from biota (mainly vegetation) and outputs through respiration. Carbon becomes sequestered in the soil when the inputs are greater than the outputs (Powlson et al., 2008). Likewise, soil can become a source of carbon when outputs exceed inputs through rapid biotic turnover and human interference (Malhi and Grace, 2000).

SOM contributes ~60% of the pedological carbon pool (Post et al., 2001; Lal, 2004). Originating from vegetation in the form of root exudates and root, leaf and wood litter as well as dead soil fauna and microbial exudates, SOC is decomposed within soil to either be released as CO_2 and CH_4, leached through the soil profile into waterways or

stabilised into either labile or recalcitrant pools. The labile pool accounts for between 60 and 80%, which decomposes rapidly, with a residence time <10 years. The remaining fraction (20-40%) is split between an intermediate (10-100 years) and stable pool (>1000 years), (Trumbore et al., 1996; Lützow et al., 2006; Lorenz et al., 2007). This stable pool is controlled by spatial inaccessibility, the chemical structure of the OM and the availability of mineral surfaces and metal ions in which to complex (Sollins et al., 1996; Six et al., 2002; Lützow et al., 2006).

Several factors within soil affect the turnover of SOC (Dungait et al., 2012). These include chemical structure (Filley et al., 2008), soil temperature (Amundson et al., 1989; Trumbore et al., 1996) and moisture (Amundson et al., 1989), pH (Anderson and Domsch, 1993), clay content (Feller and Beare, 1997), nutrient status (Berg, 2000), microbial communities (Amundson et al., 1989; Anderson and Domsch, 1993), and overall site conditions including climate, aspect, morphology and vegetation (Filley et al., 2008). Traditionally, SOC has been divided into 'labile' and 'recalcitrant' pools depending upon the residence time within soil. However, components of these pools thought to be reserved to one or the other have more recently been found in opposing pools (for example carbohydrates and lignin, respectively), contradicting this theory (Lützow et al., 2006; Kleber et al., 2011). This signifies that SOC is still more complex than our current understanding and that great heterogeneity exists within soils.

Global variability between soil types and thus SOC stocks is considerable, which means estimations are often difficult or unreliable (Eswaran et al., 1993). This is largely due to differences in latitude, climate, micro-climate, vegetation inputs, bedrock and microbial communities. Such high variability is reflected in the soils of the Amazon region (Batjes and Dijkshoorn, 1999) due to its heterogeneous environment and vast scale. Even within the relatively small country of Guyana, this heterogeneity has been noted (Eden, 1964; Suggett, 1964), and is therefore likely to affect SOC stocks spatially. Objective 1 and 2 of this study address the heterogeneity of soils and their SOC stocks in savannah and rainforest ecosystems. Such heterogeneity is also likely to affect molecular SOM composition; the literature surrounding key SOM components is reviewed in the following sections.

1.2.4 Carbohydrates

Carbohydrates are the primary resource of metabolic energy in living organisms, and are created during photosynthesis by plants therefore incorporating carbon, hydrogen, oxygen and using primary energy from solar radiation. They come in both simple (monosaccharides), intermediate (oligosaccharides) and more complex, longer chain forms (polysaccharides), (Brett and Waldron, 1996).

In the tropics, there are few studies on carbohydrates as compared to other latitudes and studies on SOM content (Nacro et al., 2005). Those that have been conducted include those in native grasslands and savannahs of Costa Rica (Guggenberger and Zech, 1999), the savannah soils of the Congo and Senegal (Larré-Larrouy, 1997; Sall et al., 2002) and in the soils of a forest and savannah mosaic landscape in the Côte d'Ivorie (Nacro et al., 2005). Less attention has been paid to looking at natural ecosystems than those under management (Nacro et al., 2005). However, carbohydrates could be important for soil quality: through the formation and subsequent stabilisation of aggregates, controlling soil microbial activity and plant growth (Puget et al., 1998; Nacro et al., 2005).

Despite their chemical structure which is comparatively simple compared to lignin and tannin, and, thus, more easily degraded (Derrien et al., 2006), they account for 10-20% of SOM (Cheshire, 1979; Amelung et al., 1996; Puget et al., 1998). Through calculating residence times (based on differences in the peak area and isotopic content), their sugar carbon atoms have also been found to date older than those of the bulk SOC, and thus may be important in contributing to the recalcitrant SOM pool (Gleixner et al., 2002; Kogel-Knabner, 2002). Their significance in SOM as microbial necromass has recently been reported (Miltner et al., 2012), and a shift in focus from the importance of lignin and its inherent chemical recalcitrance, to microbial turnover of SOM and carbohydrate microbial necromass, has taken place (Glaser et al., 2004; Kleber, 2010; Thevenot et al., 2010; Dungait et al., 2012). Several factors may account for this recalcitrance, stemming from the surrounding conditions of the carbohydrates, such as physical / physio-chemical preservation and incorporation into humic substances (Derrien et al., 2006; Lützow et al., 2006) or microbial recycling (Nacro et al., 2005).

In the analysis of complex biomaterials such as soil, TMAH with thermally assisted hydrolysis and methylation (THM) pyrolysis-gas chromatography/mass spectrometry (GC/MS) analysis has proven useful in identifying some carbohydrates trapped within SOM (Fabbri and Helleur, 1999; Schwarzinger, 2003; Tanczos et al., 2003; Estournel-Pelardy et al., 2011). Thus, it is able to identify a cellulose pool that may be otherwise unseen from acid hydrolysis (Estournel-Pelardy et al., 2011). Therefore, the use of this technique to identify possible complex carbohydrate mixtures in soils along a savannah-rainforest boundary may provide interesting insights into their recalcitrance in the tropics. Objective 3 relates to the identification of carbohydrates in SOM and their amounts in the range of soils studied.

1.2.5 Lignin

Lignin derives its name from the Latin word for wood (*lignum*). It comprises a structural component of plants, lending rigidity to their forms as well as coating xylem cell walls to create hydrophobicity and prevent water loss (Campbell and Sederoff, 1996). As a part of the cell wall in vascular plants, lignin is interconnected with cellulose and hemicellulose (Sarkanen, 1971; Campbell and Sederoff, 1996). This biopolymer constitutes a third to the organic carbon content of the biosphere (Boerjan et al., 2003) and is second most abundant to cellulose and hemicellulose in vascular plant species (Crawford, 1981; Gold, 1989). It has a highly complex and variable macromolecular structure (Figure 1.1). In woody plants, lignin can contribute 30% to mass, whilst non-woody plants show lesser amounts (Adler, 1977).

The structure of lignin itself is comprised of three main components (monolignols), namely *p*-coumaryl, coniferyl and sinapyl alcohols (Figure 1.2). These monolignols undergo oxidative co-polymerisation during biosynthesis. On enzymic dehydrogenation, they are broken down into *p*-hydroxyphenyl (P), guaiacyl (G) and syringyl (S) units (Adler, 1977). As the contribution of these components in lignin varies widely, they can be used as biomarkers to distinguish between different vascular plant types and sources: for example gymnosperms, angiosperms and non-woody plants, such as grasses (Higuchi, 1980). Characteristics of gymnosperms (softwood) are G dominated lignins, wioth low levels of P units found to be less susceptible to degradation than S or C phenols, whilst angiosperms (hardwood) are primarily composed of S as well as G, with trace levels of P units (Sarkanen, 1971; Boerjan et al., 2003). In contrast, non-woody

species are dominated by cinnamyl (P) lignins known as *p*-coumaric acid and ferulic acid, with smaller quantities of G and S lignin derivatives (Clifford et al., 1995).

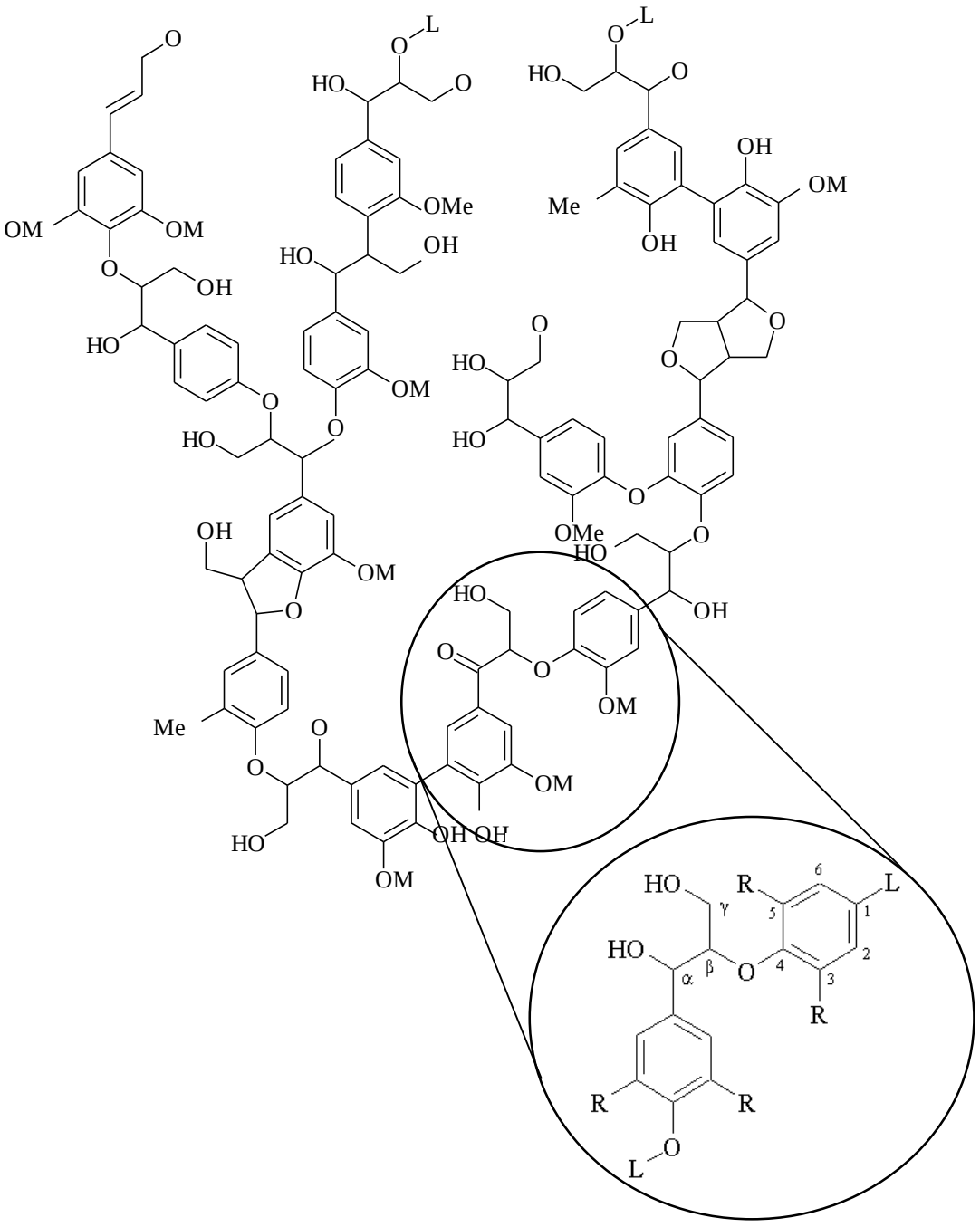
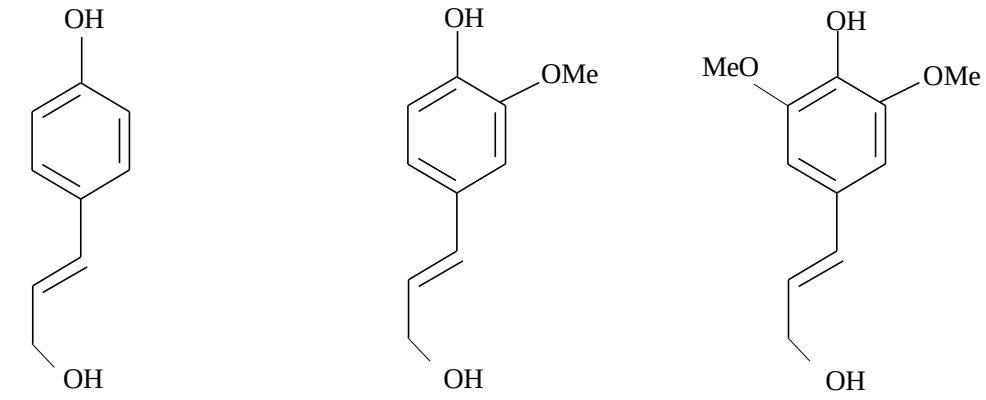


Figure 1.1 A schematic representation of lignin (reproduction of Dorrestijn, 2000; L = lignin biomacromolecule). The dominant β-O-4 linkage in the lignin macromolecule is highlighted in the open circle.



p-coumaryl alcohol coniferyl alcohol sinapyl alcohol

Figure 1.2 A schematic of the three main lignin precursor alcohols: p-*coumaryl, coniferyl and sinapyl (adapted from Dorrestijn, 2000).*

As a complex structure, lignin consists of various linkages between the monolignols described above. These monolignols are connected mainly via arylglycerol- β -aryl ether (β-O-4) links as seen in Figure 1.3. In hardwood and softwood, β-O-4 accounts for 60% and 46% of the monolignol links respectively (Dorrestijn et al., 2000). This covalent bond occurs between the carbon in the β position of an alkyl side-chain and the phenolic oxygen atom in the fourth position of the phenol ring (Adler, 1977). Other linkages include carbon-carbon bonds in the form of β-5, β- β, β-1 and 5-5, which are stronger than the ether linkage. The latter are found mostly in softwood (30% compared to 21% in hardwood). This is due to the greater abundance of guaiacyl and thus availability of C5 on the phenol ring for coupling (i.e. other C atoms are already occupied by functional groups, such as hydroxyl and methoxy groups), (Dorrestijn et al., 2000; Boerjan et al., 2003).

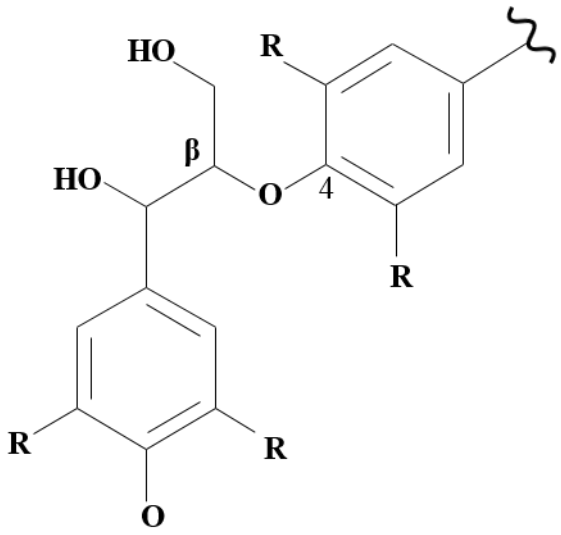


Figure 1.3 The β-O-4 bond most common in monolignols, (reproduction from Dorrestijn et al., 2000).

Due to the complexity and variability in linkages within the lignin biopolymer and its high molecular weight, it has previously been considered recalcitrant (Crawford, 1981). Such recalcitrance was assumed to lead to its stability and possible accumulation in SOM, compared to less structurally complex molecules such as cellulose. However, studies such as Gleixner et al. (2002) found opposing results, in which lignin was not present in soils despite being detected in maize inputs, yet polysaccharides were major pyrolysis-GC/MS products in the investigated soils. It has also been suggested that lignin actually has a faster turnover rate than bulk SOM (Dignac et al., 2005; Heim and Schmidt, 2007). Lignin degradation is fastest under aerobic conditions (Kiem and Kögel-Knabner, 2003; Dignac et al., 2005; Bahri et al., 2006; Kalbitz et al., 2006; Marschner et al., 2008; Klotzbücher et al., 2011; Dungait et al., 2012), where white rot fungi are one of the most efficient degraders (Gold, 1989; Hatakka, 1994). Robertson et al. (2008) demonstrated significant lignin oxidation in relation to enzymatic activity and fungal growth during white rot decay of wheat straw. Lignin degrading fungi, (specifically species *Buergenerula spartinae, Phaeosphaeria typharum* and *Leptosphaeria obiones*), have also been found in anaerobic environments (Benner et al., 1984). The three main groups of lignin degrading fungi (basidiomycetes) are soft rot, brown rot and white rot (Kirk and Farrell, 1987). White rot are the only complete degraders of lignin, performing full mineralisation of lignin to water and CO_2 (Gold, 1989). Robertson et al. (2008) reported that the white-rot species *Pleurotus ostreatus* degraded lignin from wheat straw after 21 days after the rapid activation of the enzyme

peroxidase. The residues of white-rot degradation can be identified through extensive side-chain oxidation and aromatic ring cleavage (Umezawa and Higuchi, 1987). Brown rot fungi do not show a high degree of side-chain oxidation, however, but demethylate the methoxyl groups of lignins, leading to the production of hydroxylated phenyl (catechol derivatives), (Anders et al., 1988; Enoki et al., 1988; (Filley et al., 2000). The main processes of fungal degradation can be seen in Figure 1.4.

Some species of bacteria (*Streptomyces* and *Nocardia*) have also been found to degrade lignin (Sørensen, 1962; Antai and Crawford, 1981; Crawford et al., 1983; Godden et al., 1992; Trojanowski, 2001). In addition to biotic degradation, abiotic factors may also play a role in the breakdown of lignin, for example through photo-oxidation (Hernes and Benner, 2003; Gallo et al., 2006; Frouz et al., 2011) and leaching (Hernes et al., 2007).

As the main source of lignin degradation, the conditions in which fungi can be active are important in determining its breakdown. Temperature, moisture, pH and substrate availability all affect fungal activity (Amundson et al., 1989; Donnelly et al., 1990; Andersson and Nilsson, 2001). Different species may colonise the range of niches these edaphic parameters provide, leading to variations in degrader communities and activity, which could influence lignin degradation rates. For example, Blagodatskaya and Anderson (1998) reported greater fungal respiratory activity than bacterial under low pH. If the fungi assessed in the latter study is of a lignin-degrading variety, this could indicate greater lignin degradation is acidic conditions. Within heterogeneous environments on the savannah-rainforest boundary, these differences in abiotic and biotic factors may result in variations in lignin composition and degradation. This aspect will be assessed through Objective 3 (Section 1.3). So far, the majority of studies conducted on lignin composition in soils has been focussed primarily in temperate latitudes (Kalbitz et al., 2006; Heim and Schmidt, 2007; Nierop and Filley, 2007; Mason et al., 2009; Swain et al., 2010), where the function and degradation of lignin is still not completely understood (Thevenot et al., 2010).

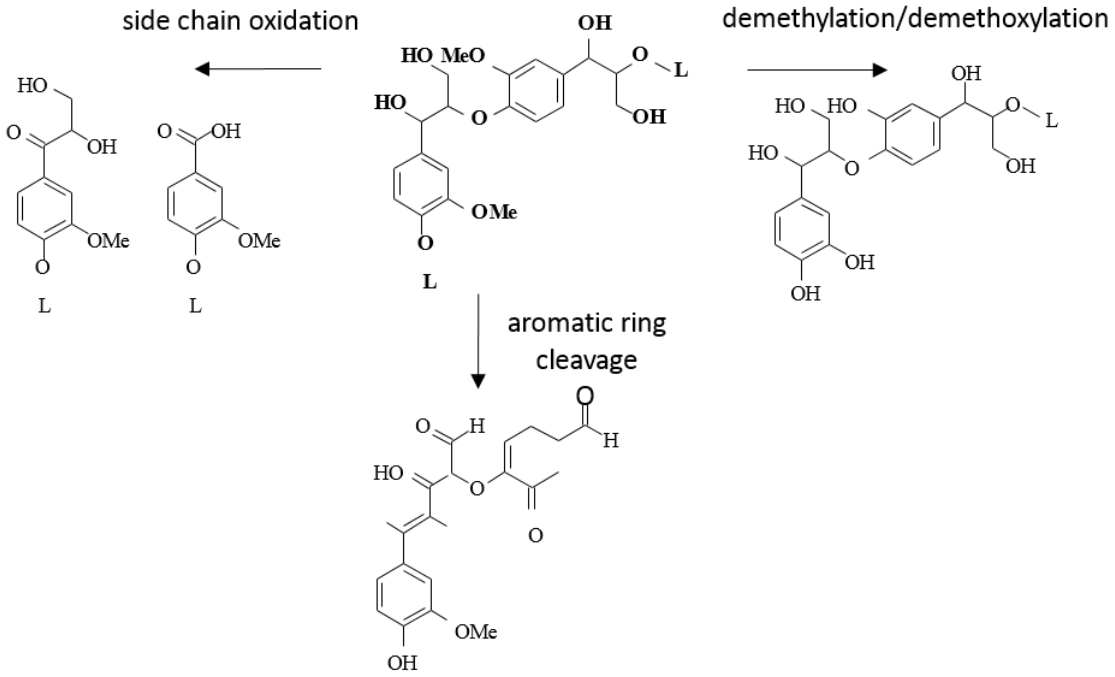


Figure 1.4 The main fungal degradation alterations of guaiacyl lignin (adapted from Filley et al., 2000; L = lignin biomacromolecule).

1.2.6 Lignin Characterisation

Adler (1977) gives a thorough reflection on the history of lignin and its analysis, showing that initially research was aimed towards its structure and biosynthesis. The primary method employed during such studies of lignin at this time was the cupric oxide (CuO) oxidation method (Hedges and Parker, 1976; Hedges and Mann, 1979; Hedges et al., 1982; Kögel, 1986). From the latter method, a series of lignin reaction products are formed, which include six vanillyl (V) and syringyl (S) phenols in aldehyde, ketone and carboxylic acid form, as well as ferulic and *p*-coumaric acid (C) which are *p*-hydroxyl substituted cinnamic acids (Hedges and Parker, 1976). These structures are formed from the three main monolignols as described in Section 1.2.4, and correspond to phenols G, S and P, respectively. Their methylated forms can be seen in Figure 1.5. Parameters were derived from these products in order to assess the vegetation type from the sample, these consisted of the ratios S/V, C/V and [Ad/Al] for the degradation state, as well as the overall lignin yield (Λ). The latter ratios can, however, be influenced by leaching and sorption to minerals from which elevated ratios in deep soil layers may result (Hernes and Benner, 2003; Hernes et al., 2007). This stems from the differing surface reactivity and solubility of OM and lignin, causing

fractionation during leaching and sorption (Kaiser and Guggenberger, 2000; Aufdenkampe et al., 2001)

Due to the fact that this early method did not provide any structural information on the lignin under assessment (Dijkstra et al., 1998), new methods were sought in order to do this. The CuO technique meant that the original propyl side chain was destroyed during the process, thus not allowing the interpretation as to whether the lignin phenols are intact (Hatcher et al., 1995; Filley et al., 2000). Aside from this, the technique is very time consuming (Hatcher et al., 1995).

A more efficient technique which allows for the structural assessment of lignin in samples was thus later developed, and is now in greater use than the traditional CuO method. This new technique involves high temperature pyrolysis followed by gas chromatography and mass spectrometry (Py-GCMS) and has been used to assess lignin in soils (Dijkstra et al., 1998; Vancampenhout et al., 2009). Lignin is treated thermally without the presence of oxygen, yielding smaller and more volatile fragments which allow for detection by the GC (Kaal and Janssen, 2008). In order that benzenecarboxylic acids could also be released by this pyrolysis process (Saiz-Jimenez, 1994; Klingberg et al., 2005), an in-situ derivatising agent was added to prevent decarboxylation of these products during pyrolysis (Challinor, 1989). This agent is tetramethylammonium hydroxide (TMAH) which methylates the lignin phenols making them GC amenable by forming the methyl ethers of carboxylic and hydroxyl groups (Challinor, 1989; Kaal and Janssen, 2008), so that the compounds are identified by interfacing the GC with a mass spectrometer (GC-MS). This method can also be carried out offline, through heating both the TMAH and sample in a sealed glass tube and analysing the products after cooling (Vane et al., 2001).

During the process, thermal bond dissociation leads to the fragmentation of lignin into compounds with reduced molecular mass, giving a reflection of how the original polymer was composed (del Rio et al., 1996). Clifford et al. (1995), highlighted that the TMAH reaction is primarily a thermally assisted chemolytic degradation, as opposed to a pyrolytic bond cleavage and thus the term ‘thermally assisted hydrolysis and methylation’ (THM) has now been widely employed. At present, TMAH is the most widely utilised derivatising agent to use in conjunction with the THM method (>90%) (Shadkami and Helleur, 2010). Tetraethylammonium hydroxide (TEAH) and

teratbutylammonium hydroxide (TBAH) have also been used for lignin analysis, but were found to produce lower yields of polycarboxylic acids than TMAH and only partially alkylate the phenolic hydroxyl groups (Lehtonen et al., 2003).

1.2.7 THM in the presence of TMAH

The use of the THM procedure allows the β-O-4 bonds of the lignin macromolecule to be broken, (Hatcher et al., 1995), through the formation of an unstable cyclic epoxide structure (Filley et al., 1999). These linkages are known to be of the most common in the lignin structure, but the actual proportion may vary from species to species (Pandey and Kim, 2011). Using THM can also indicate how many linkages are left intact in the macromolecule (McKinney and Hatcher, 1996; Klotzbücher et al., 2011). As the full process of THM is yet not fully understood, it has not been possible to assess the quantitative recovery of lignin with this method (Kaal and Janssen, 2008). Despite this, it is currently the best method for the analysis of humic substances (Saiz-Jimenez, 1994).

Using TMAH in conjunction with the THM method has been found to be most appropriate for fresh plant material, plant litter and soil surface horizon analysis, due to its specific bond cleavage, which may already be lost in extensively coalified lignin (Hatcher, 1990; Filley et al., 1999). The total monomer yield released during lignin depolymerisation from such samples relies upon the extent of alteration of the lignin macromolecule, as well as the proportion and type of the β-O, β-5 and β-β linkages. This will assert influence over the degradation rates of lignin components guaiacyl, syringyl and cinnamyl (Vane et al., 2001). The method allows for a wide suite of lignin phenols to be produced, these include all eight of those also produced in the earlier CuO method, in addition to *threo/erythro* 1-(3,4-dimethoxyphenyl)-1,2,3-trimethoxypropne (G14/15) and *threo/erythro* 1-(3,4,5-trimethoxyphenyl)-1,2,3-trimethoxypropane (S14/15) (Hedges and Parker, 1976). Lignin parameters used for assessing the degradation state and total lignin phenols can also be applied using the relevant compounds (Section 1.2.6), as in the CuO method (Hedges and Mann, 1979; Hatcher et al., 1995; Klotzbücher et al., 2011).

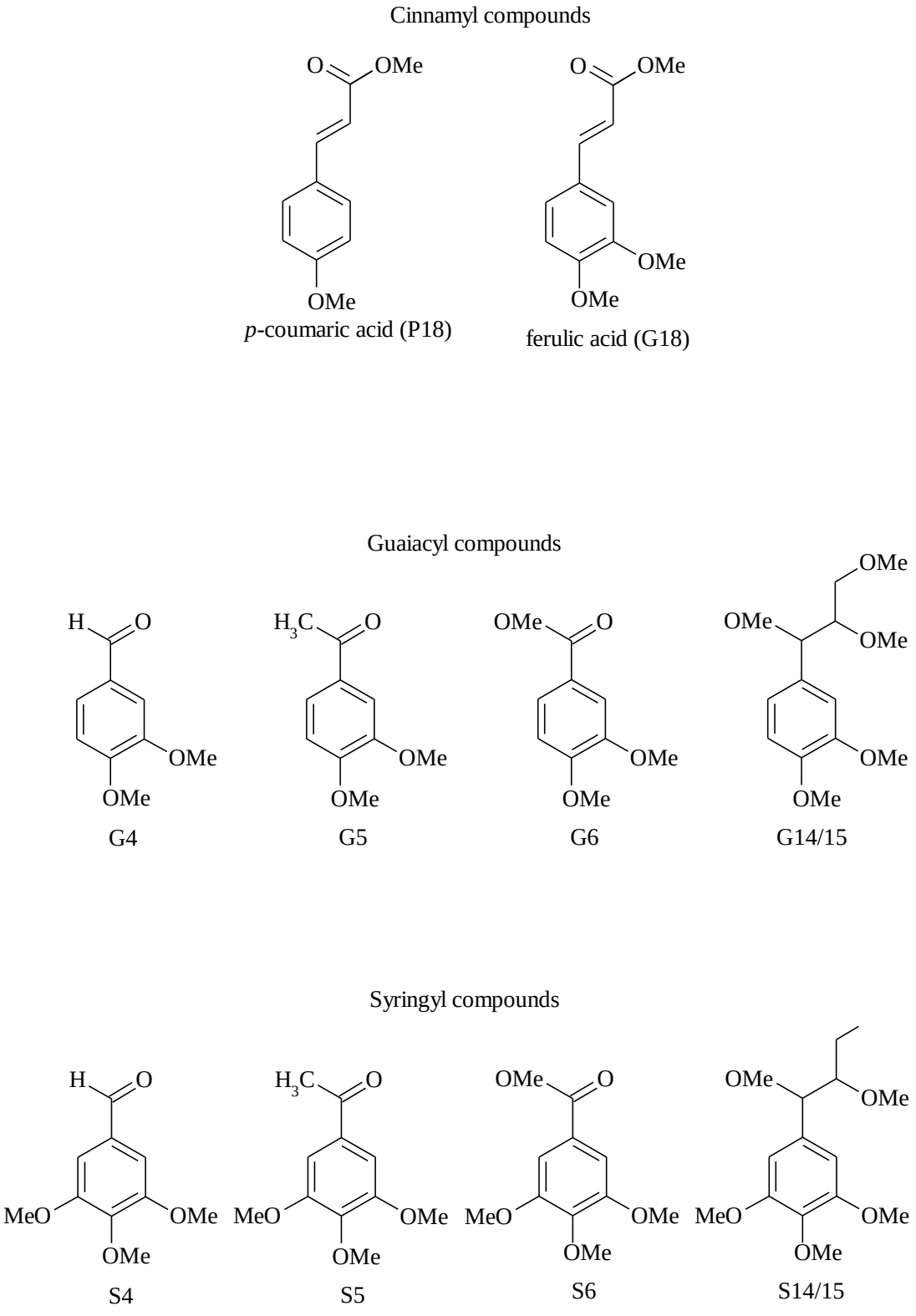


Figure 1.5 Dominant permethylated aromatic compounds released by TMAH thermochemolysis (adapted from Filley et al., 2006).

1.2.8 ^{13}C-labelled TMAH

Despite being the most suitable current method for the molecular analysis of SOM components, the THM in the presence of TMAH has limitations. One of its most significant is the inability to be able to distinguish between the methoxyl groups that have been added during the THM process and those that were originally present (Filley et al., 2000). Thus, microbial degradation cannot be recognised in addition to the compound potentially not being lignin derived. In 1999, the method was developed in order that the aforementioned shortcomings could be rectified; this was via the use of ^{13}C-labelling of the TMAH agent, so that phenolic methylated groups also showed ^{13}C (Filley et al., 1999). This then clearly allows for the recognition of lignin, altered lignin and non-lignin phenols (Filley et al., 1999).

Since its introduction, it has been applied with success to the analysis of SOM, DOM and biopolymers (Filley et al., 2000; Filley et al., 2002; Frazier et al., 2005; Filley et al., 2006; Nierop and Filley, 2007; Mason et al., 2009; Swain et al., 2010). On calculation of the lignin parameters Λ, [Ad/Al] and Γ after the use of ^{13}C-labelled TMAH, a significant alteration was shown as compared to the parameters that were analysed with just TMAH, however S/G and C/G ratios remained similar (Nierop and Filley, 2007). In a study of sandy soil profiles beneath oak woodland the total yield of lignin was reduced by 16-46% after the use of ^{13}C-labelled TMAH, the reduction owing to the distinction between lignin and tannin and poly-hydroxyl compounds, which were contributing to the original signal (Nierop and Filley, 2007).

The similarities between the lignin source and tannin source lie in the compounds syringic acid and gallic acid, respectively: these two compounds are both recognised as 3,4,5-trimethoxybenzoic acid methyl ester (also known as S6). S6 gives a molecular weight of 226 when methylated with unlabelled TMAH. However, once methylated with ^{13}C-labelled TMAH, a higher molecular weight of 228 is produced owing to the methylation of its two hydroxyl groups; whilst gallic acid gives a weight of 230 as its four hydroxyl groups are methylated. This allows the distinction between lignin and tannin source when assessing the mass spectra of the compound, using the percentage of ^{13}C-labelled methyl groups (Filley et al., 1999; Filley et al., 2006), see Figure 1.6. Despite the success of the distinction, there is one limitation of the method which means

that gallic acid cannot be distinguished from fully demethylated syringic acid, the former being the main building block of hydrolysable tannins (Nierop and Filley, 2007).

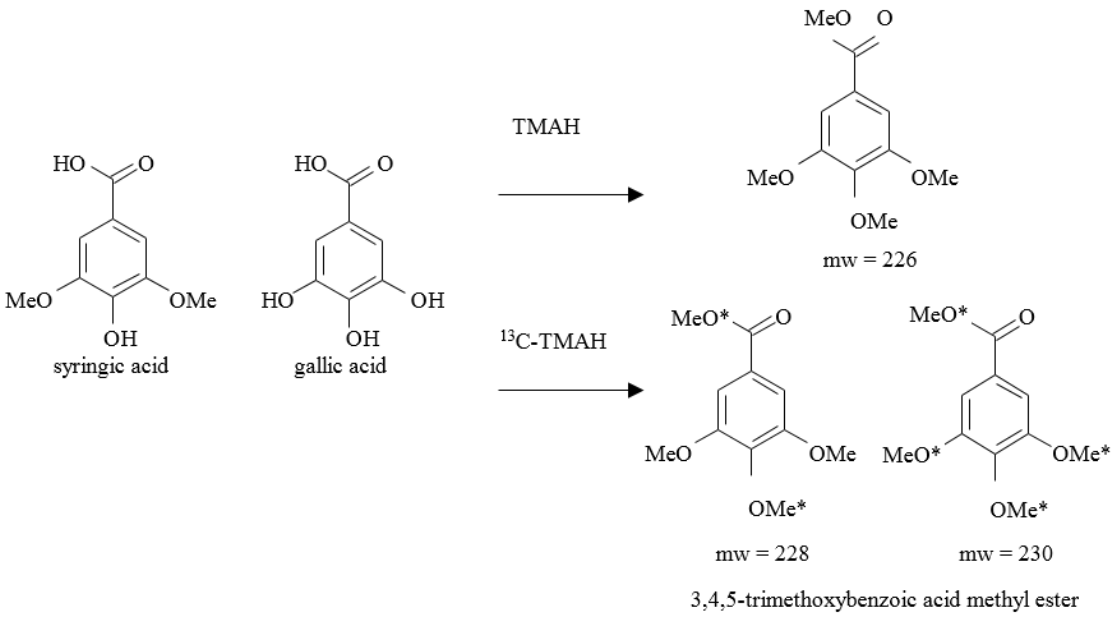


Figure 1.6 A reaction schematic reproduced from Swain, 2013 shows how ^{13}C-labelled TMAH distinguishes between the lignin syringic acid and tannin gallic acid during methylation. Asterisks highlight where ^{13}C-labelled TMAH has added methyl groups.

As well as distinguishing between the origins of S6, ^{13}C-labelled TMAH can also distinguish between the origins of guaiacyl phenol G6 and cinnamyl phenol G18. The former compound can be confused between that of lignin origin known as 3-hydroxy-4-methoxy benzoic acid methyl ester, (or that generated by the Cannizzaro reaction: see below), and that of microbially demethylated lignin or protocatechuic acid, known as 3,4-dihydroxybenzoic acid methyl ester: both are methylated as 3,4-dimethoxybenzoic acid methyl ester (Nierop and Filley, 2007).

G18 has two possible sources: 3-(3-methoxy, 4-hydroxy-phenyl)-3-propenoic acid methyl ester, from lignin; and 3-(3-dihydroxyphenyl)-3-propenoic acid methyl ester, from microbially demethylated lignin or caffeic acid. Both of these sources are methylated to give *trans* 3-(3,4-dimethoxyphenyl)-3-propenoic acid methyl ester (Nierop and Filley, 2007).

As mentioned above, G6 may also be affected by the Cannizzaro type reaction, whereby a disproportionation of aldehyde occurs. The reaction produces methoxybenzoic acid methyl ester and methoxybenzyl alcohol methy ether (Hatcher and Minard, 1995). Potentially, this could be of particular concern for the lignin parameter [Ad/Al] which indicates the oxidation state.

As there is no singly used THM method, for example through the range of temperatures used, it may be difficult to form comparisons between the literature (Klingberg et al., 2005). Despite this there are many positive advantages in using the technique, as previously described, and it has been noted as the best and fastest thus far developed for the analysis of substances such as soil organic matter (Saiz-Jimenez, 1994; Kaal and Janssen, 2008). Thus far, only a small number of recent tropical soil studies have employed pyrolysis-GC/MS as a method for assessing lignin characteristics (Buurman et al., 2007; Buurman and Roscoe, 2011; Stewart et al., 2011), whilst others have used the CuO method (Neufeldt et al., 2002; Wilcke et al., 2008). Therefore, it is important to undertake new studies via pyrolysis-GC/MS in order to create more comparable studies across tropical regions.

1.2.9 Tannin

As secondary metabolites, tannins are thought to play a wide range of roles in vascular plant adaptations, for example, herbivore defence, competitive advantage, nutrient cycling and litter decomposition (Kraus et al., 2003 and references therein). They therefore have a strong effect upon soil degradation dynamics, such as in reducing fauna decomposition of plants (Loranger et al., 2002; Coq et al., 2010), as well as limiting microbial activity (Schultz et al., 1992). In tropical plants they have been found to be particularly abundant, more so than in temperate tree species (Coley and Aide, 1991; Hallam and Read, 2006). Therefore, these compounds may prove to be a significant component of SOM in savannah and rainforest soils. Their amounts in vegetation samples will also be assessed to further corroborate evidence for the source and importance in these ecosystems.

Tannins are defined as water soluble polyphenolic compounds (Haslam, 1988), and are the fourth most abundant plant biomacromolecule behind cellulose, hemicellulose and lignin; thus accounting for a significant contribution to C in terrestrial biomass (Hernes and Hedges, 2000). The leaves and bark of tree species may consist of up to 40% tannin

in dry weight, which may exceed that of lignin (Kraus et al., 2003). There are two groups of tannin which consist of hydrolysable (gallo- and/or ellagi-) tannins (HT) and condensed tannins (proanthocyanidins) (CT) (Hernes and Hedges, 2004), see Figure 1.7. HT's are specific to dicot angiosperms, whilst CT's are found in both dicots and monocot gymnosperms (Bate-Smith, 1977; Haslam, 1988). The composition of CT's consists of flavan-3-ols connected via C-C bonds, and their monomers can be identified by the quantity of OH groups on the B-ring. For example, procyanidins (PCs) have di-hydroxy rings, whilst prodelphinidins (PDs) have tri-hydroxy B rings. HT's are divided into two groups: gallotannins, consisting of gallic acid; and ellagitannins, consisting of hexahydroxydiphenic acid esters, connected via ester linkages to a central sugar moiety (glucose). The structure of tannin is complex and diverse, similarly to lignin, and is thus variable across plant species (Kraus et al., 2004).

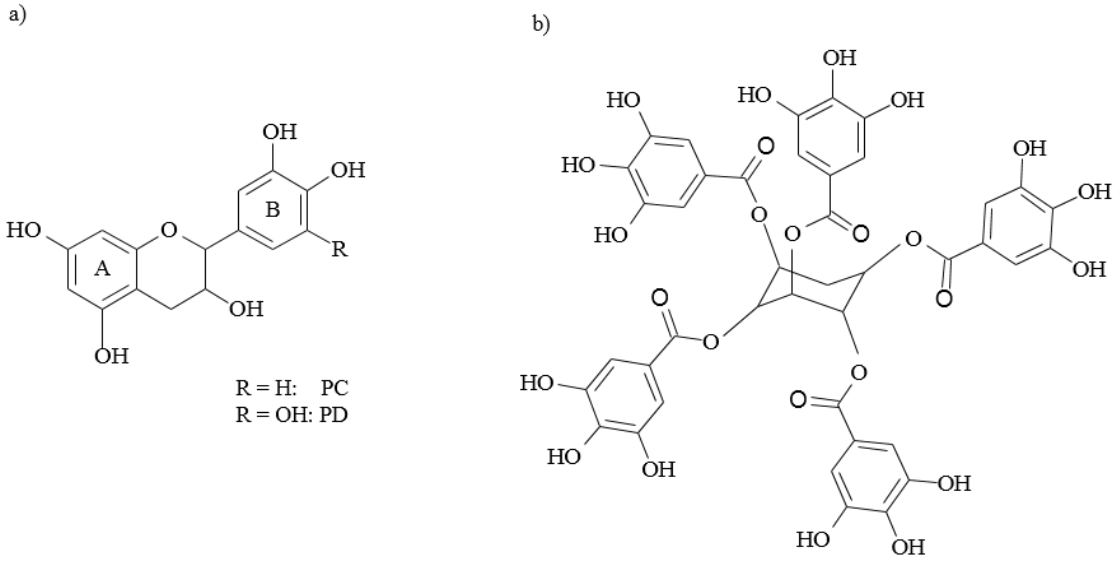


Figure 1.7 The structures of a) condensed and b) hydrolysable tannins (CT and HT). R = H: epicatechin, R=OH: epigallocatechin (reproduced from Behrens et al., 2003; Nierop et al., 2005). Where PC = procyanidins and PD = prodelphinidins.

Due to their effect in soils, tannins may be important for estimating long-term decomposition rates (Loranger et al., 2002). However, this may be site specific due to their ability to be leached through soil profiles if the right conditions exist, depending upon precipitation, temperature and availability of clay minerals for complexing (Kraus et al., 2003). In seasonal wet periods and rainstorm events on the savannah-rainforest boundary, significant leaching of these tannins may occur. High temperatures in this region may also induce high microbial activity (Zogg et al., 1997). Conversely, if

complexing minerals are present, tannins may be stabilised (Gu et al., 1994). To date, the fate of tannins in soils is still largely unknown (Nierop et al., 2005). However, it is now possible to assess the extent of tannins alongside lignin in soil via the use of ^{13}C-labelled TMAH with pyrolysis-GC/MS, which enables the discrepancy between the effect and fate of these distinct macromolecules in soil organic matter decomposition (Filley et al., 1999). The fate of tannins in soils from the tropical plants assessed in this study (as of Objective 3) will provide more information about the effects of site specific conditions on their degradation state.

1.2.10 SOM analysis in the tropics

Although many studies, especially those concerning climate change, have assessed the SOC of tropical soils due to their potential as either large carbon sinks or sources, few have considered the molecular composition of this SOC. Studies conducted in other regions of the world have shown the importance of understanding SOM composition for ecological purposes, such as nutrient cycling, plant physiology and microbial populations (Hättenschwiler and Vitousek, 2000). Those that have been conducted in tropical and arid regions have revealed interesting differences between biomes and sub-environments, linked to plant type, land use and environmental conditions (Guggenberger et al., 1995; Filley et al., 2008; Stewart et al., 2011; Carr et al., 2013). Many of the studies on molecular SOM in tropical regions have focussed upon agricultural systems, as opposed to or in comparison to natural ecosystems (Guggenberger et al., 1995; Olk et al., 2002; Rumpel et al., 2007; Filley et al., 2008). However, it is also important to consider natural ecosystems which may be susceptible to climate change and future land use pressures (Hammond and ter Steege, 1998) as these ecosystems may hold significant amounts of carbon in their soils (Batjes and Dijkshoorn, 1999), and their molecular SOM characteristics may be important for discerning climate or land use-induced changes (Swain et al., 2010).

1.3 Aims and Objectives of this Thesis

With current IPCC (2013) predictions forecasting a potentially destabilising annual increase in temperature and changes to precipitation patterns in eastern Amazonia, it is vital to understand how SOC stocks may be altered. It may be possible for large releases

of CO_2 currently held within the soil to create positive feedbacks into the global carbon cycle (Cox et al., 2000). Studies which connect molecular SOM characteristics to bulk SOC and above ground C stocks are essential to better characterise the overall C cycle in terrestrial systems. Given the lack of molecular SOM data from pristine soils on the climate sensitive savannah-rainforest boundary, it is critical to ascertain bulk SOC and its molecular composition in this region, in order to accurately estimate its stocks and fate. Assessing the composition of SOC is not trivial, however. Molecular components are influenced by numerous abiotic and biotic factors that may change over short distances and with fluctuations in climatic conditions (e.g. seasonality), which ultimately decide their fate. Thus, the overarching aim of this study is to characterise bulk and molecular SOC on the savannah-rainforest boundary. It will therefore provide a baseline for comprehending the factors influencing it, which may be altered with modifications to local weather patterns caused by wider climate change. Several sub-environments on two savannah-rainforest boundaries in central Guyana have been assessed. Investigation into the SOC stocks on both of these boundaries, and the molecular organic carbon characteristics on one boundary, have been undertaken.

The study was sub-divided into three sections each with its own objectives, as follows:

1) Field sites and preliminary soil classifications (Chapter 3).
   This first sub-section included an assessment of the two field sites (Wowetta and Surama) in Central Guyana, with respect to the heterogeneous vegetation sub-environments and their respective soils. This encompassed a distance of 2.5 km into the savannah and the rainforest at either side of the boundary.
   The specific objectives were:

a) complete reconnaissance surveys of both vegetation and soil types in the two areas of Wowetta and Surama in order to establish the range of sub-environments and soil types present for the most representative sampling sites,
b) to assess soil properties in the field and laboratory in which to make a preliminary soil classification for each site under the updated Food and Agriculture of the United Nations World Reference Base 2014.

2) Soil carbon stocks of sub-environments on the savannah-forest boundary of Wowetta and Surama in Central Guyana (Chapter 4).

This sub-section investigates the soil organic carbon stocks, using horizon depth, bulk density and total organic carbon measurements, within each sub-environment soil profile, to a maximum depth of c. 3 m in both Wowetta and Surama.

The specific objectives were:

a) to estimate the SOC stocks in full soil profiles (which extended up to a maximum of c. 3 m depth) of the range of sub-environments and associated soil types found on the savannah-rainforest boundary in the tribal lands of Wowetta and Surama,
b) to establish which sub-environments have greatest SOC stocks,
c) to compare the SOC stocks estimated to existing SOC data of the Amazon region and the wider tropics, in order to validate historical datasets against the contemporary data presented in this study.

3) Surface soil and vegetation input and organic molecular chemistry of SOM along the Wowetta transect (Chapter 5).

This sub-section utilised THM in the presence of ^{13}C-labelled TMAH to assess the state of lignin in surface (0-6 cm) soils of each sub-environment along the Wowetta savannah-rainforest boundary. The analysis also allowed for identification of tannin input, as well as carbohydrates. The specific objectives were:

a) to identify key vegetation biopolymers (e.g. lignin and tannins) within surface soils and vegetation inputs across the transect,
b) to identify carbohydrates which may be present within the surface soils and vegetation inputs,
c) to compare the degradation state of lignin across the sub-environment surface soils and the relative contributions of tannins, as well as carbohydrate contents,
d) to compare molecular SOM results (lignin) to bulk SOC results to assess differences between sites on the Wowetta transect and possible correlations between the two measurements.

Chapter 2. **Methodology**

2. Methodology

2.1 Overview of sampling techniques

Two areas were selected for sampling on the savannah-rainforest boundary in central Guyana, detailed information of this area and sampling sites is given in Chapter 3. These included one on the tribal lands of Wowetta, and a second on the tribal lands of Surama. This was done to account for changes and differences within sub-environments and their respective soils between an area of open savannah (Wowetta) and enclosed savannah (Surama). Prior to sampling a thorough two week reconnaissance was conducted to identify as many soils and vegetation types in the area as possible. The reconnaissance revealed that the greatest changes in soil type coincided with those in vegetation. Therefore, representative sites in both areas were identified through selecting a site at each vegetation type (sub-environment) found on the reconnaissance. Biomass samples (fresh shoot / leaf litter and wood litter) were sampled in triplicate from the surface of the soil pit and the surrounding area (within 0.25 m of the soil pit). Where no litter layer existed, such as in the savannah grassland sites, fresh shoots had to be sampled. Fresh grass shoots were also sampled in areas of savannah with trees in addition to leaf litter, as the grass itself did not form a litter layer. Soil was also sampled in triplicate from each horizon (soil layer) to a maximum depth of ~3 m. Samples were also taken in triplicate from 0-6 cm from the surface of each soil profile, in order to check for unseen differences in organic matter (e.g. via similar colouration) between the surface soil and underlying horizons. The thickness of 6 cm was chosen as the bulk density corer was 6 cm in diameter, and thus allowed a bulk density measurement to be made from this surface thickness. The latter measurement then also allowed the organic carbon stock (OC) to be calculated for the initial 6 cm soil depth. All samples were stored in sealed amber glass jars (glass was used to avoid plastic contamination). As soon as possible after being sampled, these jars were placed in a freezer in order to avoid any out of situ degradative changes. During sample collection, soil profile descriptions were taken and recorded according to Payton (2010). These included soil texture (‘hand texture method’), soil colour (Munsell Colour chart) structure, boundary depth of each horizon and full sampling depth of the soil profile (see Appendix D for field descriptions). Water table depth from the surface was also recorded using measuring tape as soon as the water table had been breached. Bulk density sampling,

volumetric water content and pH measurements are detailed below in Section 2.2, 2.3 and 2.7.

2.2 Bulk density (D_b)

Bulk density includes soil solids and pores within a given volume of soil sampled: it is the 'mass unit' of dry soil. During field work, a metal core with a known volume of 100 cm^3 was used in order to take an undisturbed soil sample from a cleaned soil pit surface. This was done in triplicate at each site, excluding the pisoplinthic forest (PF) and pisoplinthic savannah grassland (PSG) where the pisoplinthic material (hardened aggregates of iron oxide) prevented accurate sampling (see Chapter 3 for site and soil descriptions). Where sampled, stones were minimal and so no correction must be made to account for this. These samples were weighed, oven dried at 105 °C for 48 hours, reweighed and placed back in the oven for 4 hours before reweighing to check dryness. Accuracy was in accordance with the British Standard for bulk density measurements (maximum 0.1 g change in weight between oven drying episodes). Using the above measurements the equation below was used to calculate the bulk density:

Equation 2.1Bulk density

$$Db = Wd / Vs$$

D_b = bulk density in cm^3,

W_d = weight of oven dry soil (g)

V_s = volume of soil (solids and pores) (cm^3)

2.3 Volumetric water content (θ)

This calculation involves the use of measurements from Section 2.2: weight of oven dry soil, known volume of bulk density sample and bulk density (D_b), (Rowell, 1994). Firstly, the gravimetric water content needs to be calculated:

Equation 2.2 Gravimetric water content

$$\frac{mass\ of\ water\ in\ soil\ (g)}{mass\ of\ oven\ dry\ soil\ (g)} = gravimetric\ water\ content$$

This measurement can then be used to calculate the volumetric water content (θ), as below:

Equation 2.3 Volumetric water content

$$\theta = gravimetric\ water\ content\ x\ bulk\ density$$

2.4 Standard Laboratory Procedures

Dichloromethane (DCM) and methanol (MeOH) were used for cleaning equipment and extracting soil and vegetation samples. These were supplied as laboratory grade solvents by LSS Ltd. (UK) and then distilled on a 50 plate Oldershaw column. Glassware and (accelerated solvent extraction) ASE extraction equipment was cleaned by soaking in Decon 90, scrubbing and then rinsing multiple times with tap water, followed by 15 MΩ.cm deionised water. After drying in an oven at 60 ˚C, glassware was then rinsed multiple times in DCM and MeOH and then in the solvent to be used three times.

Glass wool and quartz tubes used to contain samples to be run during analysis with both on-line thermally assisted hydrolysis and methylation (THM) in the presence of tetramethylammonium hydroxide (TMAH) as well as flash pyrolysis gas chromatography-mass spectrometry (Py-GCMS) were respectively extracted with DCM:MeOH (93:7; v:v) in a Soxhlet apparatus for 24 h and rinsed with DCM before use.

2.5 Sample preparation

An aliquot of all samples was taken in order to conduct analysis upon. The remainder of the sample was kept frozen, in case of future use. All biomass and soil samples were then freeze dried and ground to a fine fraction (<0.25 µm). Soil samples were ground using either an agate ball mill or an agate pestle and mortar. Biomass samples were ground using liquid nitrogen in a cryomill. All samples were passed through a <0.25 µm sieve to verify the particle size. Subsequently, samples were extracted using ASE as detailed in Section A. 2. Appendix A. This method was chosen for use after an experiment comparing it to Soxhlet with reference to efficiency, reliability and cost (see below).

2.6 Total organic carbon (TOC) and carbon storage (OC)

TOC was determined using 0.1 g of freeze dried, sieved and ground sample. 1 mL of hydrochloric acid 4.0 mol/L, was added to each crucible, which was allowed to drain from the sample for 4 hours. The samples were then placed in the oven at 60 to 70 °C for 16 – 24 hours. The Leco CS230 Carbon/Sulphur Analyser was calibrated prior to use and the organic carbon content of a reference soil measured. A blank was used with each sample set.

The organic carbon content was calculated as follows:

Equation 2.4 Organic carbon percentage on oven dry basis

$$Organic\ carbon, \% = \frac{C_s - C_{bl}}{D}$$

Where C_s is the measured carbon percentage of the sample, C_{bl} is the measured carbon percentage of the blank and D is the dry matter factor.

After calculating both TOC and D_b it was then possible to calculate the weight of organic carbon (t C ha-^{1}) of soils by horizon, using the following formula:

Equation 2.5 Organic carbon (OC)

$$OC = d\ x\ Db\ x\ TOC$$

Where d = depth (cm), D_b = bulk density (g cm^3), TOC = total organic carbon (%).

Through using TOC as a percentage, the conversion from g per cm^2 to t per ha, is avoided. This conversion has caused confusion and errors in the past (Cannell and Milne, 1995).

Where horizons were missing bulk density data, due to an inability to sample them (e.g. water saturation or depth of horizon), bulk densities were estimated using surrogate values determined by pedotransfer functions or rules (Bouma and van Lanen, 1987), as used in Batjes and Dijkshoorn (1999). The primary surrogate option was to calculate the mean bulk density of the soil profile and use this value for missing horizons within the profile. The secondary option (if only one bulk density value was available for a profile) was to use a mean soil profile bulk density value of a corresponding soil type and the one available horizon.

2.7 pH

A suspension of soil was made through weighing 5 ± 0.1 mL freeze dried, seived and ground soil (Section 2.5) in a screw-cap bottle with 25 mL water from a measuring cylinder. This was then shaken for 1 hour. After this, the suspension was left for between 1-3 hours in order to equilibrate. A pH electrode and meter was calibrated prior to use using standard buffer solutions, after which the pH-H_2O of the suspension was then measured and recorded in the laboratory.

2.8 X-Ray Diffraction (XRD)

X-ray diffraction was undertaken on a subset of samples in order to have a preliminary view of the minerals present within the study sites, for the purpose of aiding a preliminary soil classification.

X-ray diffraction was carried out using a PANalytical X'Pert Pro Multipurpose Diffractometer (MPD) fitted with an X'Celerator* detector. Diffraction data was acquired by exposing powder samples to copper-Kα X-ray radiation, generated from a copper (Cu) anode supplied with 40 kV and 40 mA, which has a characteristic wavelength (λ) of 1.5418 Ångstroms. Data sets were collected over a range of 2-70º 2θ with a step size of 0.0334º 2θ and nominal time per step of 250 seconds, using the scanning X'Celerator detector (hence the seemingly long counting time per step). Fixed anti-scatter and divergence slits of ¼º and ¼º were used together with a beam mask of 10mm and all scans were carried out in 'continuous' mode.

Samples were prepared by packing approximately 500 mg of dry milled material into 16-mm diameter steel sample wells. A spinning stage was used during analysis to maximise the randomisation of crystallites contributing to the diffraction pattern.

Phase identification was carried out by means of the X'Pert accompanying software program PANalytical HighScore Plus V3.0 in conjunction with the ICDD Powder Diffraction File 2 Database (1999), ICDD Powder Diffraction File 4 - Minerals (2012), the American Mineralogist Crystal Structure Database (March 2010) and the Crystallography Open Database (February 2012; www.crystallography.net). Comparisons of observed data sets with reference patterns were carried out on a 'best-fit' basis.

* The X'Celerator is an ultra-fast X-ray detector that uses RTMS (Real Time Multiple Strip) technology. It operates as an array of a hundred channels which can simultaneously count X-rays diffracted from a sample over the range of 2θ angles specified during a scan. The X'Celerator is therefore able to give produce high quality diffraction data in a significantly shorter time period than an older style diffractometer would require.

2.9 THM in the presence of unlabelled TMAH and ^{13}C-labelled TMAH

In the presence of unlabelled and ^{13}C-labelled tetramethylammonium hydroxide, on-line thermally assisted hydrolysis and methylation (THM) was performed using a pulsed mode open pyrolysis system specifically a CDS 1000 pyroprobe unit (Chemical Data Systems, USA) fitted with a platinum coil and a CDS 1500 valved interface.

Sample weight (mg) was defined based upon total organic carbon (TOC %) (~1 mg for organic soil and ~20 mg for mineral soil samples) which depended upon the sub-environment. The amount needed was weighed into a quartz pyrolysis tube plugged with extracted silica wool to securely hold the sample in place. Immediately prior to pyrolysis, 3 µl of 5α-androstane at a 1 mg/ml concentration with dichloromethane was injected onto the sample. 10 µl of unlabelled or ^{13}C-labelled TMAH (25% w/w) was also injected immediately before pyrolysis. Each quartz tube was inserted into the platinum coil of the pyroprobe and then heated 610 °C for 10 s (20 °C / ms temperature ramp). The platinum resistance-heated coil was interfaced with an HP5890 gas chromatograph with an open split and thermochemolysis products were separated on a 60 m HP5-MS column (0.25 mm internal diameter, 0.25 µm film thickness). Helium was used as a carrier gas due to its inert properties, at a flow rate of 1 ml/min. A solvent delay of 10 minutes was used. The GC oven was programmed from 50 to 220 °C at a rate of 1.5 °C / min, then isothermally held for 1 minute and finally raised to 320 °C at a 15 °C / min rate and held for 16 minutes. Compound detection was carried out using a HP 5973 mass selective detector in full scan mode (m/z 50-700).

Compound identification was based upon the NIST98 spectral library of that >95% and known retention times and ion fragmentation (Hatcher et al., 1995; del Rio et al., 1996; Filley et al., 2006). Compound amounts within each sample were calculated using compound weights against the internal standard.

Good peak separation was allowed by a slow increase in temperature of 1.5 °C / min from 50 °C to 220 °C in the GC oven. This ensured complete peak separation and accuracy in the estimated peak area based on the internal standard (IS). The high temperature used (610 °C) has been proven to be effective at cleaving condensed tannins (CTs) into characteristic thermochemolysis products (Nierop et al., 2005).

Samples were run using the same conditions on the py-GC MS in order for reproducibility of peak retention times and areas. These conditions included solvent type, temperature, contact time of sample with both IS and TMAH and the ratio of sample to TMAH and IS. Through this, direct comparisons of different sites can be made.

Samples were run via THM with both TMAH and ^{13}C-labelled TMAH in order to assess the contribution of non-lignin source to the phenolic signal.

2.10 Aromatic hydroxyl contents

In order to determine the contribution of non-lignin sources to the phenolic signature, the aromatic hydroxyl contents were calculated. The calculations from the work of Filley et al. (1999), Filley et al. (2006) and Mason et al. (2009) were used and are given in Appendix B. An initial assessment of samples with unlabelled TMAH allowed for the individual compounds to be identified and the appropriate baseline fragment ion ratios determined. These ion ratios are required in accurately calculating the percent of the ^{13}C addition with the use of ^{13}C-labelled TMAH. From the Wowetta sampling transect, triplicate solvent extracted surface soil and biomass samples from each site were analysed when using unlabelled TMAH, and duplicate when using ^{13}C-labelled TMAH, due to the restricted amount of derivatising agent.

The process of THM with ^{13}C-labelled TMAH allows for the acidic oxygen functional groups of the products to be methylated by the ^{13}C-labelled methyl groups. This then reflects the number of acidic oxygen functional groups present on the individual products, which is indicated through the additional molecular ion for each product, as compared to that of the unlabelled TMAH product.

2.11 Temperature calibration of pyroprobe

In order to make sure a constant temperature was used for all sample runs, each time the pyroprobe coil was renewed, the pyroprobe was recalibrated to 610 ˚C inside the quartz tube. The temperature inside the pyroprobe differed from the programmed temperature due to the insulating properties of the glass tube and glass wool in which the sample was placed. The pyroprobe was calibrated according to Bashir (1999). Where possible, however, the pyroprobe was calibrated by the manufacturing company to 610 ˚C prior to use.

Calibration was performed using 5 different salts of known melting points. These salts were inserted into the quartz tubes with glass wool used to plug each end. Each tube was then placed into the coil of the pyroprobe and inserted into a brass heating block of a 340 ˚C constant temperature. Gas flow rate of helium carrier gas was maintained at 2-10 mL/min. See Table 2.1 for the salts used and their expected melting points:

Table 2.1 Salts used for calibration and expected melting points

Salt	Expected Melting Point (°C)
PbCl2	501
LiCl	605
CsCl	646
KI	681
KCl	770

A glass window was used to observe melting points and initial and fully melted temperatures were recorded. This was repeated three times in order to calculate an average to plot. The temperature was initially set to that lower of the expected melting point and increased until the melting point was observed. The actual and observed melting points were then plotted on a graph so that the observed temperature for the pyroprobe could be determined.

2.12 Mass yields and lignin parameters

Mass yields from thermochemolysis products were determined through the use of THM in the presence of TMAH. The internal standard (IS) 5α-androstane was used with an assumed relative response factor (RRF) of 1 (for example as used in Richoz (2012)), which allowed semi-quantitative measurements of the differing amounts of thermochemolysis products across the sites and down the soil profiles in each horizon. Measurements were semi-quantitative as an internal standard was not used during solvent extraction of samples, see Section 2.13.

Several lignin parameters were used to assess composition and characteristics between sites and depths. Lambda (Λ) is the sum of all of the 8 dominant lignin derived phenols (G4 + G5 + G6 + S4 + S5 + S6 + G18 + P18) normalised to 100 mg of OC (Hedges and Mann, 1979; Hedges et al., 1982; Kögel, 1986). Individual compound yields used in calculating Λ allow changes within relative degradation dynamics to be assessed. S/G and C/G ratios were also used; these are weight ratios of total syringyl and cinnamyl phenols compared to total guaiacyl phenols. S/G reflects the relative abundance of angiosperms (S/G > 0) to gymnosperms (S/G = 0) and so is used for assessing vegetation inputs. These ratios are calculated through the following: OC-normalised syringyl amounts (S4 + S5 +S6) / OC-normalised guaiacyl amounts (G4+G5+G6), (Hedges and Mann, 1979). C/G ratio indicates the amount of non-woody tissue as the compound cinnamyl is only produced in significant amounts by non-woody vascular plants. This is calculated through the sum of normalised amounts of cinnamyl (P18 and G18) / normalised amounts of guaiacyl, (G4 + G5 + G6), (Hedges and Mann, 1979).

2.13 THM Reproducibility

The accuracy for manual peak integrations was tested to ensure that results were reproducible. This was done by repeating the integrations of 4 peaks within one TIC five times. Results showed that percent relative standard deviation (RSD) ([std dev/av]*100) varied from 0.42 to 1.50%.

These RSD values show that reproducibility is relatively high and thus repeatability is not a concern within this project, as has been expressed in previous work (Kaal and Janssen, 2008).

2.14 Statistical analysis

Statistical difference tested for via methods such as General Linear Model (GLM) and Kruskal Wallis may prove unreliable when sample size is small relative to the variability in the data. Due to the relatively small sample size in this study (3 field replicates of all samples and 2 instrument replicates of THM thermochemolysis analysis) the statistical difference of values between sites was evaluated using the standard error of the mean (SE). This is represented on graphs as error bars (±) throughout the thesis.

Chapter 3. **Field sites and soil classifications**

3.1 Introduction

Due to the potential for deforestation and land use alterations with predictions of a warmer and drier climate in eastern South America in the next century and beyond (IPCC, 2013), it is vital that detailed contemporary soil surveys of vulnerable regions are undertaken and that the biomes are monitored over time to document change. The range of soil types and their characteristics (such as texture, water content and organic matter content) need to be measured in order to improve understanding and predictions of changes that may consequently take place (Raich and Potter, 1995). For example, large releases of CO_2 may occur as vegetation inputs, soil cover and microbial communities change (Nobre et al., 1991; Zogg et al., 1997; Laurance and Williamson, 2001).

Therefore this chapter aims to:

a) Conduct a literature review on the existing knowledge of climate, vegetation, geology and soil types in the study areas,
b) Undertake a ground-truthing reconnaissance survey of the study areas in order to evaluate a sampling strategy and sampling sites that best represent the area and,
c) Describe the sub-environments sampled and detail the soil characteristics measured, giving a preliminary soil classification to each site under the Food and Agriculture Organisation of the United States (FAO) guidelines (IUSS, 2014).

3.2 Existing knowledge of the study area

3.2.1 Location

The study area is located in Guyana, South America. Specifically, it is within the Northern Rupununi region of central Guyana (Figure 3.1: inset). The Rupununi region is situated in the north eastern part of the Takutu Basin – a Mesozoic graben between parallel faults which form the Pakaraima Mountains to the north and Kanuku Mountains to the south. This basin is 280 km long and 40 km wide, stretching from Guyana into Brazil. Within this basin the Northern Rupununi savannahs border rainforest to the north, east and south-east of Guyana. The study sites are within the tribal lands of Wowetta at 4°01’02.5”N, 59°04’06.0”W and Surama at 4°08’30.4”N, 59°03’56.6”W. Wowetta lies on the very north eastern savannah-rainforest boundary of the Rupununi. Surama is in a 5 square mile enclosed area of savannah to the north of the vaster expanse of the Rupununi and the Takutu Basin (Figure 3.1). Although the area of savannah in Guyana is relatively small, it is understudied, particularly in relation to SOC characteristics, and its pristine quality provides a unique opportunity to investigate this at such a climate sensitive interface.

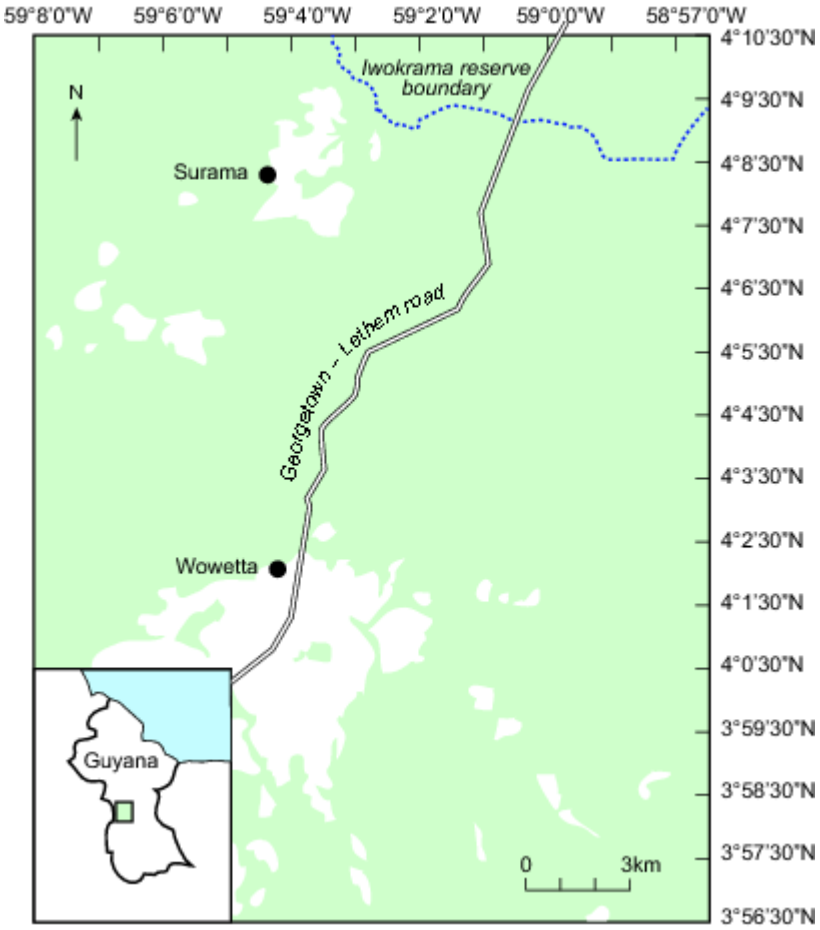


Figure 3.1 Location of study sites Wowetta and Surama in Central Guyana. White areas indicate the Northern Rupununi savannah and green areas indicate rainforest. Inset: the location of the study areas within Guyana (Google Maps 2014).

3.2.2 Climate

The savannah is classified as a drier tropical wet-dry climate than the rainforest, and has one wet (April to August) and one dry season (September to March). The rainforest has a tropical wet climate with two wet and dry seasons throughout the year. The first forest wet season corresponds to the savannahs, and the second lasts from mid-November to February. The dry season is longer than the wet within the savannah (7 months compared to 4) and has high temperatures but lower humidity than in the wet season (Suggett, 1964; Bovolo et al., 2012).

The most current precipitation data ranges from 1400-1800 mm annually, whilst annual temperature variations are lower (25 – 27 °C), indicating the stronger influence of precipitation on the savannah-rainforest boundary (Bovolo et al., 2012). Maximum

temperatures occur in April and October post-equinox and relative humidity is high at 70 % (Bovolo et al., 2009). Recent IPCC (2013) predictions of a hotter (0.5 – 2 °C increase) and drier (up to -10 % decrease in precipitation) climate within much of South America, including Guyana, in the next 50+ years carries the consequential risk of forest die-back and potential savannah expansion. Bovolo et al. (2012) highlighted that the savannah dry season varies spatially and in intensity between years, likely due to ENSO and rain-shadow effects from the Guiana Highlands.

Consistent winds throughout the year mean that temperatures are lowered. During the wet season soils become flooded and are heavily leached. They are subsequently hardened by dry conditions in the dry season (Suggett and Braun 1964). The seasons are better distributed within the forest where it is more humid due to greater canopy cover, and dews appear all year round (Suggett and Braun 1964). Due to these seasonal conditions, it is expected that soil type and SOM characteristics will differ between savannah and rainforest: greater SOM degradation may occur where leaching is most pronounced, whilst lesser SOM degradation may occur where soils remain wettest throughout the year.

Land use and land use change may also affect soil type, however pristine areas have been targeted for assessment in this study. Intense precipitation pulses during the wet season may cause effective leaching and runoff of SOM (Pereira et al., 2014), whilst exposure to sunlight in savannahs during the dry season may favour photo-degradation (Austin and Vivanco, 2006). Microbial community composition and activity will also be affected by these wet and dry seasons, through suppression of activity during anaerobic periods and increased activity in dry periods (Freeman et al., 2001). This may cause variation in SOM degradation extent depending upon soil type or water retention. The current chapter will identify soil types and characteristics, whilst Chapters 4 and 5 will explore the SOM characteristics and discuss such abiotic and biotic influencing factors of sample sites.

3.2.3 Geology

The following sections review studies undertaken at the Guyana Lands and Surveys Commission (GLSC), the Guyana Geology and Mines Commission, Iwokrama research base and the University of Guyana. This entailed sourcing materials on geology, soils

and vegetation in the study area. It also includes further research undertaken via journals accessed on the internet, which are referenced in the following passages.

Figure 3.2 shows the location of the Takutu Basin within central Guyana, which encompasses the Northern Rupununi savannahs, including the Wowetta field site. This basin developed within the heart of the Precambrian Guyana shield. A generalised geological map of the Takutu Basin and surrounding Archean Guyana shield can be seen in Figure 3.3. The second field site of Surama is located to the north of the basin in the surrounding Precambrian metavolcanics and Iwokrama formation. The latter formation consists of felsic volcanics and associated subvolcanic granite, dating from around 4000 – 2000 million years ago.

Figure 3.2 Location of the Takutu Basin in central Guyana (adapted from Crawford et al. (1985).

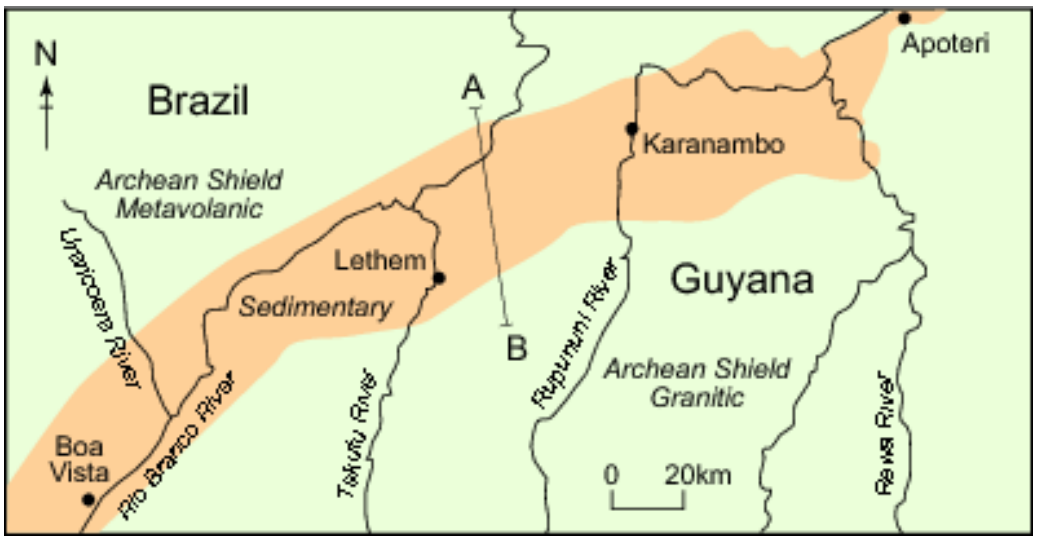


Figure 3.3 Basic geological map with A-B cross section of the Takutu Basin relating to Figure 3.4 (adapted from Crawford et al. (1985).

Including the Precambrian Guyana shield, there are seven known layers of geology within the Takutu Basin. These layers can be seen in Figure 3.4. Daniel and Hons (1984) describe the Precambrian crystalline bedrock complex as consisting of granites, gneiss, amphibolites, rhyolites and quartz, in addition to metamorphic rocks of both volcanic and sedimentary origin. In the Northern Rupununi savannahs and Kanuku Mountains, this complex dates to 2700 million years BP. Above this lies the Apoteri formation:1700 m of mafic volcanics in the form of grey tholeiitic basalt, dating to the Jurassic and potentially older (i.e. Proterozoic) ages. This basalt accumulated to a significant thickness, but was likely eroded and potentially faulted prior to the next formation. Deposition of the Manari formation over the Apoteri through alluvial and lacustrine environments subsequently took place. This formation consists of grey-brown shale, siltstone and non-marine carbonates and are c. 300 m thick, dating to the early Jurassic. This was also eroded following deposition and shows evidence of block faulting. The c. 1200 m thick Pirara formation overlies this, which formed during arid climatic conditions of the early Jurassic allowing evaporites to be extensively deposited. In a mix of lagoon and shoreline non-marine environments, interbedded halite and shale accumulated. Minor occurrences of limestone and marl also constitute this formation. Towards the edges of the basin, the deposits grade into fine-grained clastics. This is covered by c. 3000-5000 m of late Jurassic sedimentary rocks of the Takutu formation, consisting of reddish brown mudstone, shale and sandstone and thin limestone (Sinha, 1968). The primary and secondary source rocks of these deposits are thought to be the

Kanuku (southern) and Makarapan (north eastern) horsts respectively. Berrangé (1977) details the 'Nappi Laterite formation', which stretches over the Mesozoic graben of the northern savannahs. This formation overlies 3-4 km of sediment and is of the Eocene age (Sinha, 1968). Dating from the Oligocene to Pleistocene, the Northern Savannahs formation of clastics sits above the Nappi Laterite. Holocene sand, silt and clay sediments of the Rivers formation are presently accumulating in the basin.

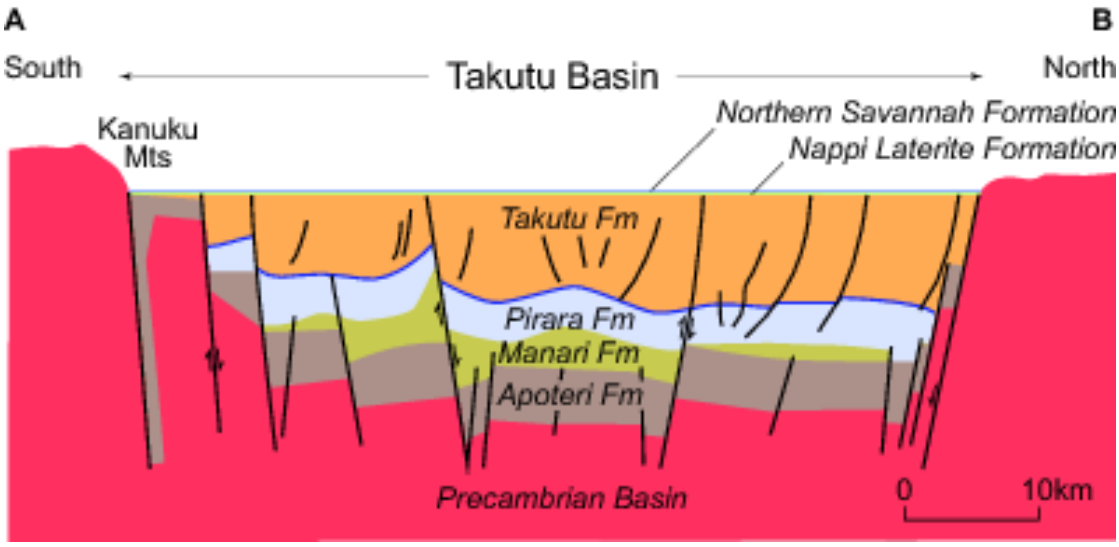


Figure 3.4 Geological cross section of the Takutu Basin, showing several layers of geology (adapted from Crawford et al. (1985).

3.2.4 Soils

The influence of the Nappi Laterite formation can be seen within the soil development of the soil types previously recorded, as detailed below. Leaching of the parent rock described above during the Eocene would have led to the predominance of insoluble iron and aluminium ions constituting the laterite formation. This would have occurred under high temperatures and a wet-dry seasonal climate, with rain water percolating soluble ions, and dry conditions bringing the dissolved ions back to the surface via capillary action. With the return of the wet season, the soluble ion salts deposited on the surface were then removed via surface run-off, leaving the insoluble ions. Accumulation of sesquioxides in wet areas resulted in the formation of plinthite. After a drop in the ground water level, potentially by a lowering of the rock beneath, this plinthite irreversibly hardened to form laterite. Due to a high erosion resistance, relief inversion of the landscape developed. This has resulted in the present-day gently undulating

landscape of laterite and seasonally waterlogged flats (Sinha, 1968; Daniel and Hons, 1984).

Such weathered rock is an important feature of the ferralsols mentioned below. The Northern Savannahs and Rivers formations lying above the Nappi Laterite are also key features within the soil formation: these sandy deposits led to poorly developed regosols discovered in the area.

Within the study region two soil groups have been distinguished and mapped by Suggett and Braun (1964): 'soils of the Youthful Pediplain' and 'old sandy terrace and residual sand soils'. In 2005, Guyana Lands and Surveys Commission created an updated map in which the two aforementioned soil units are included (GLSC, 2005). These two soil groups are described below.

'Soils of the Youthful Pediplain' create a matrix within which the soils described below exist.. These soils have formed from the erosion and weathering of crystalline rocks and are covered by high forest vegetation. They are classed as red-yellow ferralsols and low humic gleysols.

The 'Old sandy terrace and residual sand soils' are deep and unconsolidated sands deposited from rivers and crystalline rock erosion and are largely undeveloped. These residual soils form a gently undulating landscape and may have small wetter areas with a higher clay percentage within them where depressions meet high water tables. They are classed as regosols (brown quartz sand), and characteristically have little structure or profile development due to a high proportion of sand. Excessive drainage occurs leading to droughty conditions in the dry season but flooding may also ensue in the wet season. Humus staining can be evident from greyish brown colouring at the surfaces, with a gradual change down the profile. These soils are extremely acidic and infertile (pH 4 – 4.5).

3.2.5 Vegetation

Forests in the study region are broadly characterised as 'lowland tall, evergreen, seasonal forest' on the Vegetation Map of Guyana (Huber, 1995). Species such as *Goupia* and *Manikara* exist here. Non-flooded tall, evergreen forest also grows, with species such as *Eschweilera, Licania, Catostemma* and *Chlorocardium.* Dry, deciduous

forest borders the savannah and is characterised by the species *Goupia glabra, Couratari, Sclerolobium, Parinari, Apeiba, Peltogyne, Catostemma, Spondias mombin* and *Anacardium giganteum* (ter Steege, 2000). Forests may also degrade into Muri-scrub (*Humiria balsmaifera*) where fires and floods are frequent (ter Steege, 2000).

Eden (1964) found that throughout the Rupununi savannahs there exists a mosaic of different vegetation types. These include wooded savannah, herbaceous savannah with grass or sedge dominance, sedge swamp, palm swamp, galleria (river bank) forests and forest islands. More recent studies have noted lowland shrub savannah within in the Northern Rupununi (Huber, et al., 1995). Here grass species such as *Trachypogon, Axonopus* and shrubs *Curatella* and *Byrsonima* can be found (ter Steege, 1998) addition to sedges such as *Rhynchospora* and *Bulbostylis* (ter Steege, 2000). These shrubs along with others have been suggested to be fire-climax vegetation, i.e. this plant community is maintained by the occurrence of fire (ter Steege, 2000).

3.3 Reconnaissance survey

3.3.1 Local knowledge

Before conducting a reconnaissance survey of the savannah-rainforest boundary around Wowetta and Surama, meetings were held with local residents to gain local current and historical land use knowledge of these areas. Specifically, areas were discussed in which residents knew anthropogenic activity (such as agricultural practices) had not been taking place. Areas where this activity was currently occurring and had occurred within recent history (c. 50 years) were also discussed and some of these sites were also visited with local farmers. This was done to verify that the sites chosen were not under direct current or recent anthropogenic influence, so that the data collected was known to be as unaffected by human influence as was possible.

3.3.2 Ground truthing

A two week ground truthing reconnaissance survey was subsequently carried out within the tribal lands of Wowetta and Surama. The reconnaissance areas were chosen as advised (as above) by local residents, in order to ensure that a representative sampling of the savannah-rainforest boundary at these two areas was undertaken (Figure 3.5). This survey entailed using landscape form and vegetation types to evaluate where soil type and edaphic conditions may also change.

Soil testing was done using an Eijkelkamp auger to a maximum of 2 -3 m depth to provide an overview of the soil profile. Texture (hand test), colour (Munsell colour chart), boundary type of each horizon, the depth of each horizon, water table height and occurrence of plinthic material, (preferred term of FAO for hard or soft laterite material, formed through continual wetting and drying cycles), were deciphered and recorded in the field. They were then used to assess the study area and choose representative sites for sampling.

The savannah sub-environments described in Eden (1964) were found: wooded savannah, herbaceous savannah with grass and sedges, palm swamp and forest islands. These sub-environments are noticeable mainly through vegetation type, however, elevation or slope may also alter edaphic conditions and so soil was tested at both vegetational changes and changes in slope and elevation. Where possible, vegetation species were identified by local field assistants.

The occurrence of soft and hard plinthic material was common to all sites investigated and forms a major part of the soil formation. These findings are consistent with the Nappi Laterite Formation (Berrange, 1977) and the undulating laterite landscape formerly described in Section 3.2.4 (Sinha, 1968; Daniel and Hons, 1984; ISSS, 1998). The soft plinthic material identified highlights the intermediate stage of laterite formation, in that the accumulated sesquioxides have not hardened to laterite in all the sites investigated. Quartz sand overlying plinthic material was also common (particularly in savannah sites) and thus was a similarly important part of the soil formation. This is consistent with the North Savannah and Rivers formation overlying the Nappi Laterite.

Soils varied depending upon landscape position in both areas and were either sub-orders of plinthosols or gleysols as according to the specifications of FAO World Reference Base for Soil Resources (IUSS, 2014). These soil types are similar to those cited in the literature (Section 3.2.4), in that gleysols were also found. However, the other major group of soil classified were plinthosols, conversely to the ferralsols found by Suggett and Braun (1964) and as mapped by GLSC (2005). Many characteristics of these two soil types are the same, however plinthosols have >15% volume of weakly-cemented concretions, nodules or mottles compared to ferralsols, which have lower concentrations (FAO, 2014). It is also likely that this difference in soil type allocation may also be due to the revisions of the soil classification system in recent years. The regosols described in earlier work bear a close resemblance to the upper horizons of some of the plinthosols described in this study (GLSC, 2005). The difference in soil type allocation may be because no plinthic material was found in those soils classified as regosols. However, in this study, evidence of plinthic material was found throughout the reconnaissance survey within the soil profile.

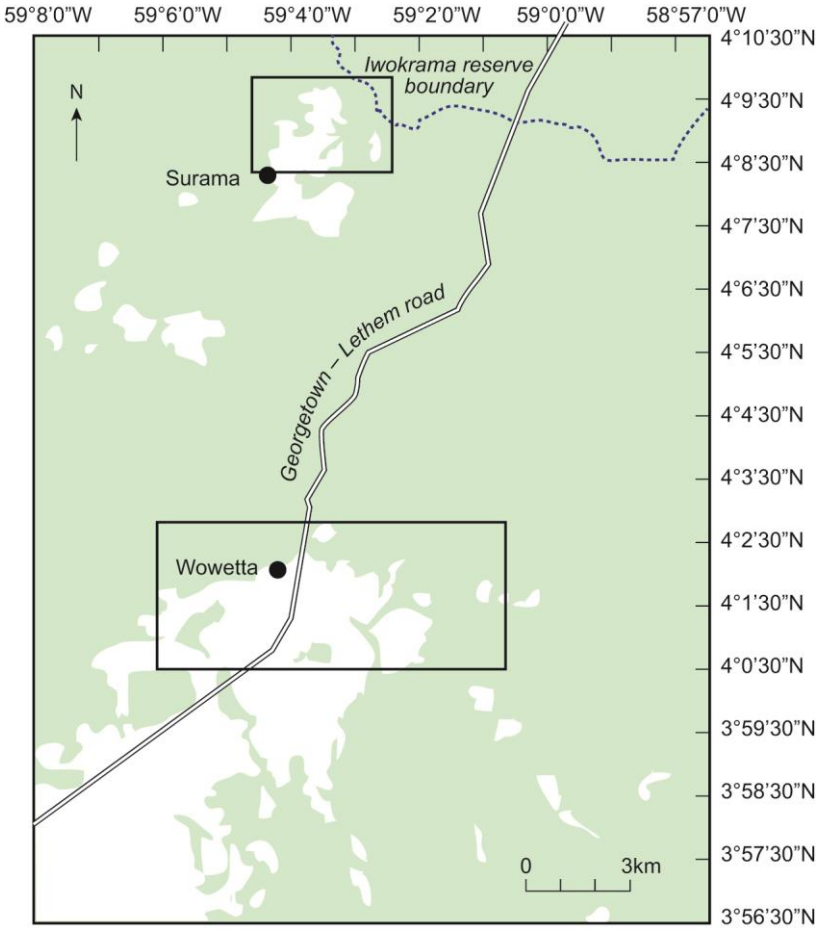


Figure 3.5 Reconnaissance areas within black boxes at Wowetta and Surama. White areas indicate savannah and green areas indicate rainforest.

3.4 Sampling methodology

Eighteen sample pits were excavated in February 2012. Full details of the sampling methodology for soil and vegetation can be found in Chapter 2, Section 2.1.The properties of each soil profile are fully detailed in Appendix D.

The occurrence of precipitation was noted prior to and during sampling as this can affect properties such as the depth of the water table. It was noted that heavy rain occurred within a week of sampling, however, no precipitation occurred on days when samples were taken. Sampling was undertaken at the beginning of the dry season, starting at Wowetta and finishing at Surama.

It must be noted that the measurement of the water table is not static and will alter depending upon season and length of time since a rain event. For example, after a rain event or during the wet season the water table may be higher. Therefore this measurement is a guideline rather than being absolute. This may have implications in understanding soil organic matter (SOM) content and degradation, i.e. there may be changes in microbial activity with differences in soil water content at different times of year. Ideally it would be measured throughout the year and averaged for a more accurate value, however this was not within the scope of study. The appearance of mottled horizons from iron oxide redox reactions may thus also change with water table depth throughout the year.

The depth of occurrence of either the pisoplinthic or the plinthic horizon from the surface was also measured. These two plinthite forms were found throughout the study areas and indicate the presence of iron oxide undergoing redox reactions through wetting and drying cycles (as previously mentioned in Section 3.3.2). Some sites consisted of horizons with ≥ 40% volume of concretions or nodules of iron oxide ≥ 2mm (pisoplinthic) or mottled colours indicating redox reactions of iron oxide (plinthic horizon). A moisture probe was also taken into the field, however this equipment failed to work (probably due to high humidity) and therefore no results could be collected for this measurement.

These measurements were taken in order to provide a good basis for soil type identification as according to the guidelines of the FAO World Reference Base for Soil

Resources 2014 (IUSS, 2014). Total organic carbon (TOC) and pH measurements in the laboratory (see Chapter 2) further verified these field identifications, however, due to time constraints, laboratory measurements as recommended in van Reeuwijk (2002) could not be undertaken.

3.4.1 Wowetta transect sample sites

Using the information gained from local residents and from the reconnaissance survey, sites were selected along a 5 km transect from the savannah into the rainforest within the Wowetta area. A transect was chosen as the best sampling method as it allowed the greatest number of sub-environments to be included within the study. These sub-environments vegetation grades from areas of pure grassland (SG) to isolated savannah trees (ST) and denser canopies of woodland (SW). Palm trees and other savannah tree species grow alongside grasses and sedges in swampier areas (SS). The savannah is dissected by tongues of rainforest island (FI) extending from the main body of the rainforest or occurring as isolated groups. The transition zone (T) between savannah and rainforest at these areas and at the main body of rainforest is abrupt.It is marked on this transect by a border of muri scrub (c. 5 m tall). This vegetation establishes post-fire and it is known that a natural fire occurred in this area in 2005 (personal communication with local indigenous community, 1st February 2012). The rainforest section of the transect consists of the three sub-environments: swamp forest (SF); mixed tree species forest (MF); and pisoplinthic mixed tree species forest (PF). The rainforest has a high, but not emergent level, canopy (c. 30-50 m tall). The occurrence of palm trees was noted in SF, as in SS. A mixture of shrub level plants also exist beneath the canopies of the rainforest sites.

The location of these sub-environments along the 5 km transect are shown below in Figure 3.6 via GPS points. A photograph of each sub-environment is then depicted in Appendix C to show the differences between vegetation.

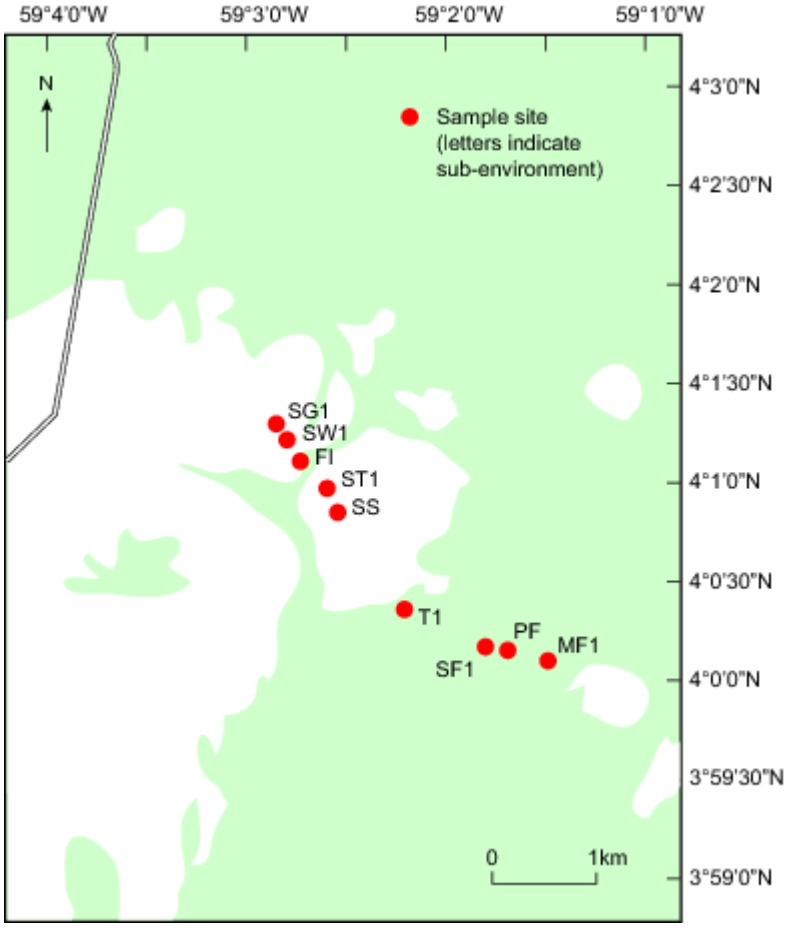


Figure 3.6 Wowetta transect GPS sampling points in closed red circles with specific site abbreviations labelled adjacently. GPS points have an error of up to 10 m. Green indicates rainforest vegetation, white indicates savannah.

3.4.2 Surama sampling sites

Due to the size of the savannah in Surama and the close proximity of local residents, it was not feasible to create a transect of savannah and rainforest sites. This is because the areas of most pristine savannah and rainforest are not aligned but are dispersed throughout the landscape. Where it was impossible to guarantee savannah sites were chosen without recent or current land use, certain sites within the rainforest have been set aside as a reserve for eco-tourist attractions in which no anthropogenic activities such as burning or agriculture are allowed within the last 50 years (oral communication with residents). Thus using local knowledge, sites were chosen from the most pristine savannah sites and the rainforest reserve.

The sites consist of four forest, four savannah and one transition site. Savannah sites include savannah grassland (SG), savannah grassland on a pisoplinthic mound (PSG), savannah tree (ST), and savannah wood (SW). It should be noted that the vegetation found on the pisoplinthic mounds found at both Wowetta (forest) and Surama (savannah) show that these mounds do not result in a specific vegetation type. The transition (T) site at Surama has vegetation consisting of tall grasses and shrub trees, but no muri scrub as in Wowetta. As muri scrub is often found post-fire, the lack of its presence may suggest the lack of recent fire along the savannah-forest boundary. No savannah swamp was found in this area, as opposed to Wowetta. Forest sites consisted of swamp forest (SF), mixed tree species forest (MF), a kokret palm forest (PKF) and a forest dominated by mora tree species (MRF). The tree species identified here were known, and thus identified, by the accompanying field ranger.

The location of these sub-environments along the 5 km transect are shown below in Figure 3.7 via GPS points. A photograph of each sub-environment is then depicted in Appendix C to show the differences between vegetation.

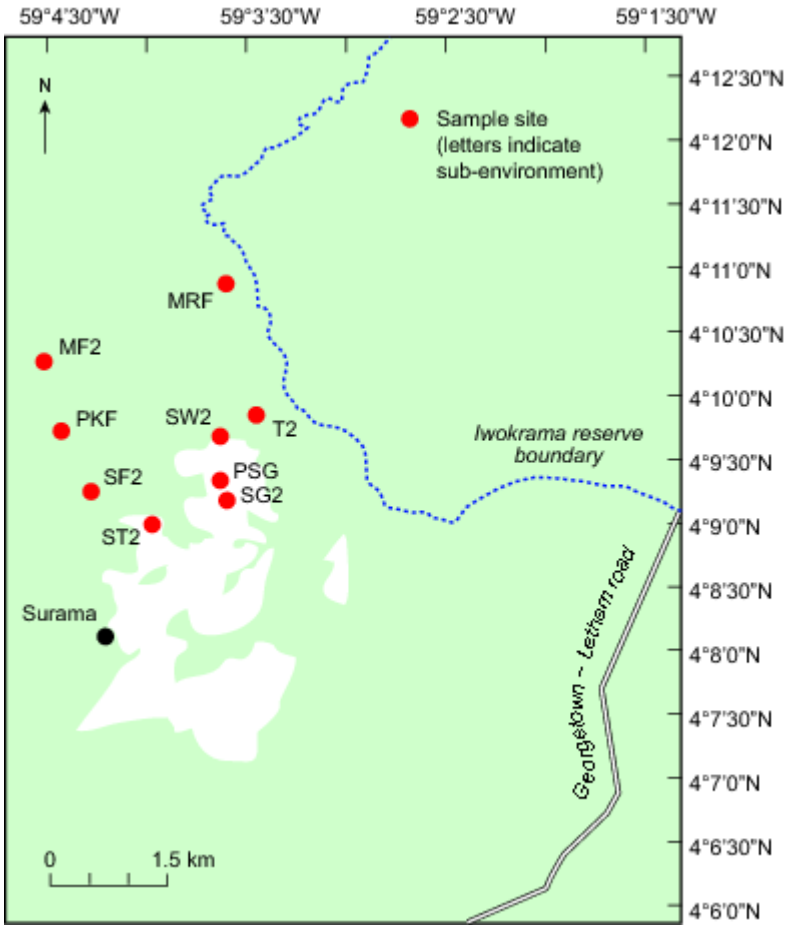


Figure 3.7 Surama transect GPS sampling points in closed red circles with specific site abbreviations labelled adjacently. GPS points have an error of up to 10 m. Green indicates rainforest vegetation, white indicates savannah.

3.5 Soil classification results

Table 3.1 shows the defining properties of each soil profile sub-environment in both Wowetta and Surama. For each soil type, the defining properties are highlighted in bold. Below are descriptions of these soil types relating to their sub-environments, as of Table 3.1.

3.5.1 Haplic plinthosols

Haplic plinthosols include savannah soil SG2, SW2 and ST2 and forest soils PKF, MRF and MF2. The haplic features of SG2, SW2 and ST2 include plinthic material 54, 50 and 200 cm from the surface with ≥50% mottling (mix of reductimorphic greys and oximorphic reds/yellows of iron oxides from repeated wetting and drying) and low activity clays (kaolinite). Although plinthic material should start within 100 cm of the surface to classify as a plinthosol, ST2 had no other defining features which would indicate a different soil type, and so is classified as a plinthosol despite a deep plinthic horizon. KPF, MRF and MF2 have plinthic material evident from the surface to the bottom of the soil profiles.

3.5.2 Albic plinthosols

Savannah soils SG1, SW1 and ST1 are all albic plinthosols due to the dominant albic horizon in these profiles. The albic horizon is generally leached of organic matter and minerals (other than quartz) and can be recognised through a light colouring (which varied from white to pale brown), low TOC and acidic pH. TOC in this horizon ranges from 0.03% to 1% and pH from 4.29 – 5.66 in SG1, SW1 and ST1 (see Appendix D). Although the surface horizons of these soils do not affect their classifications, it is worth noting that there are apparent differences between SG1 and SW1 / ST1. These differences can be seen through the following three descriptions and measurements (as given in Appendix D): the surface horizon colouring of dark greyish brown (SG), and dark brown / very dark greyish brown (SW / ST); pH 4.49 (SG1), 3.93 (SW1) and 3.9 (ST1); and TOC 0.7% (SG1), 1.61% (SW1) and 2.86% (ST1). This is likely caused by vegetation differences of grass and wood, where the latter causes a greater organic matter input into the surface horizons of SW1 and ST1 than SG1. The potential implications of these differences are discussed in Section 3.7.

Table 3.1 Defining soil properties for classification at each sub-environment. ND = not determined.

Soil Classification						
	Plinthosols				*Gleysols*	
	Haplic	Albic	Acric	Pisoplinthic	Humic	Plinthic
Sub-environments	SG2, SW2, ST2, T2, KPF, MRF, MF2	SG1, SW1, ST1	MF1	PF, PSG	FI, SF1	SS, T1, SF2
Defining properties						
Albic horizon thickness (cm)	N/A	**30 - 88**	N/A	N/A	N/A	N/A
Acric horizon depth from surface (cm)	N/A	N/A	**23**	N/A	N/A	N/A
TOC (%) surface horizon	<2	<3	<2	<3	**6 - 13**	**<2 - 5.5**
Water table depth from surface (cm)	N.D	>100	80	N.D	**25**	**25 - 50**
Plinthic material depth from surface (cm)	**0 - 50**	**60 - 100**	**42**	**0**	220, 265	**0 -54**
Gley feature depth from surface (cm)	N.D	N.D.	N.D	N.D	**25**	**0 -25**
Area of mottling in horizon (%)	**≥50**	**≥50**	**≥50**	**≥50**	≥50	**≥50**
Dominant texture	Loamy sand	Sand	**Silt loam - clay**	**Sandy pisoplinthic**	**Clay**	**Sandy loam**
Minerology	N.D.	**Quartz / kaolinite / strengite**	**Quartz / kaolinite / strengite**	N.D.	Quartz / kaolinite	**Quartz / kaolinite / strengite**

3.5.3 Acric plinthosol

MF1 is an acric plinthosol. This soil profile increases rapidly in clay content with depth (silt loam – sandy loam - clay), lending the 'acric' prefix. Plinthic material is apparent at 42 cm from the surface and is the dominant soil profile property.

3.5.4 Pisoplinthic plinthosols

PSG and PF are both pisoplinthic plinthosols, despite a difference in vegetation. Pisoplinthic material exists throughout the profile of both soils. Due to the hard nature of this material it was not possible to sample further than 40 and 70 cm depth with a spade and auger respectively.

3.5.5 Humic gleysols

Sites FI and SF1 are both humic gleysols. 'Gley' indicates soil saturation with water, resulting in reducing conditions (shown through grey colouring of the surface and an Eag horizon as well as a water table 25 cm from the surface). They show similar characteristics in respect to water table height (both 25 cm from the soil surface), colour (black grading to grey with mottles) which indicates that they are gleysols. Both also have a predominantly clayey texture with a black coloured humified surface horizon. Average TOC at the soil surface differs between the sites, with FI being roughly half of SF1 (FI: 6% and SF1: 13%). However, the high TOC of the surface horizons in both sites results in the 'humic' status of both. This humic status which is lacking in other sub-environments may have implications for the soil organic carbon stock and molecular characteristics, and is further discussed in Section 3.7 and Chapters 4 and 5.

3.5.6 Plinthic gleysols

SS and T1 are plinthic gleysols due to the presence of gleyed and mottled features. This is the overriding feature of this soil type. Plinthic material is also apparent where fluctuations in the water table has allowed mottling (oxidation and reduction) of iron oxides from the plinthite of ≥15% volume. This evidence is corroborated by the presence of the mineral strengite albeit in small amounts compared to quartz (see Appendix E). This plinthic material occurs at >100 cm from the surface, and thus this soil is primarily a gleysol with plinthic material.

SF2 is a plinthic gleysol due to evidence of yellow mottling within a grey coloured matrix at 6 cm depth from the surface of the soil, resulting from a fluctuating water table. The water table is breached at 270 cm depth from the soil surface. Plinthic material is apparent in the mottling and is the second most dominant feature of this soil after gleying, resulting in a 'plinthic' prefix.

3.6 Soil profile schematics

Figure 3.8 and Figure 3.9 are schematics of each sub-environment found and sampled in Wowetta and Surama, respectively. Each sub-environment occurred more than once, however, only one of each type was sampled in each area. The figures also show the field measurements taken. These include the height of the water table, full depth of profiles and individual horizons, and the texture and colour of each horizon. Each horizon is classified as according to these features (shown in letters adjacent to the profile). They also show the vegetation type above the profiles. Each profile is labelled with correspondence to the sites named above.

Both schematics illustrate the undulating landscape of dry pisoplinthic mounds (sites PF and PSG, where the water table was not breached) and wetter plinthic depressions (SS, T1, SF1 & 2 and MRF). The influence of quartiztic material can be seen in the paler, dominantly sandy and eluviated (Ea) horizons of the savannah soils. Mixing between sand from river alluvium and clay from plinthic material is evident in the sub-horizons of most profiles, and is evident in the texture type (e.g. from loamy sands to sandy clays). Transition (T1) and forest sites (FI, SF1 & 2, MF1 & 2 and MRF) typically have a dominance of clay over sand in their soil profiles. However savannah sites (SG1 & 2, SW1 & 2, ST1 & 2 and SS), typically have a greater sand content. Sites with high water tables (FI, SS, T1 and SF1), and / or dense vegetation cover (all latter sites and SW1 & 2 and ST1 & 2) have dark surface horizons. Mottling from fluctuating water tables were apparent in most soil profile sub-surface horizons.

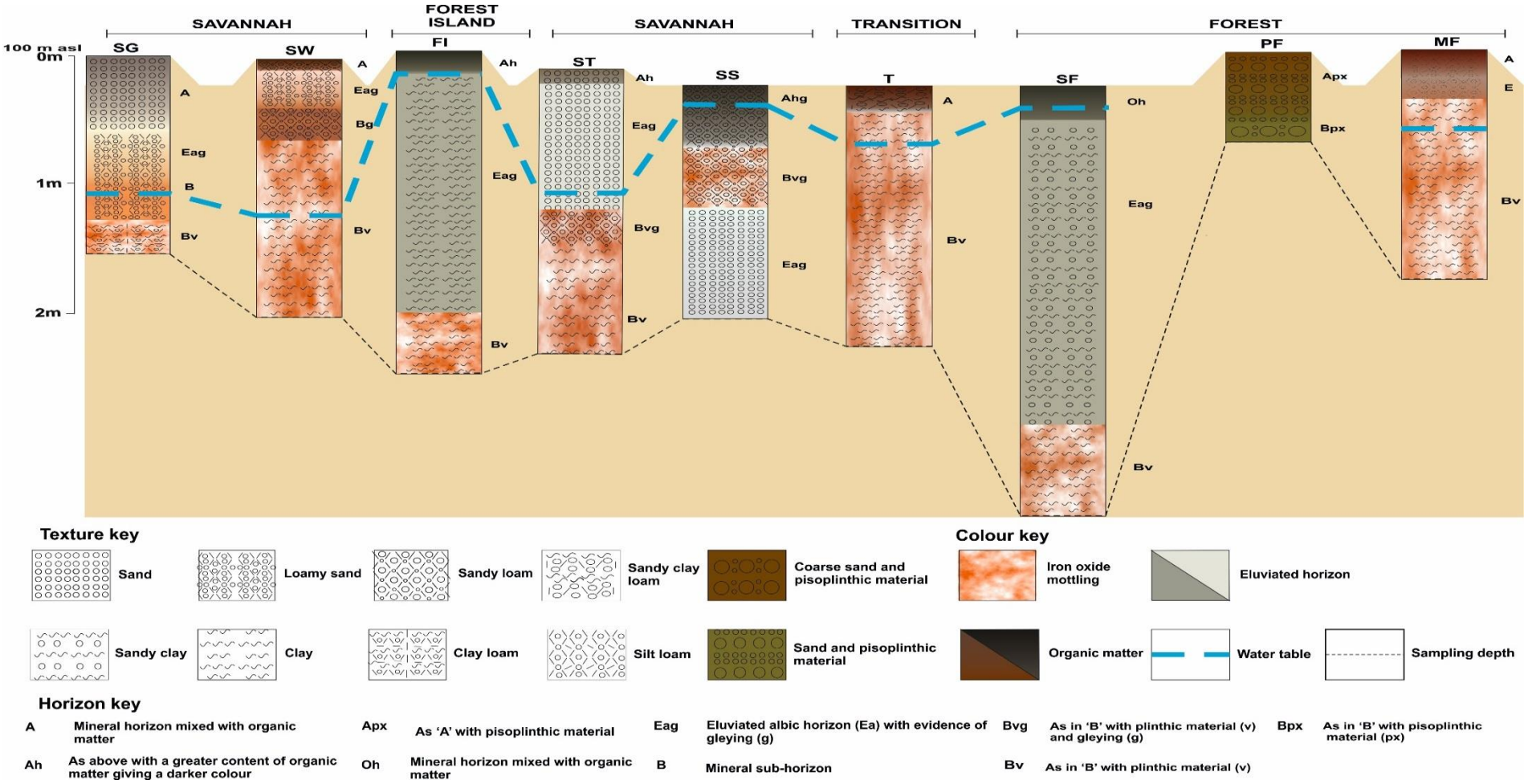


Figure 3.8 Schematic of the sequence of sub-environments sampled along the 5 km transect in Wowetta.

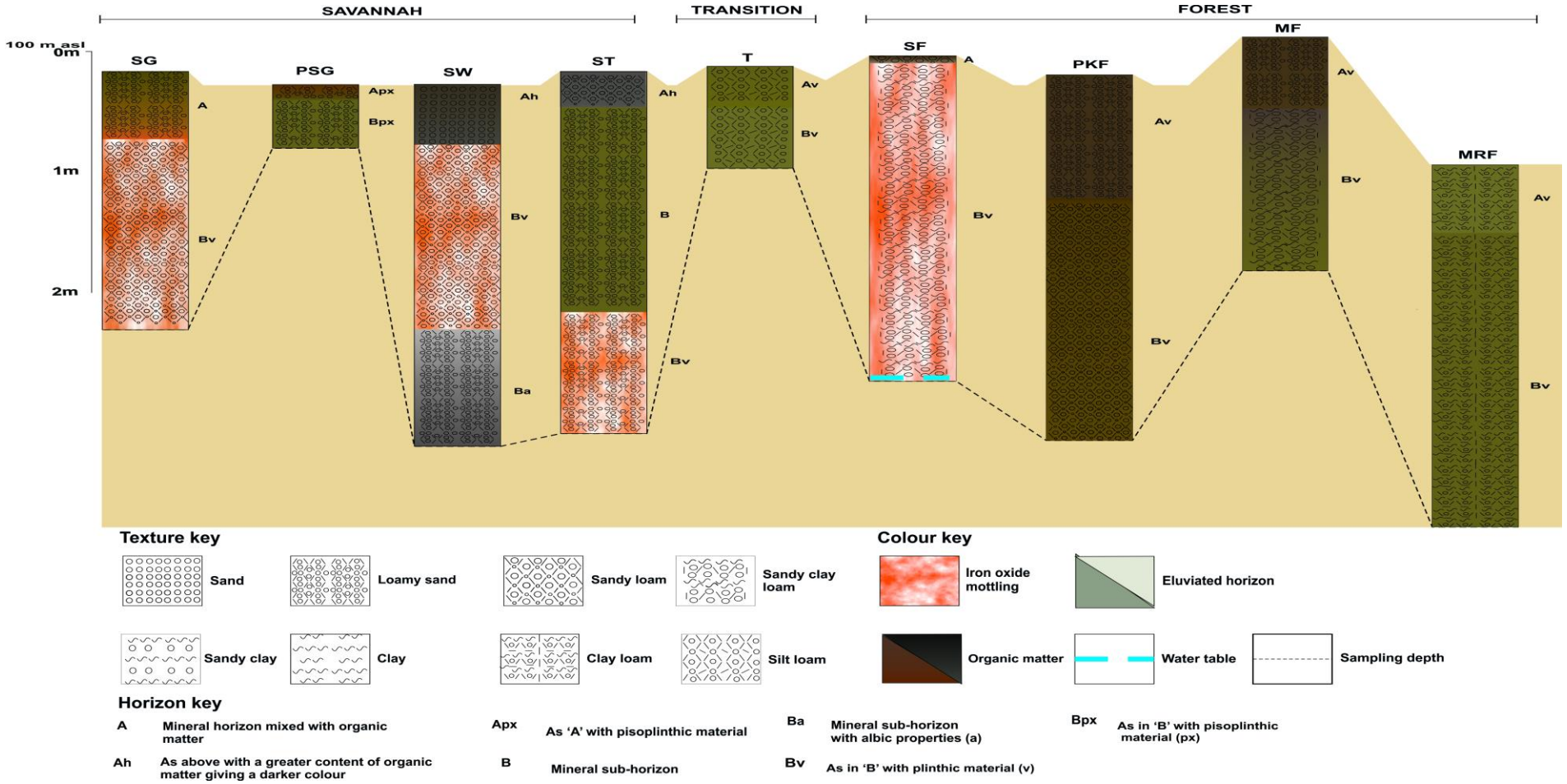


Figure 3.9 Schematic of soil profiles at each sub-environment sampled in Surama.

3.7 Discussion

As seen in Section 3.5 the same soil types may exist in either savannah, transition or forest biomes. This indicates that vegetation is not the predominant factor in soil formation and classification. . From the results presented it is evident that plinthite is a strongly influential factor upon the major soil group, which results in the majority of sub-environments being plinthosols. The high temperature, wet-dry seasonal climate of Guyana which initially weathered the underlying Takutu sedimentary and Iwokrama volcanic bedrock into plinthite during the Eocene is therefore an integral part of the soil formation. As of previous studies in this region which detail similar soil types, this influence of plinthite is to be expected (Suggett, 1964). Similarly, the landscape position and hydrology is another major factor in soil type: gleysols occur in depressions where the water table is near the soil profile surface.

The factors that cause soil types to have different sub-groups or prefixes to their classifications (e.g. haplic plinthosol and albic plinthosol) are caused by a mixture of environmental and vegetation differences between sub-environments. For example, humic gleysols (SF1 and FI) have a humic prefix due to a high amount of organic matter in the surface horizons caused by both high water content (see Chapter 4, Section 4.3.5) and vegetation inputs (observation), whereas plinthic gleysols SS, T1 and SF2 have dominant plinthic properties due to drier soil profiles, and a lower vegetation input at SS. However, haplic, albic and acric plinthosols are a result of parent material features. For example, the presence or eluviated absence of iron oxides, results in haplic and albic soil sub-groups respectively. The sandy texture and the rapid textural change with depth (sandy loam – clay) results in albic and acric soil sub-groups respectively.

Differences caused by vegetation between soil profiles are most apparent in the surface soil. For example, as evident in Figure 3.8 the surface soil of SG1 is lighter in colour, less fibrous in texture, less acidic and lower in TOC than SW1 and ST1. These differences are likely caused by less vegetation inputs into SG1 soil than SW1 and ST1: greater vegetation inputs may cause a darker colouring, higher TOC and greater acidity if the quality of these inputs is poor (i.e. lignin, tannin and cutin rich). Filley et al. (2008) showed that woody encroachment into savannah grassland on sandy loam surface soils, over 14 - 105 years, caused an enrichment in cutin and suberin aliphatics

in non-aggregated particulate organic matter (POM). They concluded that woody encroachment led to a rapid accumulation of biochemically recalcitrant molecules in the POM. Creamer et al. (2011) identified that old woody stands have greater surface soil organic carbon (SOC) than grasslands. Despite this SOC accrual, a greater proportion of SOC was mineralised to CO_2 than in grasslands, indicating a lack of biochemical recalcitrance. It was speculated that this greater respiration may be due to increased microbially available OM inputs from the woody encroachment; thus, the location of the SOC within the soil matrix is more important than its chemical structure. SOC accrual under woody encroachment was therefore attributed to changes in microbial community structure and activity due to soil moisture limitations, (Jackson et al., 2002; Huxman et al., 2005), release of microbial inhibitors from plants, (Weidenhamer and Callaway, 2010) and resulting enzyme suppression (Waldrop et al., 2004). Therefore, these observed differences caused by vegetation within surface soils may indicate more complex differences in SOC and molecular characteristics between soil types and sub-groups. Of particular importance, as noted by Creamer et al. (2011), are the differences in microbial community and activity, which are influenced by the specific site conditions, and in turn are very influential on the SOM characteristics. The SOC and molecular characteristics of individual sub-environments will be assessed in Chapters 4 and 5 respectively.

Surface soils of SF1 and FI also showed differences relative to other soils; with darker colours and highest TOC's (Figure 3.8). This indicates that SOC may be highest and potentially molecular compounds are preserved at these sub-environments (this is further assessed in Chapters 4 and 5). However, in the surface 25 cm above the water table, where the soil is not completely saturated, there may be degradation of organic compounds due to more aerobic conditions (Amundson et al., 1989; Austin et al., 2004). Potentially, degradation rates may be higher than at drier sites such as the savannah soils, in which water limitation may prevent microbial activity (Huxman et al., 2005).

The sub-set of soils that were tested for mineralogy show that low activity clay (kaolinite) and quartz are the main minerals present (see Appendix E). This may result in a lack of ability to stabilise organic matter in the profile (Martin and Haider, 1986), leading to low TOC seen throughout profiles (except at surface soils of SF1 & 2 and FI) and, thus, overall low SOC stocks. Weaker strengite signals have also been identified in

most surface soils (except SF1).. The effects of this mineralogy are further discussed alongside SOC data in Chapter 4. The low activity clay in these soils may also indicate that organic molecules such as lignin, tannin and carbohydrates have a low ability to be stabilised, which will be investigated in Chapter 5.

Despite the knowledge of a fire in 2005 along the Wowetta boundary, and previous studies identifying charcoal and thus evidence of fires in central Guyana rainforest (Hammond and ter Steege, 1998), no such evidence of fires within soil profiles was observed whilst sampling. A thin dark coloured layer was observed on some savannah soils close to the transition (T1) site at the boundary, however this was not observed at the T site itself. This suggests that evidence of the 2005 fire has since been lost in the soil and is only apparent in the resulting Muri scrub. However, molecular evidence may reveal lasting fire-induced characteristics of SOM, as discussed in Chapter 5.

3.8 Conclusions

The soils between the two study areas of Wowetta and Surama show the same main orders of classification (plinthosols and gleysols) but with some differences in sub-orders of classification. Sub-orders of classification ranged between haplic, albic, acric and pisoplinthic for plinthosols. This depends upon typical features (haplic), the presence of eluviated material (albic horizon), a rapid textural clay increase with depth (acric) or pisoplinthic material. Gleysol sub-orders varied between humic and plinthic, depending upon either high surface TOC or the depth of a plinthic horizon from the surface of the soil profile.

The majority of soils are plinthosols, which show the predominant influence of geological weathering processes (resulting in an accumulation of iron, aluminium and quartz) on soil formation. The few exceptions that are gleysols, (swamp forest sites in both areas and the forest island and savannah swamp) also highlight the impact of hydrology on soil formation. Low activity kaolinite clay and the mineral strengite indicate a low ability to complex organic matter in the soil. Low TOC in most surface horizons (and throughout profiles, see Appendix D) highlights the inability of these soils to retain organic matter. Differences in the properties of surface soils, caused by local vegetation, may have implications for SOC and molecular quality and degradation extents between sub-environments, as investigated in Chapters 4 and 5.

Chapter 4. **Soil carbon stocks of sub-environments on the savannah-rainforest boundary of Wowetta and Surama in central Guyana**

4.1 Introduction

Chapter 1 highlighted the significance of soil organic carbon (SOC) stocks within the Amazon, encompassing Guyana. It also described the importance of these stocks in relation to climate change and population pressures, particularly on the savannah-rainforest boundary. Chapter 3 then divulged the variability of environment and soils on the savannah-rainforest boundary, which may lead to variability within SOC stocks. This chapter aims to estimate the SOC stocks in full soil profiles, (up to a maximum of c. 3 m depth), in the range of sub-environments and associated soil types found on the savannah-rainforest boundary in the tribal lands of Wowetta and Surama, (see details in Chapter 3). It thereby creates a baseline of SOC data for these areas.

Specifically, the objectives were to:

a) assess how SOC changes in soil profiles with depth,
b) establish which sub-environments hold most SOC,
c) compare the SOC stocks estimated to existing data of the Amazon region, and the wider tropics in order to validate these historical values against new data.

4.2 Methodology, sites and samples

This chapter encompasses data from both Wowetta and Surama sub-environments, sampled at the beginning of the dry season between January and February 2012. Data of bulk density, total organic carbon (TOC), soil organic carbon (SOC) stocks and volumetric water content from full soil profiles were used in this chapter. For full details on sampling, measurements and statistics as well as site descriptions, please refer to Chapter 2 and Chapter 3 respectively.

Soil samples were taken per horizon in order to understand where most organic carbon is held within each profile. Sampling each horizon means that important pedogenetic information about the soil profile is retained, which is beneficial when studying soil processes in the context of carbon storage (Grüneberg et al., 2010). Differences in carbon stocks within or between profiles can be better understood via this method, as it is easy to relate the amount of carbon to individual horizon characteristics, such as texture. Horizon thickness is also crucial for estimating accurate carbon stocks. However, this method makes comparisons between soil types more difficult as the type of horizon may differ, in which case sampling per square metre may be more suitable (Grüneberg et al., 2010). Despite this, it is possible to convert horizon-based measurements to square metre-based measurements. In a similar method to the sampling approach in VandenBygaart et al. (2007) and Palmer et al. (2002), conversion has been done using the thickness of each horizon contained within each metre, for example within 0-1 m: Ah = 10 cm, A = 60 cm and B = 30 cm. The full thickness of the B horizon is actually 50 cm, but the remaining 20 cm is beyond the first metre, and so is factored into the second metre (1-2 m) estimation, but not in 0-1 m. This conversion has been employed during the discussion of this chapter in order to compare the stocks estimated in this study to those in previous studies.

4.3 Results

4.3.1 Vegetation contribution to TOC

Figure 4.1 shows the differences in average TOC between sites along the sampling transect in Wowetta of wood litter, fresh shoot / leaf litter and soil profiles. SG (savannah grassland) had no wood litter / trees present at the site and hence has no values for this vegetation component.

Wood litter across the transect is relatively similar, varying by ~5%. Fresh shoot / leaf litter varies slightly more: ~10%. These differences may be due to variations in plant composition and degradation rates between sites. Sites SW, ST and SF have the highest summed vegetation TOC inputs (~94%) and site SG has the lowest (44%), due to the lack of wood litter input.

Average soil profile TOC also varies across sites by ~12%, which is most likely due to differences in vegetation input quantities and degradation rates. Site SF has the highest average soil profile TOC (12%) whilst SG has the lowest (0.7%). Despite SW and ST having vegetation inputs with some of the highest TOC, they have some of the lowest soil profile TOC (~2%).

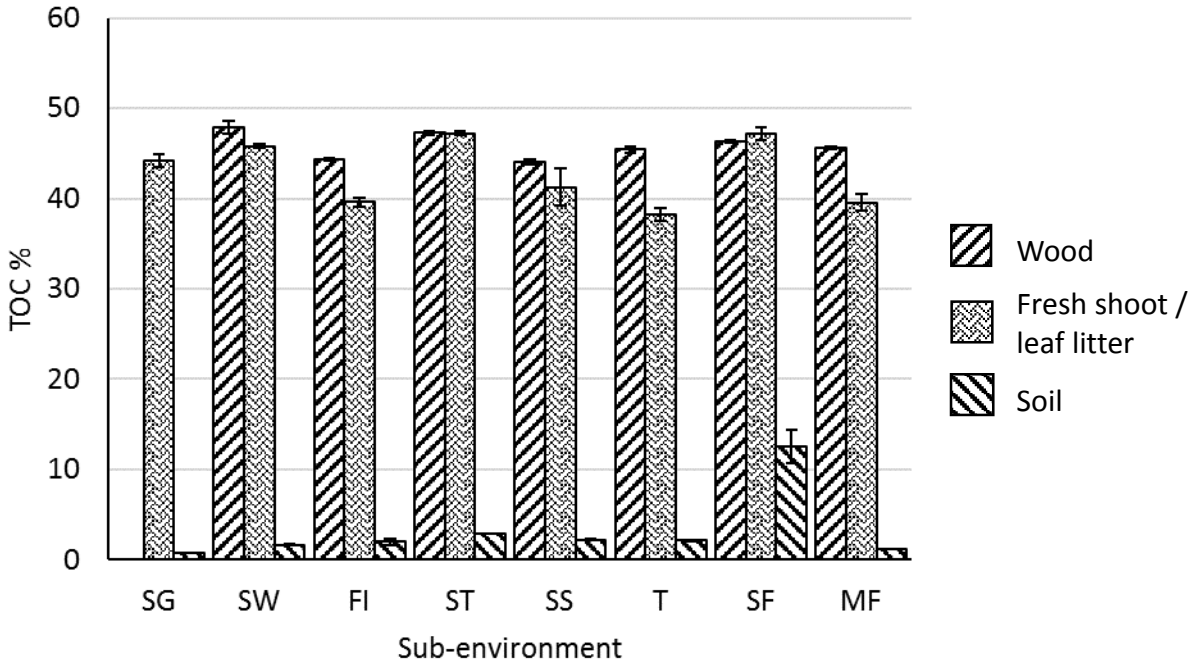


Figure 4.1 Average wood litter, fresh shoot / leaf litter and soil profile TOC % across the sampling transect in Wowetta, of 3 replicates with standard error (S.E.) bars shown. SG: savannah grassland; SW: savannah woodland; FI: forest island; ST: savannah tree; SS: savannah swamp; T: transition; SF: swamp forest; MF: mixed tree spp. forest.

4.3.2 Soil profile TOC

TOC for each soil horizon and site at both Wowetta and Surama are shown in Table 4.1 and Table 4.2, respectively. TOC decreases down the profile in all soils, for example, in SG1 from 0.91% to 0.03% and from 16% to 0.1% in SF1 soil at Wowetta, and in SG2 from 0.97% to 0.29% and from 5.5% to 0.71% in SF2 at Surama. The sharpest decreases are between the surface horizons and the underlying sub-horizons, which is likely due to the degradation of vegetation inputs from the surface (Hättenschwiler and Jørgensen, 2010).

At 0-6 cm, site SF1 and 2 had the highest TOC in both Wowetta and Surama (16% and 5.5% respectively). The surface TOC of FI is almost half that of SF1 (7%), despite being of the same soil classification, and is further evidence to corroborate high variability.

All other sites have much lower TOC at 0-6 cm (<3%). These values may reflect a low vegetation input or relatively fast organic matter turnover in the surface. Between Wowetta and Surama corresponding sub-environments with TOC <3% were similar: SW1 and 2 are 1.61 and 1.63% respectively, ST1 and 2 are 1.76 and 1.62% respectively, for example. However some differences do occur, such as T1 (1.42%) and T2 (0.52%) highlighting soil variation within similar sub-environment, as in the swamp forest sites.

Table 4.1 Full soil profile TOC%, bulk density and OC storage for sub-environments in Wowetta, parentheses indicate standard error of triplicates.

	Boundaries (cm)	TOC (wt %)	D_b (g cm^3)	OC (t C ha^{-1})		OC (% total)
			Area 1 - Wowetta			
Savanah grassland (SG1)						
Leaf litter /shoots		44.15				
Wood litter						
0-6 cm	6	0.91	1.66	9.08	(0.20)	16.02
A	52	0.49	1.69	43.06	(5.04)	75.97
Eag	103	0.03	1.98	3.03	(0.08)	5.34
B	120	0.02	1.78	0.61	(1.77)	1.07
Bv	137	0.03	1.78	0.91	(1.77)	1.60
Total				56		
Savannah woodland (SW1)						
Leaf litter /shoots		45.78				
Wood litter		47.83				
0-6 cm	6	1.61	0.93	9.02	(0.49)	12.46
Ah	9	1.61	0.93	13.48	(0.88)	18.61
Eag	39	0.20	1.32	17.16	(0.75)	23.70
Bg	64	0.52	1.93	18.37	(1.59)	25.37
Bv	200	0.04	1.63	14.38	(0.93)	19.86
Total				72		
Forest island (FI)						
Leaf litter /shoots		39.63				
Wood litter		44.6				
0-6 cm	6	7.3	0.70	30.77	(1.26)	11.93
Ah	27	5.71	1.30	200.42	(1.26)	77.69
Eag	220	0.10	1.30	24.31	(1.26)	9.42
Bv	280	0.03	1.30	2.45	(1.26)	0.95
Total				258		
Savannah tree (ST1)						
Leaf litter /shoots		47.20				
Wood litter		47.25				
0-6 cm	6	1.76	0.93	9.88	(0.03)	7.77
Ah	10	1.76	0.93	16.37	(1.77)	12.88
Eag	98	1.00	1.12	98.25	(0.62)	77.33
Bvg	125	0.06	1.50	2.56	(0.06)	2.01
Total				127		

	Boundaries (cm)	TOC (wt %)	D_b (g cm^3)	OC (t C ha^{-1})		OC (% total)
			Area 1 - Wowetta			
Savannah swamp (SS)						
Leaf litter /shoots		41.28				
Wood litter		44.05				
0-6 cm	6	2.62	1.13	17.83	(0.85)	11.18
Ahg	54	1.83	1.33	131.65	(2.79)	82.58
Bvg	105	0.05	1.23	2.97	(1.82)	1.86
B2	200	0.06	1.23	6.98	(1.82)	4.38
Total				159		
Transition (T1)						
Leaf litter		38.25				
Wood litter		45.43				
0-6 cm	6	2.58	1.12	17.30	(0.30)	17.24
A	26	1.61	1.54	64.74	(6.84)	62.61
Bv	264	0.08	1.59	21.37	(3.20)	20.67
Total				103		
Swamp forest (SF1)						
Leaf litter		47.23				
Wood litter		46.3				
0-6 cm	6	16.17	0.61	59.02	(5.59)	15.18
Oh	27	8.82	0.96	228.61	(55.56)	58.78
Eag	265	0.30	1.30	92.14	(20.81)	23.69
Bv	333	0.10	1.30	9.14	(20.81)	2.35
Total				389		
Mixed forest (MF1)						
Leaf litter		39.52				
Wood litter		45.62				
0-6 cm	6	1.16	1.24	8.53	(0.04)	12.02
A	23	1.12	1.49	38.38	(1.63)	54.12
E	42	0.11	1.65	3.45	(0.10)	4.86
Bv	195	0.08	1.68	20.56	(0.38)	28.99
Total				71		

Table 4.2 Full soil profile TOC%, bulk density and OC storage for sub-environments in Surama, parentheses indicate standard error of triplicates.

	Boundaries (cm)	TOC (wt %)	D_b (g cm^3)	OC (t ha^{-1})		OC (% total)
			Area 2 - Surama			
Savannah grassland (SG2)						
0-6 cm	6	0.97	1.30	13.96	(0.65)	8.86
A	54	0.78	1.54	67.86	(1.88)	43.07
B	213	0.29	1.59	75.73	(2.73)	48.07
Total				158		
Savannah woodland (SW2)						
0-6 cm	6	1.63	1.17	4.81	(0.08)	4.04
A	50	1.05	1.40	34.07	(0.87)	28.62
Bv	203	0.38	1.28	74.52	(0.48)	62.59
B2	220	0.26	1.28	5.66	(0.48)	4.75
Total				119		
Savannah tree (ST2)						
0-6 cm	6	1.62	1.44	7.60	(0.27)	7.10
A	30	0.82	1.54	5.99	(1.75)	5.59
B	154	0.31	1.62	35.92	(2.32)	33.53
Bv	300	0.23	1.53	57.62	(1.45)	53.78
Total				107		
Transition (T2)						
0-6 cm	6	0.52	1.35	11.41	(0.74)	11.54
A	36	0.47	1.53	53.91	(14.26)	54.52
B	94	0.48	1.44	33.57	(1.41)	33.95
Total				99		

	Boundaries (cm)	TOC (wt %)	D_b (g cm^3)	OC (t ha^{-1})	OC (% total)	
		Area 2 - Surama				
Swamp forest (SF2)						
0-6 cm	6	5.45	1.11	36.30	(0.87)	11.30
A	270	0.71	1.48	284.93	(10.13)	88.70
Total				321		
Palm forest (KMF)						
0-6 cm	6	0.91	1.36	7.42	(0.15)	6.06
A	109	0.63	1.76	114.93	(3.07)	93.94
Total				122		
Mora forest (MRF)						
0-6 cm	6	2.51	1.05	9.88	(0.34)	4.91
A	56	0.55	1.48	47.60	(2.10)	23.65
B	301	0.27	1.45	143.78	(6.08)	71.44
Total				201		
Mixed forest (MF2)						
0-6 cm	6	1.32	1.25	15.80	(2.03)	9.24
A	60	0.56	1.51	49.12	(4.35)	28.71
B	332	0.38	1.58	106.18	(11.23)	62.06
Total				171		

4.3.3 Soil profile SOC stock

The change in SOC with profile depth is displayed in Table 4.1 and Table 4.2 and is given in the unit's t C ha^{-1}. Figure 4.2 shows the percentage of SOC in each soil horizon relative to the total profile SOC in a) Wowetta and b) Surama. TOC (wt %) was converted into SOC taking into account individual horizon thickness and bulk density. Unlike the general decrease with depth in TOC described above, SOC does not always decrease in deeper horizons.

In Wowetta, despite most soils showing an SOC depth decrease (SG1, SW1, FI, T1 and SF1), some soils show increases in the deepest horizons (SW1, ST1 and MF1). This is due to horizon thickness, as TOC and bulk density decrease with profile depth. For example, MF1 SOC decreases from 38 - 3 t C ha^{-1}, until the deepest horizon which measures 17 t C ha^{-1}, this horizon depth measures 153 cm compared to the overlying horizon which is 19 cm. ST1 shows an increase in SOC in the Eag horizon (98 t C ha^{-1}), also due to a greater horizon thickness than the overlying (16 t C ha^{-1}) and underlying horizons (3 t C ha^{-1}).

In Surama only T2 shows an SOC decrease down the profile (from 54 to 34 t C ha^{-1}), whilst SG2, SW2, ST2, MF2 and MRF increase in SOC stock with depth. Again this is due to horizon thickness as TOC decrease with depth and bulk density either decreases or remains similar with depth. For example, the deepest horizon in MF2 is 272 cm thick with an SOC of 106 t C ha^{-1} compared to its overlying horizon of 66 cm and 49 t C ha^{-1}.

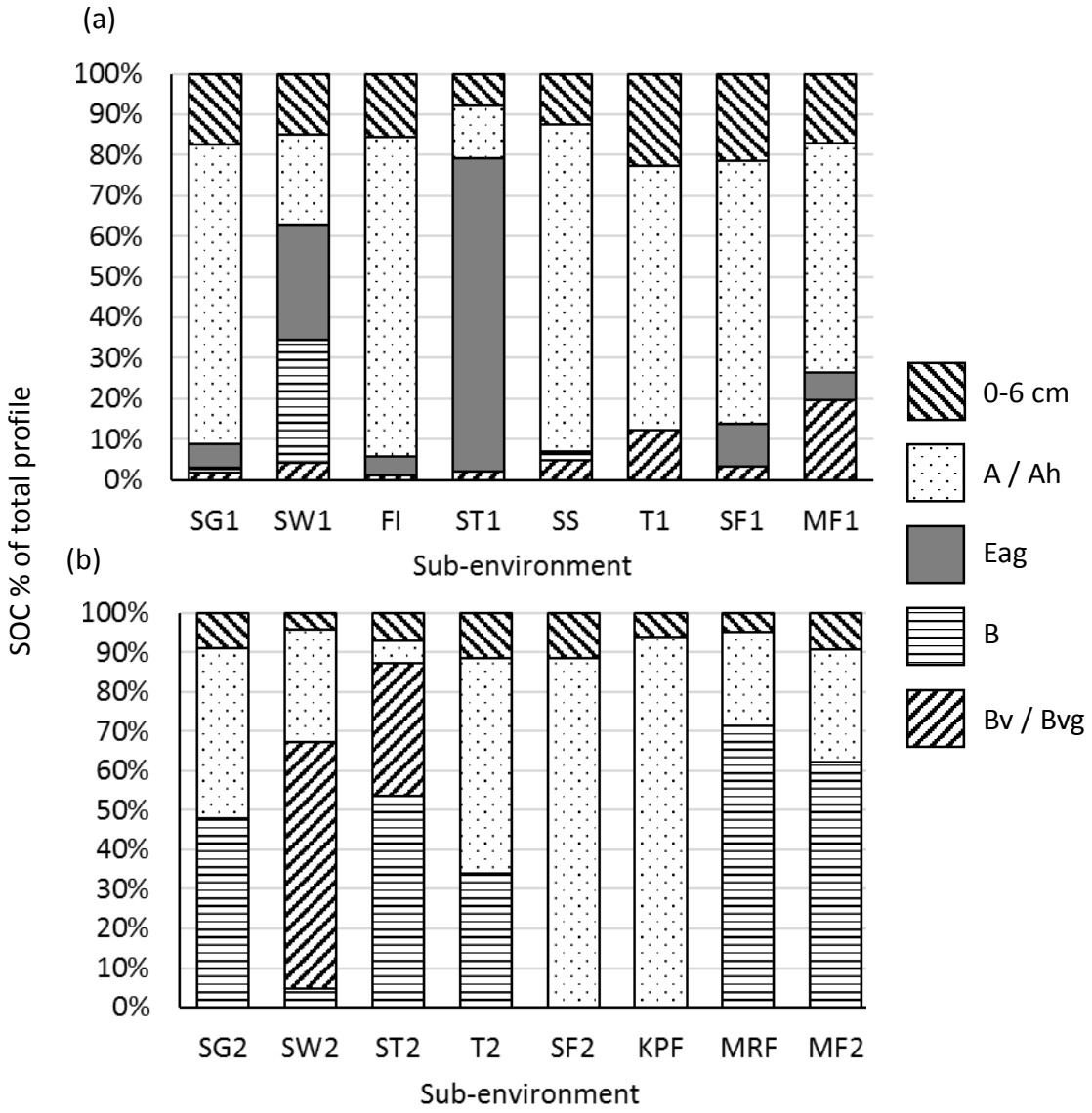


Figure 4.2 Percentage of each soil horizon SOC relative to the total soil profile SOC in (a) Wowetta and (b) Surama. SG: savannah grassland; SW: savannah woodland; FI: forest island; ST: savannah tree; SS: savannah swamp; T: transition; SF: swamp forest; MF: mixed tree spp. forest; KPF: kokret palm forest; MRF: mora forest.

4.3.4 Relative proportion of SOC stock of each sub-environment in Wowetta and Surama

Figure 4.3 and Figure 4.4 show the relative proportions of each sub-environment to the total SOC stock of the sites sampled in either Wowetta or Surama. These relative proportions were checked against an average soil profile depth of all sites in Wowetta and Surama respectively, which revealed that despite differences in depth between sites, proportions remain largely the same (data not shown). The main difference is that both SF sub-environments have an average of 8% greater proportions, emphasizing the importance of TOC and water content (Section 4.3.5).

Both SF sub-environments have the largest proportions of SOC at 31% and 25% for the sampling areas of Wowetta and Surama, respectively. The sites with the second greatest proportions of SOC differ between areas: FI (21%) in Wowetta; and MRF (16%) in Surama. SS has the third greatest SOC proportion in Wowetta (13%). MF2 & SG2 (13 and 12% respectively) have the third largest SOC in Surama.

Sites with the lowest proportions of SOC (<10%) are MF1, SG1, ST1 & 2, SW2, T1 & 2 and PKF. Thus these are mainly savannah sub-environments, however, both transitional sites and two forest sites also account for <10% SOC relative to their sampling areas.

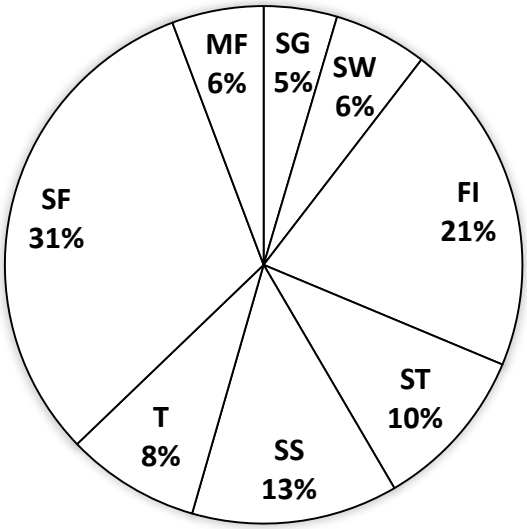


Figure 4.3 The relative proportion of each soil profile SOC to the total SOC in Wowetta sites.

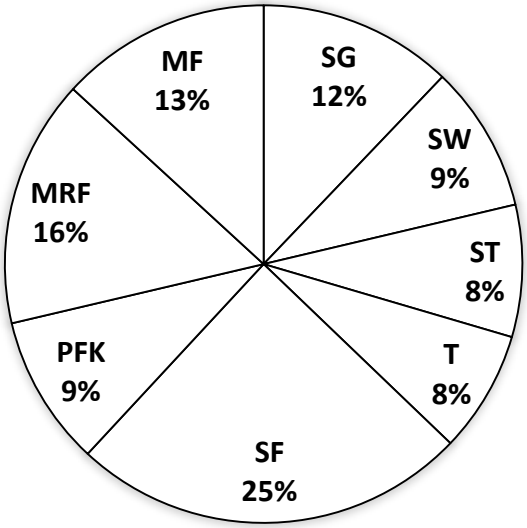


Figure 4.4 The relative proportion of each soil profile SOC to the total SOC in Surama sites.

4.3.5 Volumetric water contents of the soil profiles

Volumetric water content (θ) has been calculated from bulk density measurements (Chapter 2: Section 2.3, Equation 2.3), in order to verify that the sub-environments SF1 & 2 and FI which have a water table at 25 cm depth, do have a higher soil moisture content than those with water tables deeper than 25 cm when sampled. Figure 4.5 and Figure 4.6 show the average profile θ for each site at Wowetta and Surama, respectively.

Figure 4.5 shows that sites SF1 and FI have similar volumetric water contents (75% and 67%, respectively), but are higher than all other sites. This reflects the SOC for these sites. SS has the next highest θ at 58%. SW1, ST1, T1 and MF1 sites have some of the lowest θ across the transect (~40%). SG1 has the lowest θ at 27%, which corresponds to it also having the lowest SOC. Figure 4.7 shows a strong positive correlation between θ and SOC ($R^2 = 0.9$), emphasising that highest θ is related to highest SOC.

Figure 4.6 shows that SF2 also has the highest θ in Surama at 41%. However, due to variability in the soil of MF2 and PKF (illustrated through the SE bars), these soils may be similar to SF2 in θ, (35% and 34% on average). These θ values are significantly lower than those found at Wowetta, however, and may have resulted in the lack of relationship found between the two parameters (data not shown). Sites MRF (28%), T2 (31%), ST2 (18%) and SG2 (26%) also show low θ values. SW2 has the driest profile, with a soil profile θ average of just 8%. All the soils in Surama were dry when sampled, as opposed to those at Wowetta. This highlights the high variability of soil properties, which are evident even within similar sub-environments. However, future work must also consider temporal and seasonal variability to verify these results.

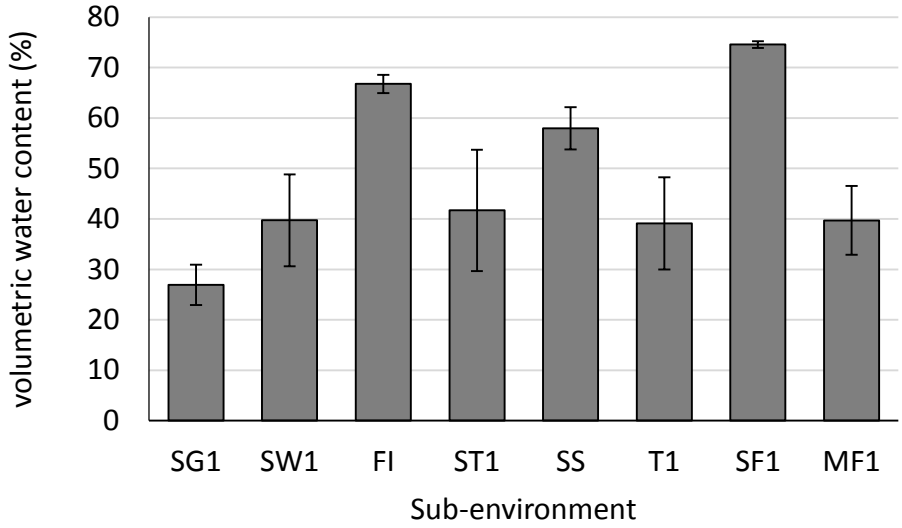


Figure 4.5 The average soil profile volumetric water content for sub-environments in Wowetta, bars show S.E. multiplied by two.

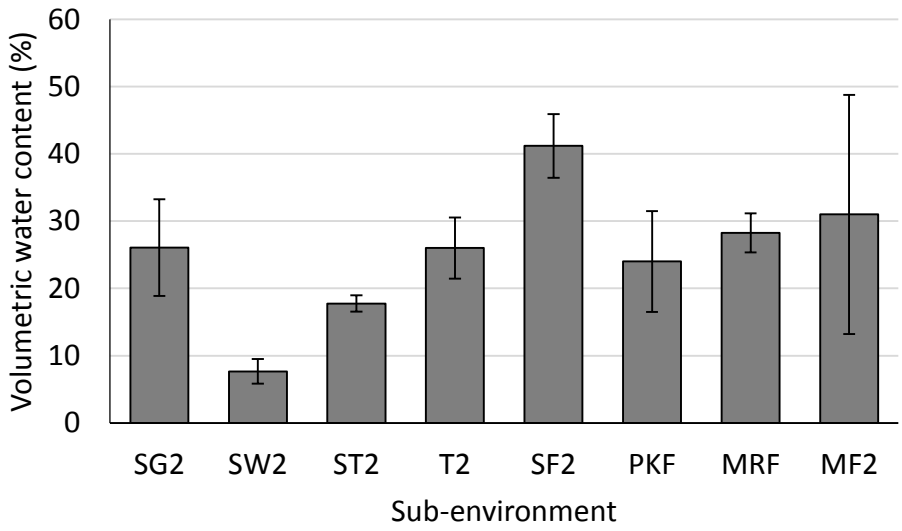


Figure 4.6 The average soil profile volumetric water content for sub-environments in Surama, bars show S.E. multiplied by two.

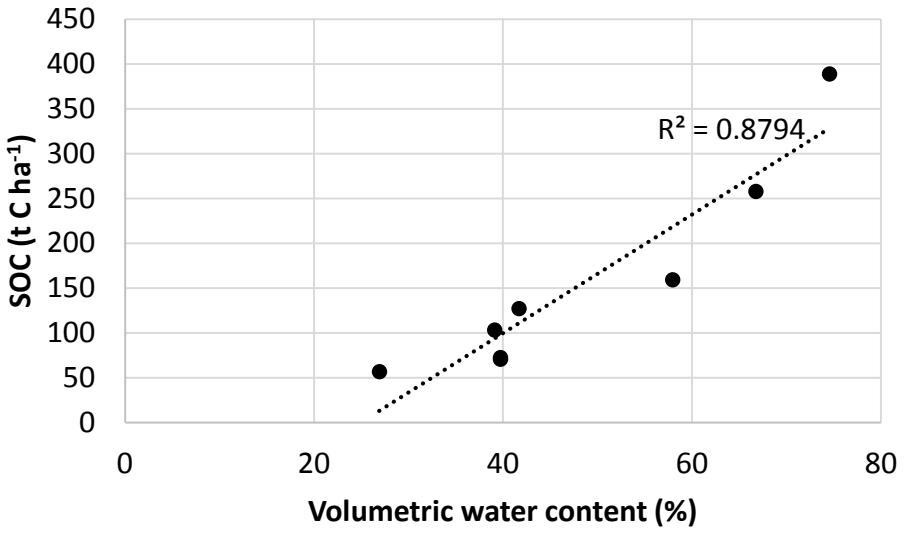


Figure 4.7 Correlation between SOC and volumetric water content. R^2 value shown.

4.4 Discussion

4.4.1 Soil profile TOC

The variations in TOC across the sub-environments are likely due to differences in decomposition rates between the soils. This may be due to several factors, such as differences in microbial communities (Macrae et al., 2013), incorporation into clay minerals and organo-mineral complexing (where organic matter is physically protected by binding to the mineral surface e.g. cation-bridging, ligand exchange, H-bonding and van der Waal forces), (Gu et al., 1994; Sollins et al., 1996; Lützow et al., 2006; Feng et al., 2014), as well as photo-degradation (Hernes and Benner, 2003; Austin and Vivanco, 2006; Gallo et al., 2006), water content (Fenner and Freeman, 2011), and pH and temperature (Donnelly et al., 1990). It should be noted that incorporation into clay minerals is unlikely given that quartz and kaolinite constitute the mineralogy of these soils (see Chapter 3, Table 3.1). The presence of the ferric iron phosphate mineral strengite detected in the soils (Appendix E) also suggests a low ability for organic matter to complex, as this mineral is known to be stable at low pH in soil (Lindsay, 1979; Haynes, 1982). Conversely, iron oxy-hydroxides were not detected, (such as hematite) which may have otherwise induced some organo-mineral complexing (Gu et al., 1994; Sollins et al., 1996; Arnarson and Keil, 2000; Lützow et al., 2006). Thus, in Bv horizons where iron oxides are evident, TOC is no higher than horizons where iron oxides are not present.

The highest TOCs are in SF1 and FI soils, which may be related to elevated volumetric water content compared to other soils, (Section 4.3.5), for a longer period during the year, suppressing microbial activity through anaerobic conditions (McLatchey and Reddy, 1998; Kwon et al., 2013). Inherent to the properties of organic matter is its ability to retain water, thus volumetric water content may also be high in these soils because of the high TOC. SF2 and FI were roughly half the TOC of SF1, (5.5%, 7% and 16% respectively). SF2 is a drier site (41% θ compared to 75% and 67% respectively, see Section 4.3.5), and was not inundated with water at 25 cm depth from the surface when sampled, as in SF1 and FI soils. Despite the surface 25 cm of SF1 not being fully saturated, it was wetter than SF2 on sampling, (see Section 4.3.5).

Recent research into peatlands has revealed that wetting and drying cycles may induce greater microbial degradation of SOM (Fenner and Freeman, 2011; Abbott et al., 2013). After dry periods, in which inhibitory phenols are depleted by the microbial activation of phenol oxidase in aerobic conditions, carbon and nutrients are released via hydrolase activity for microbial growth. This enzyme activity lasts long into the re-wetted period (months – years) after drying, at least until phenolic concentrations are great enough to significantly inhibit microbial enzymes again. Only after the phenolic content of soils is significantly reduced can hydrolase activity be initiated by microbes and, thus, decomposition take place (Fenner and Freeman, 2011). Therefore, FI and SF2 soils may undergo more significant phenolic depletion and thus resulting greater carbon degradation during both the dry and wet season, causing lower TOC's than SF1 soil. This greater depletion of phenolics may be due to more pronounced drying of soils during the dry season. Although these soils are not peatlands, it is possible that the latter processes are occurring in these relatively organic rich surface soils.

Potentially, edge effects such as a greater exposure to sunlight at FI, (being an isolated strip of forest c. 80 m wide surrounded by savannah), may also mean that organic matter degradation at FI is more rapid than at SF1, which is c. 1 km deep into the forest (Austin and Vivanco, 2006). The similarity of these two humic gleysols could indicate that this forest island was previously part of a swamp forest. It is possible that transition soils T1 & 2 also undergo greater SOM degradation from greater exposure at the boundary of the forest.

All other soils appear to show higher TOC degradation than the aforementioned SF1 and 2 and FI, with only slight variations between sub-environments. The less dense vegetation in wooded savannah sub-environments (SW1 & 2, ST1 & 2) do not appear to significantly affect TOC, as forest soils MF1 & 2, KPF, MRF and PF all have similar or lower TOC's. However, the low TOC's in forest soils may be subject to different decomposition mechanisms, such as amount of microbial activity or community type, which result in these similarly low TOC's.

Savannah sub-environments, especially grasslands SG1 & 2, are more exposed to sunlight and thus photo-degradation of soil carbon, as a result of less dense vegetation than forest sub-environments. This likely contributes to lower SOC stocks (Hernes and Benner, 2003; Gallo et al., 2006). As a result of less vegetation cover soil temperatures

may also be higher, causing greater decomposition, as long as soils are not moisture limited (Trumbore et al., 1996). In shaded savannah sub-environments such as SW and ST, higher surface soil TOCs emphasize this photo-degradative exposure and potential as a mechanism for SOM decomposition. However, the savannah grassland also has lower vegetation inputs than SW1 & 2 and ST1 & 2, which is likely to give a lower TOC.

4.4.2 Soil profile SOC stock

A comparison of individual horizon SOC between sub-environments revealed that despite very low TOC's in some sub-surface horizons (e.g. ST1 Eag 1%, MF1 Bv 0.08%, SW2 B 0.38% and MF2 B 0.38%), accounting for the bulk density and the horizon thickness in the SOC calculation (see Chapter 2: equation 2.4) resulted in significantly greater SOC stocks in these horizons, as compared to those with higher TOC. Thus, when soil profiles are sampled via metre increments, rather than by horizons, important information such as the location in the soil profile of the greatest carbon stock may be lost. This may be important when considering SOC losses from soil due to vegetation or climate change.

SOC's across sub-environments reflected TOC's in that the wettest soils (SF1 & 2 and FI) had the greatest SOC stocks (Section 4.3.4). This is likely due to the reasons discussed above (Section 4.4.1).

FI is classified as the same soil type as both SF soils (gleysol), and as in SF1 also has a high water table at 25 cm of the surface, as well as high volumetric water content (67% compared to 75%). The difference in the proportion of carbon stock between the latter site and SF1 is likely to be a lower average TOC in the profile of FI (3.28% compared to 6.35% respectively, Table 4.1 and Table 4.2). The soil profile of MRF has a higher TOC than other sites at Surama (profile average of 1.11%) which is likely to have caused a greater SOC stock than other sites.

SS has a similar soil type to that of SF1 and FI (gleysol) with a high water table, thus this site shows a relatively significant SOC stock in respect to other sub-environments. However, as a savannah site, it has a less dense vegetation cover and input (mainly grasses and sedges) and is more exposed to sunlight and thus photo-degradation (Austin and Vivanco, 2006), which may both result in a lower TOC (soil profile average

1.14%). Its sandy textured sub-surface Eag horizon also suggests that carbon is easily leached through the profile.

Soils in sub-environments MF2 and SG2 have similar SOC proportions relative to other soils in Surama. This is surprising because of their contrasting ecosystems of forest and savannah. However, both have a low TOC (0.75% and 0.68%) and a similar bulk density (1.45 g cm^3 and 1.48 g cm^3). This shows that organic matter turnover rates may be similar in both sub-environments, although this may be via different decomposition pathways such as differing microbial activity and communities or sunlight exposure.

The differences of proportional SOC between comparable sub-environments in Wowetta and Surama, such as the savannah grassland (SG1 & SG2) and mixed tree spp. forest (MF1 & MF2), are due to lower TOC, bulk density and shallower soil profile depths in Wowetta.

In both areas, the average proportional stocks of SOC are greatest in forest sites (Wowetta 19% and Surama 16%). Savannah sites account for a much lower proportional SOC average of 9% in Wowetta and 10% in Surama. The transition zones show lowest average SOC stocks, with a greater similarity to savannah sites than forests (8% in both areas). This is potentially a result of SOM degradation at the forest edge, potentially being more exposed to sunlight, wind and higher temperatures (Kapos, 1989).

Conclusively sub-environments with the greatest average TOC result in the highest SOC stocks (SF1 & 2). Furthermore, these sites also have wetter soil profiles, whilst transition sites and dry savannah sites have lower SOC stocks.

Several authors have published data of estimated SOC stocks for tropical soils beneath rainforests and savannahs via metre-depth measurements of 0-1 m, 1-2 m and 2-3 m (Kimble, 1990; Eswaran et al., 1993; Batjes and Dijkshoorn, 1999; Jobbágy and Jackson, 2000). The OC of the soil profiles in this study have been calculated by metre depth intervals in order to compare the values with these studies (see Section 4.2). Soil profiles were sampled to the maximum depth possible in this study, which ranges from 1-3 m. The latter studies have shown that soil type causes great variance between the amount of SOC, and that sampling depth is important for SOC estimates; with highly

likely underestimations from those commonly only sampled to 1 m depth (Jobbágy and Jackson, 2000). Differences in SOC have been evaluated below between ecosystems (savannah and forest), sub-environments within these ecosystems and soil types.

4.4.3 SOC comparisons by ecosystem type

Jobbágy and Jackson (2000) compared SOC stocks from a range of sub-environments (tropical deciduous forests, evergreen forests and savannahs). Comparisons between the latter data and that of SOC stocks estimated in this study may highlight differences due to the categorisation of sub-environments. For example, the sub-environments presented by Jobbágy and Jackson (2000) are broader categories than those sampled in this study.

Figure 4.8, Figure 4.9 and Figure 4.10 depict the change in OC stocks from 0-1 m, 1-2 m and 2-3 m depth in both study areas of Wowetta and Surama for the two main soil groups identified (plinthosols and gleysols).

Jobbágy and Jackson (2000) showed that OC stocks up to 3 m depth for tropical deciduous forests, evergreen forests and savannahs ranged between 230 – 290 t C ha^{-1} (23 -29 kg C m^{-2}) using values from the National Soil Characterization Database (NSCD) and the World Inventory of Soil Emission Potential Database (WISE). Within the present study, most savannah sites were shallower than 3 m on reaching the petroplinthic layer (hard, impenetrable layer, see Chapter 3). However, site ST2 reached a depth of 3 m, with a total OC of 107 t C ha^{-1}. This value is clearly below that of the range shown in Jobbágy and Jackson (2000). Most forest sites measured ~3 m before reaching the petroplinthic layer, including: FI, T1, SF1, SF2, MRF and MF2. Sites T1, MRF and MF2 all had estimates below the previous study's tropical forest estimates (103, 201 and 171 t C ha^{-1} respectively), whilst sites FI, SF1 and SF2 all had values within or above the previous study's tropical forest estimates (258, 388 and 321 t C ha^{-1} respectively). These results show that differences in data sets do exist; and thus there is a need for higher resolution sampling to cover spatial variability. Although these results are not conclusive, they indicate that sub-environments such as forest swamps and islands (SF1 & 2 and FI) should be further investigated as to the extent along the boundary and thus wider impact in the region. All of these sites had greatest OC stocks in 0-1 m of the surface, whilst 1-2 m and 2-3 m had increasingly depleted OC stocks (Figure 4.8, Figure 4.9 and Figure 4.10), which highlights the fast turnover of carbon

within these tropical soil systems. Jobbágy and Jackson (2000) also showed this depletion of OC with the latter depth intervals for tropical forest and savannah biomes.

Four of the sites studied only extended to 2 m upon reaching the petroplinthic layer, which were mostly savannah sites. These included SW1, SS and SG2 and had estimated OC stocks of 72, 159 and 158 t C ha^{-1}, these fall within the range estimated by Jobbágy and Jackson (2000) for the savannah ecosystem: 40 – 340 t C ha^{-1} for up to 2 m depth. The only forest site with soil depth up to 2 m was MF1 (all other sites extended to 3 m), this site had an OC stock of 71 t C ha^{-1}, which is within the 0-2 m SOC estimates made by Jobbágy and Jackson (2000).

Savannah sites SG1 and ST1 at Wowetta only extended to 1 m depth upon reaching the petroplinthic layer. Jobbágy and Jackson (2000) showed that estimates up to 1 m depth in savannahs ranged between 45 and 222 t C ha^{-1}, this study shows that the latter two sites have OC stocks within this range: 57 and 112 t C ha^{-1} respectively. Forest site PFK and transition site T2 at Surama also only reached a depth of 1 m, and had OC stocks of 99 and 122 t C ha^{-1} respectively. These values fall within that estimated for tropical evergreen and deciduous forests to 1 m: 66 – 290 t C ha^{-1} in Jobbágy and Jackson (2000).

Although most estimates in the present study fall within the ranges estimated by Jobbágy and Jackson (2000), some disparities do occur. Swamp forest sub-environments SF1& 2 have a greater carbon stock than previously estimated for forests, by 98 and 31 t C ha^{-1} respectively. Other forest, transition and savannah sub-environments show lower carbon stocks than previously estimated (MRF, MF1 & 2, T1 and ST2). It is apparent that forest sites show the most disparity between the datasets, whilst savannah SOC stocks largely fall within the range found by Jobbágy and Jackson (2000). This suggests a greater heterogeneity within the forest ecosystem than previously recognised or sampled, highlighting the need for contemporary and more spatially detailed estimates. As forest sub-environments had mostly lower SOC stocks than in the previous study (on average ~127 t C ha^{-1} lower at 0-3 m depth), these new estimates may reduce the average tropical forest SOC estimates shown in Jobbágy and Jackson (2000). However, the results presented here are not extensive along the savannah-rainforest boundary; thus more thorough sampling needs to verify the SOC stocks and their effect on previous data.

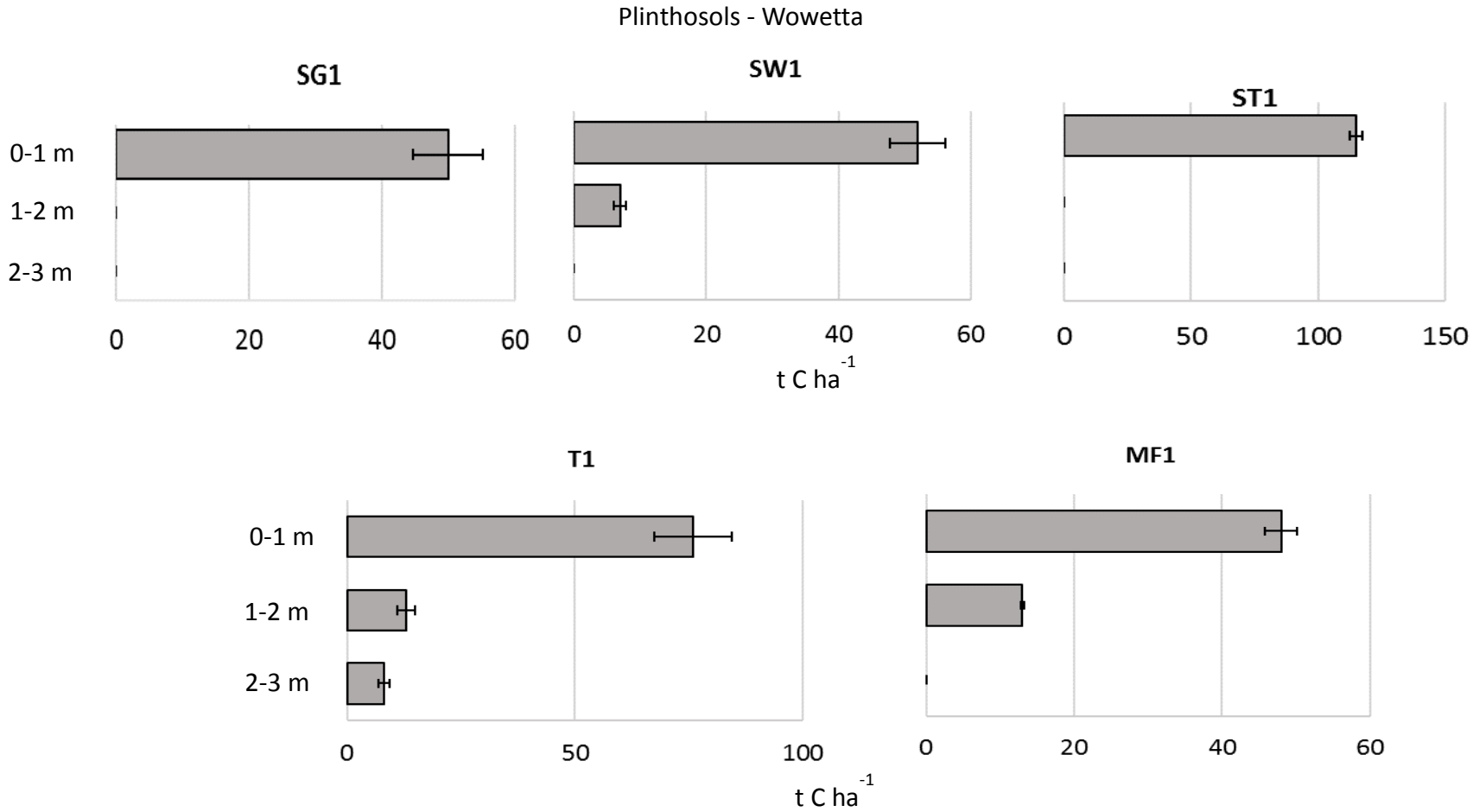


Figure 4.8 Depth profiles of OC by meter for Plinthosols in the Wowetta study area. Standard error (S.E.) bars of triplicates shown.

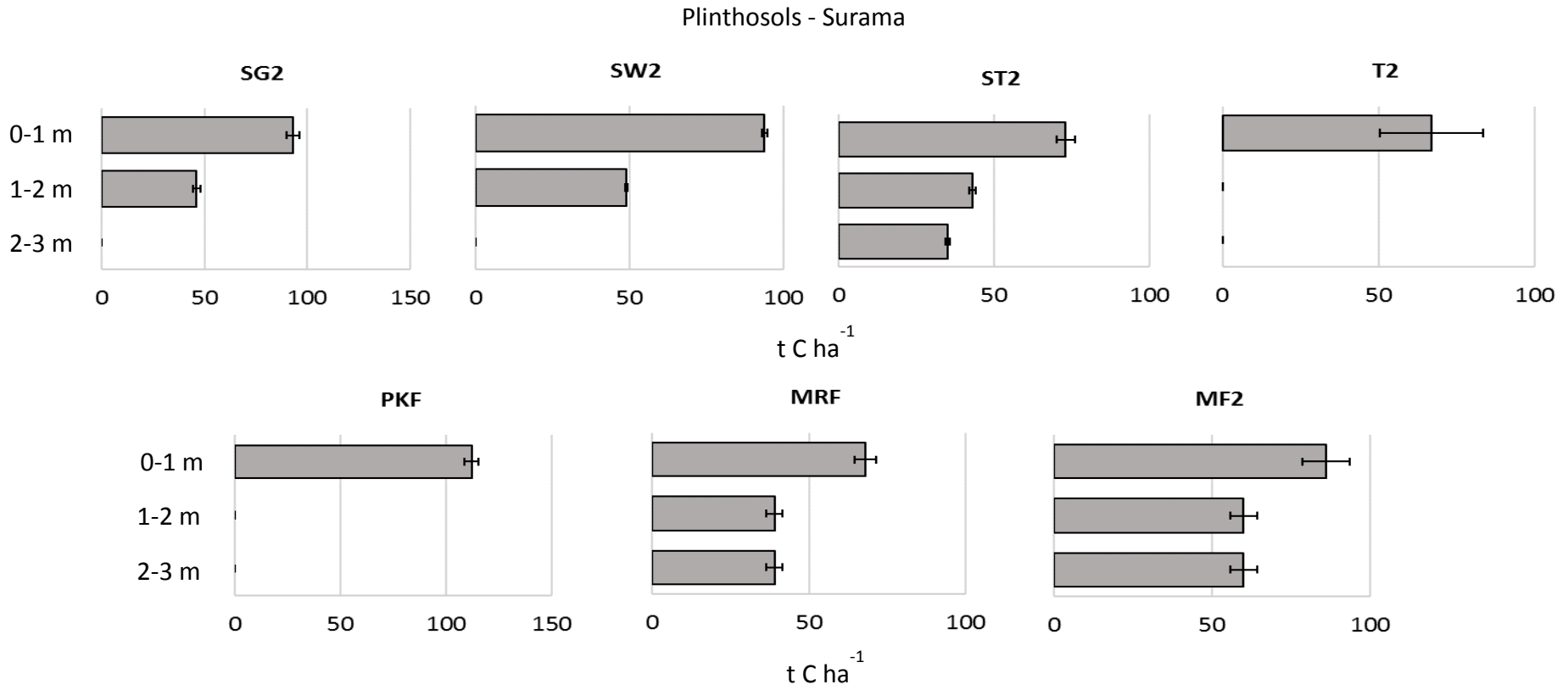


Figure 4.9 Depth profiles of OC by meter in Surama Plinthosols. S.E. bars of triplicates shown.

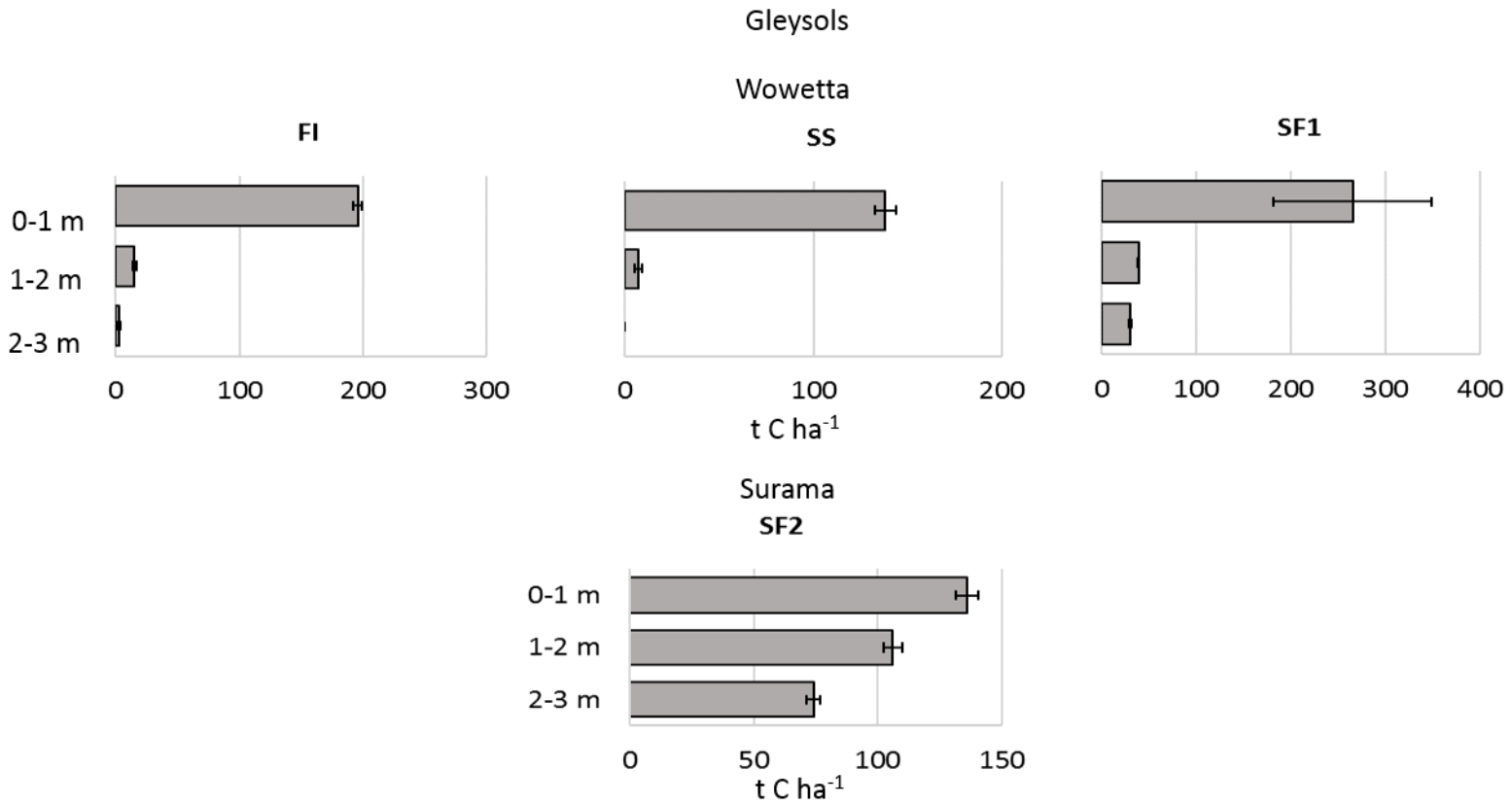


Figure 4.10 Depth profiles of OC by meter for Gleysols in both Wowetta and Surama study areas. S.E. bars of triplicates shown.

4.4.4 SOC based on soil type

The majority of soils in this study have been classified as plinthosols (see Chapter 3). A study by Batjes and Dijkshoorn (1999) compiled C data of various soil types sampled to 1 m depth of the surface in the Amazon region, from the soil and terrain project for Latin America and the Caribbean (SOTER-LAC) database. These soils were given classifications based on United Nations Food and Agriculture Organisations (FAO) standards, as done in the current study. This compilation of data can be compared to the present study of SOC in 1 m depth from the surface (Figure 4.8 and Figure 4.9).

Batjes and Dijkshoorn (1999) showed that plinthosols had a mean SOC stock of 6.6 kg C m^{-2}, which converts to 66 t C ha^{-1} (which are the units used in this study). This value has a coefficient of variance (CV) of 31%; thus SOC stocks potentially ranged between 46 and 87 t C ha^{-1}.

Figure 4.8 and Figure 4.9 show that all sub-environments have an SOC stock within the range found by the latter study, with the exception of sites ST1, SG2, SW2 and PFK. The latter soils all have higher estimates than Batjes and Dijkshoorn (1999): ranging between 99 and 152 t C ha^{-1}. This further corroborates the need for a more comprehensive sample area when estimating SOC stocks.

The other major soil type found in this study were gleysols (SS, SF1 & 2 and FI), which show much higher OC stocks than the plinthosols. Batjes and Dijkshoorn (1999) demonstrated that gleysols in the Amazon may have a mean OC stock of 126.6 t C ha^{-1} (12.66 kg C m^{-2}) up to 1 m. This has a CV of 59% and thus profiles may vary between 50 and 200 t C ha^{-1}. Figure 4.10 shows that sites SS, FI and SF2 fall within the estimated range of Batjes and Dijkshoorn (1999), however SF1 has a greater OC stock of 265 t C ha^{-1} within 1 m depth.

These results further highlight the need for greater sampling resolutions, not just with regard to ecosystem type, but also soil type. This will aid in providing more realistic values for SOC stocks in the Amazon region.

Additionally, when the full soil profiles (> 1 m depth) of the study sites are taken into account, the values found in the present study show that OC stocks can be much greater than the latter values for plinthosols and gleysols. For example, plinthosol T1 was

sampled to 264 cm and has 103 t C ha^{-1}, ST2 and MRF to 300 cm and have 107 and 201 t C ha^{-1}, respectively. Gleysol SF2 was sampled to 270 cm and has 315 t C ha^{-1}, whilst SF1 was sampled to 333 cm and has 388 t C ha^{-1}. This stresses the need for deep soil sampling where such depths exist, in order to provide more accurate measurements. The above results also highlight the importance of soil type and spatial heterogeneity in OC stocks when soil sampling.

Due to the similarities in SOC stock between sub-environments of contrasting vegetation, i.e. savannah and forest, it appears that vegetation type is a less reliable indicator of SOC stock. The results presented in this chapter indicate that soil type (gleysols) with high water tables and volumetric water content are a more effective predictor of SOC stock. However exceptions may occur, such as in the savannah swamp (SS); where despite being a gleysol, SOC stocks are not highest. This shows that vegetation does have some control over SOC stocks: greater vegetation inputs and cover may give a higher SOC stock in this soil type. This may also indicate that SS was once a swamp forest before savannah encroachment, and has since depleted in SOC. The similarity in soil type and SOC stock between FI and SF1 may also indicate that FI was historically part of the wider expanse of rainforest, which is now more exposed and subsequently experiencing losses in SOC. Soil molecular characteristics may reveal more information about such sub-environment changes, and are investigated in Chapter 5.

4.5 Conclusions

The results presented above highlight the importance of the soil properties water content and vegetation inputs to the overall profile SOC stock. Soil type is shown to be a more reliable indicator of SOC stock than vegetation, with the exception of SS, which may be in a transitional state.

Primarily, soils with both the highest volumetric water content and TOC have the highest SOC stocks (swamp forests – SF1 & 2 and the forest island - FI). Saturated soil profiles are likely to have slower OM decomposition rates from anaerobic conditions (McLatchey and Reddy, 1998; Kwon et al., 2013). Greater vegetation input quantities in rainforest sub-environments such as SF1 & 2 and FI are possibly also a contributing factor to the highest SOC stocks, however this would need to be measured to verify. In the drier soils SF2 and FI, more pronounced wet-dry cycles may lead to a greater amount of SOM decomposition through microbial enzyme activity than in SF1 soil, which is wetter and potentially has less oxygen. SG1 & 2, SW1 & 2 and ST1 & 2 soils are drier and more exposed than the latter 3, which potentially controls TOC, and results in some of the lowest TOC and SOC stocks.

In comparison to other studies on similar soil types, the depth of sampling was highlighted as important for assessing SOC stock, as previous studies that sampled to just 1 m had lower values than those sampled in this study to >1 m. Despite this, savannah and forest soils in this study had a lower average SOC stock than those for savannahs assessed in NSCD and WISE databases (soils sampled to 3 m) by Jobbágy and Jackson (2000). Additionally, a high variability was apparent from the differences in SOC stocks between the same sub-environments in Wowetta and Surama. For example, SG1 (57 t C ha^{-1}) and SG2 (158 t C ha^{-1}). This shows the need to sample a larger area in order to provide more accurate results. A greater precision in SOC stocks will provide more exact assessments of the effects of local climate change and associated weather patterns on these stocks, and the implications upon vegetation, e.g. shifts from forest to savannah with lower precipitation. If savannah with lower SOC stocks than previously estimated expands into swamp forest area, this may cause a greater loss in forest SOC than previously thought. Considering the uncertainty observed through the differences in climate model predictions of precipitation changes (IPCC, 2014), a reverse scenario to the latter may result. This would involve greater

precipitation and potential forest expansion resulting in greater SOC stocks if swamp forest areas expand into any of the savannah sub-environments assessed. MF2 and MRF also have greater SOC stocks than savannah soils and may increase these if forests expand into savannah areas. However, as can be seen in Section 4.3.4 and Figure 4.8 and Figure 4.9, if MF1 and KPF expand into savannah areas there will not be a significant change in SOC stocks. Rainforest expansion would, however, depend upon anthropogenic reactions; which may mean harvesting of timber resources as rainforests expand, thus preventing potential increases in SOC stock through SF, MF2 and MRF expansion.

The following chapter will explore the organic carbon molecular characteristics of vegetation inputs and surface soils along the Wowetta savannah-rainforest transect.

Chapter 5. **Surface soil and vegetation input organic molecular chemistry of the Wowetta transect**

5.1 Introduction

Research in lignin and carbohydrates with regards to the tropics is still young and requires further investigation, especially in view of the most recent IPCC (2013) predictions, as noted in Chapter 1. Characterising soil organic matter (SOM) allows insight into the relative components that comprise the organic fraction of the soil. This aids understanding of the interactions of SOM components with surrounding edaphic, biological and environmental conditions in savannah and rainforest regions, which are known to hold large carbon stores (Batjes and Dijkshoorn, 1999), is fundamental to increasing the reliability of future predictions and creating mitigation and land management policies for SOC storage.

Therefore, in order to elucidate the fate of lignin and carbohydrates in the potentially climate-sensitive location of Central Guyana, this chapter presents TMAH thermochemolysis products for living plant tissues, freshly-deposited litter and samples of surface soil (0-6 cm) from a 5 km transect spanning the inverted plinthic landscape of seasonally wet rainforest and savannah.

The objectives in this study are to:

a) identify the molecular chemical composition (lignin, tannin and carbohydrates) and changes between vegetation inputs and surface soils in the savannah, transition and rainforest sub-environments,
b) compare these compositions and changes between the sub-environments,
c) assess the relationship between SOC calculated in chapter 4, (which factors in total organic carbon (TOC), bulk density and horizon thickness), to these compositions and changes along the transect, to inform the relative importance to overall soil organic matter.

5.2 Methodology, samples and sites

The sampling transect of Wowetta was chosen in which to analyse soil organic matter (SOM) molecular characteristics. The sites along this transect include savannah grassland (SG), savannah woodland (SW), savannah tree (ST), savannah swamp (SS), transition (T), swamp forest (SF), pisoplinthic forest (PF) and mixed forest (MF). This transect was chosen due the greater variety in soil types along the transect than that of Surama, it also provided the transect format which clearly describes the sequential changes of sub-environments along one line from savannah into rainforest. Greater detail of these soil types and sub-environments is provided in Chapter 3.

For details of the sampling and laboratory methods, as well as the parameters and statistics used in this chapter please refer to Chapter 2, Sections 2.9 - 2.14.

5.3 Results

5.3.1 Thermochemolysis products

Figure 5.1 shows a typical distribution of thermochemolysis with TMAH products from the treatment of solvent-extracted surface soil. This reveals a range of compounds forming three groups. Methylated phenols consisting of guaiacyl (G), syringyl (S) and *p*-hydroxyphenyls (P) and the methyl esters of the cinnamyl phenols ferulic acid (G18) and *p*-coumaric acid (P18) constitute the first group. Non-lignin compounds including 1,2,4-trimethoxybenzene (1,2,4-TMB) and 1,3,5-trimethoxybenzene (1,3,5-TMB) comprise the second group. Lastly, a group of methylated carbohydrates were identified. These form two sets of products and have been distinguished as follows: (i) four methylated carbohydrates (MC1-4: *m/z* 129), referred to as MC's from here on in and, (ii) three cellulose THM products (1, 2 and 4: *m/z* 142, 156 and 154 respectively), referred to as CTP's from here on in. Abbott et al. (2013) reported the presence of four CTPs (1, 2, 3 and 4) in a Swedish peat – of this latter group three CTP's were present (1, 2 and 4) which are also as yet unassigned products (see Section 5.3.4). All other THM products are described with full compound names in Table 5.1.

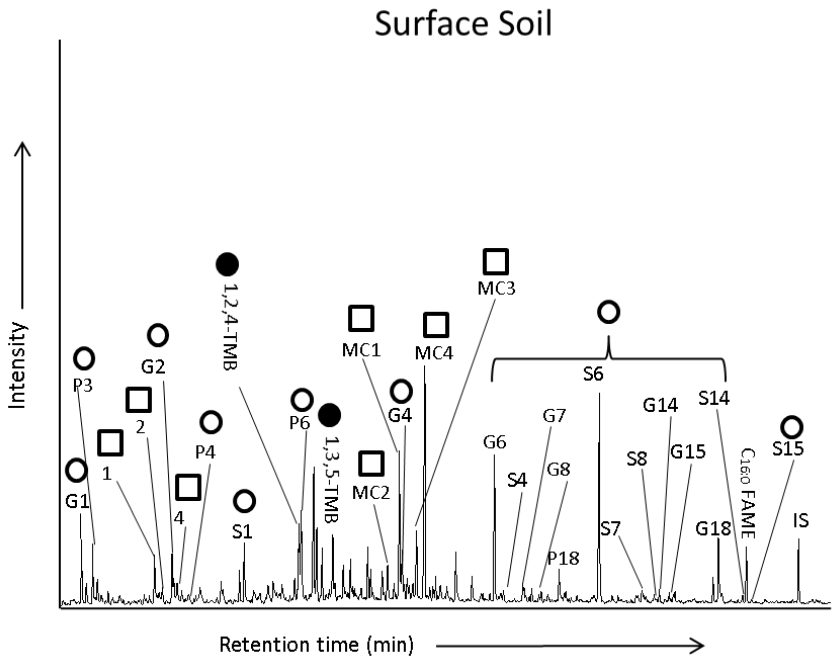


Figure 5.1 Partial chromatogram for the total ion current (TIC) of the three main groups of thermochemolysis products of surface soil. Open circles: methylated lignin phenols; closed circles: oxygenated aromatics 1,2,4-TMB and 1,3,5-TMB; open squares: methylated carbohydrates and cellulose THM products; IS: internal standard.

Table 5.1 Main three product groups and their individual compound names from the THM of surface soils, fresh shoot / leaf litter and wood litter (continued overleaf)

Group 1	Compound	Group 2	Compound
P1	methoxybenzene	1,2,4-TMB	1,2,4-trimethoxybenzene
G1	1,2-dimethoxybenzene	1,3,5-TMB	1,3,5-trimethoxybenzene
P3	4-methoxybenzeneethylene		
G2	3,4-dimethoxytoluene		
G3	3,4-dimethoxybenzeneethylene		
S1	1,2,3-trimethoxybenzene		
P6	4-methoxybenzoic acid methyl ester		
G4	3,4-dimethoxybenzaldehyde		
G6	3,4-dimethoxybenzoic acid methyl ester		
S4	3,4,5-trimethoxybenzaldehyde		
G7	cis 1-(3,4-dimethoxyphenyl)-2-methoxyethylene		
G8	*trans* 1-(3,4-dimethoxyphenyl)-2-methoxyethylene		
P18	*trans* 3-(-4-methoxyphenyl)-3 propenoic acid methyl ester		
S6	3,4,5-trimethoxybenoic acid methyl ester		
S7	*cis* 1-(3,4,5-trimethoxyphenyl)-2-methoxyethylene		
S8	*trans* 1-(3,4,5-trimethoxyphenyl)-2-methoxyethylene		
G14	*threo/ethryo* 1-(3,4-dimethoxyphenyl)-1,2,3-trimethoxypropane		
G15	*threo/ethryo* 1-(3,4-dimethoxyphenyl)-1,2,3-trimethoxypropane		
G18	*trans* 3-(3,4-dimethoxyphenyl)-3-propanoic acid methyl ester		
S14	*threo/ethryo* 1-(3,4,5-trimethoxyphenyl)-1,2,3-trimethoxybenzene		
S15	*threo/ethryo* 1-(3,4,5-trimethoxyphenyl)-1,2,3-trimethoxybenzene		

Table 5.1 continued

Group 3	Compound
1	cellulose THM product
2	cellulose THM product
4	cellulose THM product
MC1	3-deoxy-4,5,6-tri-*O*-methylgluconic acid, methyl ester
MC2	Tetra-*O*-methyl-3-deoxy-D-*arabino*-hexanoic acid, methyl ester
MC3	3-deoxy-4,5,6-tri-*O*-methylmannonic acid, methyl ester
MC4	Tetra-*O*-methyl-3-deoxy-D-*ribo*-hexanoic acid, methyl ester

5.3.2 Characterisation of savannah-rainforest transect inputs and surface soils

The distribution of thermochemolysis products in wood litter (Figure 5.2), fresh shoot / leaf litter (Figure 5.3) and surface soils (Figure 5.4) are presented for each sub-environment (SG – MF). These simplified schematics of chromatograms show that lignin phenols are the major thermochemolysis components from the depolymerisation of the solvent-extracted insoluble residues from whole plant tissues or soils. The amounts of these phenols differ between wood litter, fresh shoot / leaf litter and surface soils, as well as between sub-environments. Methylated carbohydrates (MC1-4) are detected in all samples and are more abundant in the thermochemolysis products from surface soils than vegetation samples. The amounts of THM products 1,2,4-TMB and 1,3,5-TMB as well as the cellulose products (1, 2 and 4) were low in all samples.

In wood litter (Figure 5.2) lignin phenol S6 is most prominent in sub-environments SW and ST and MF. The latter sub-environment also has a high amount of CTP1. Lignin phenol product P6 is most prominent in sub-environments SS and SF. The latter sub-environment also has a high amount of S6. Sub-environment T has relatively similar amounts of most compounds, although CTP1 is fractionally greater than other compounds. Therefore, lignin phenols mainly take precedence within wood litter samples, with some sub-environments also showing high amounts of CTP1.

In fresh shoot / leaf litter (Figure 5.3) lignin phenol S6 is most prominent in sub-environments SW, ST, SS, PF and MF. All other THM products identified are in low amounts. SF leaf litter has a clearly predominant P6 phenol compared to all other products. SG and T fresh shoot / leaf litter have predominant P18 and G18 lignin phenols. In SG fresh shoots, these are not as intense compared to other phenols such as S6 and G6 which are also relatively abundant. As with wood litter, fresh shoot / leaf litter simplified chromatograms show that lignin phenols are dominant within these extracted residue samples.

Surface soils (Figure 5.4) show a marked difference in the distribution of THM products to wood litter and fresh shoot / leaf litter. Sub-environments SG, SW, ST, SS, T and SF have more prominent, or similar amounts of methylated carbohydrates (MC1 and MC2 or 3) as the most abundant lignin phenols. Sub-environment SG has most prominent MC1 and 2 products with significantly smaller amounts of all other products. SW, ST

and SF have greater or similar amounts of MC1 and 3 to lignin phenols G6 and S6. SS has a predominant MC3 product, and similar amounts of MC1, G6, P18 and G18 which all have high amounts. T has similar amounts of MC3 to G6 and G18, which are the most prominent products. FI has dominant products of MC3, G6 and CTP1. Aside from S6 and G18, no other lignin phenols were detected in this soil. Sub-environments PF and MF show a different distribution however. Lignin phenol P6 is most prominent in both samples, whilst all other products are of much smaller amounts.

Certain lignin phenols, such as S4, S6, G4, G6 and G18 have the potential to originate from sources other than lignin. S4 and S6 can also originate from demethylated lignin (lignin which has been attacked by microbes) and tannin, G4 and G6 from vanillic acid and G18 from caffeic acid (Filley et al., 2006). Therefore the amounts of these products in Figure 5.2, Figure 5.3 and Figure 5.4 may not be purely of lignin origin. The true origin of these products has been investigated via the use of ^{13}C-labelled TMAH and is detailed in the following sections.

The difference noted here between vegetation samples and surface soils indicates the alteration of soil organic matter (SOM) from vegetation inputs to soil, and is discussed in further detail in the following sections. The average values of triplicates for each compound in Figure 5.2, Figure 5.3 and Figure 5.4 are given in Appendix G.

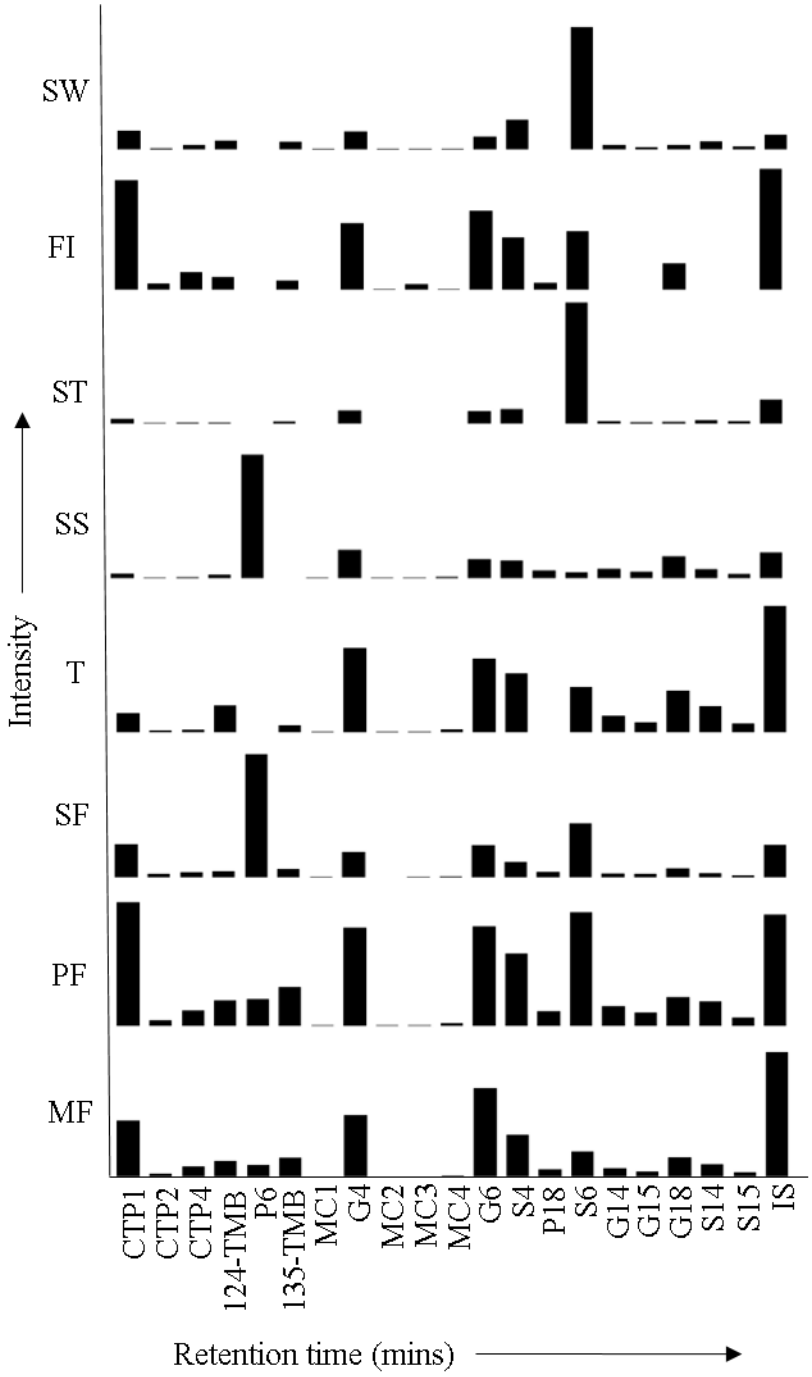


Figure 5.2 Simplified schematic of the total ion current (TIC) of thermochemolysis products from wood litter for the sub-environments SW – MF.

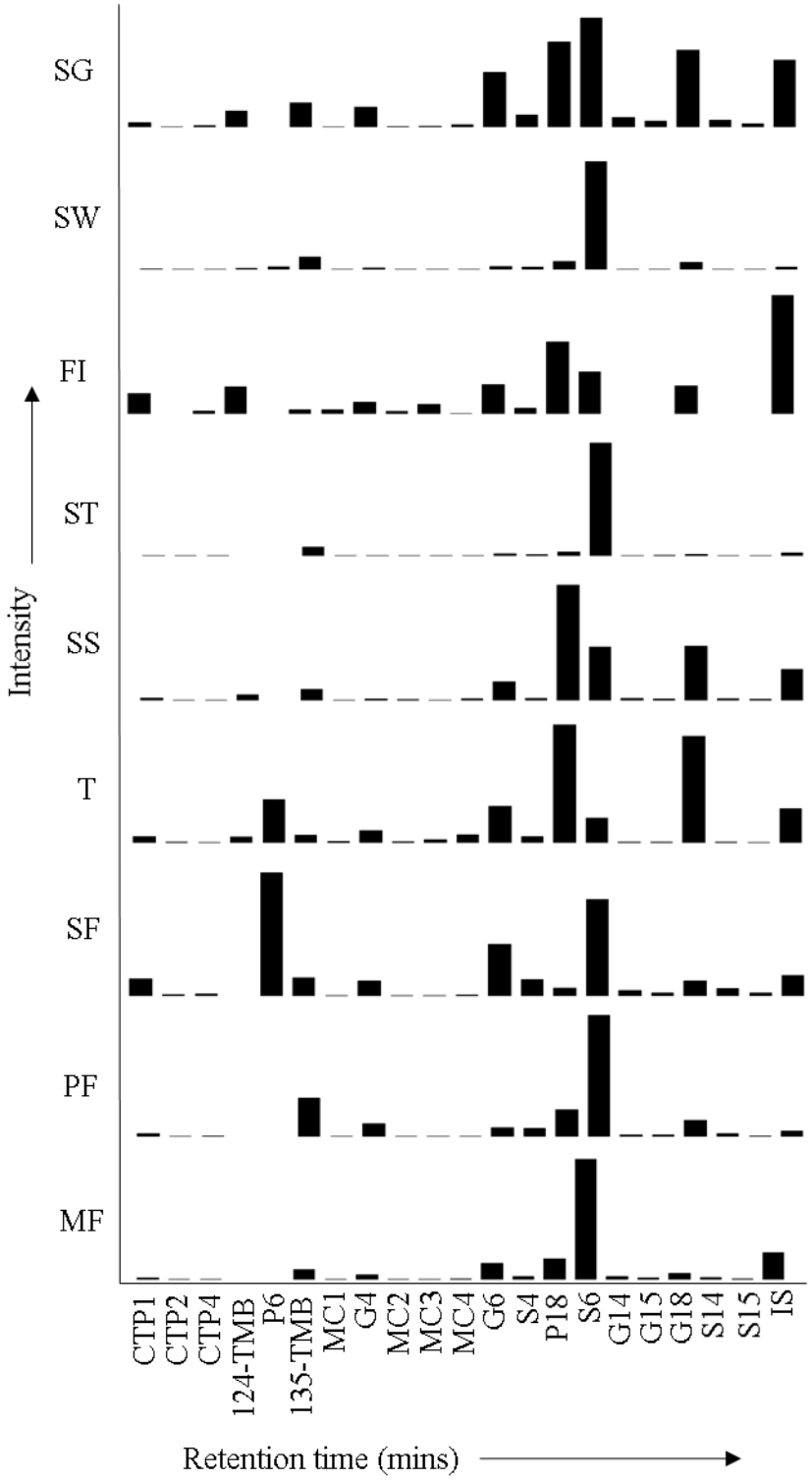


Figure 5.3 Simplified schematic of the total ion current (TIC) of thermochemolysis products from fresh shoot / leaf litter at each sub-environment across the transect (SG – MF).

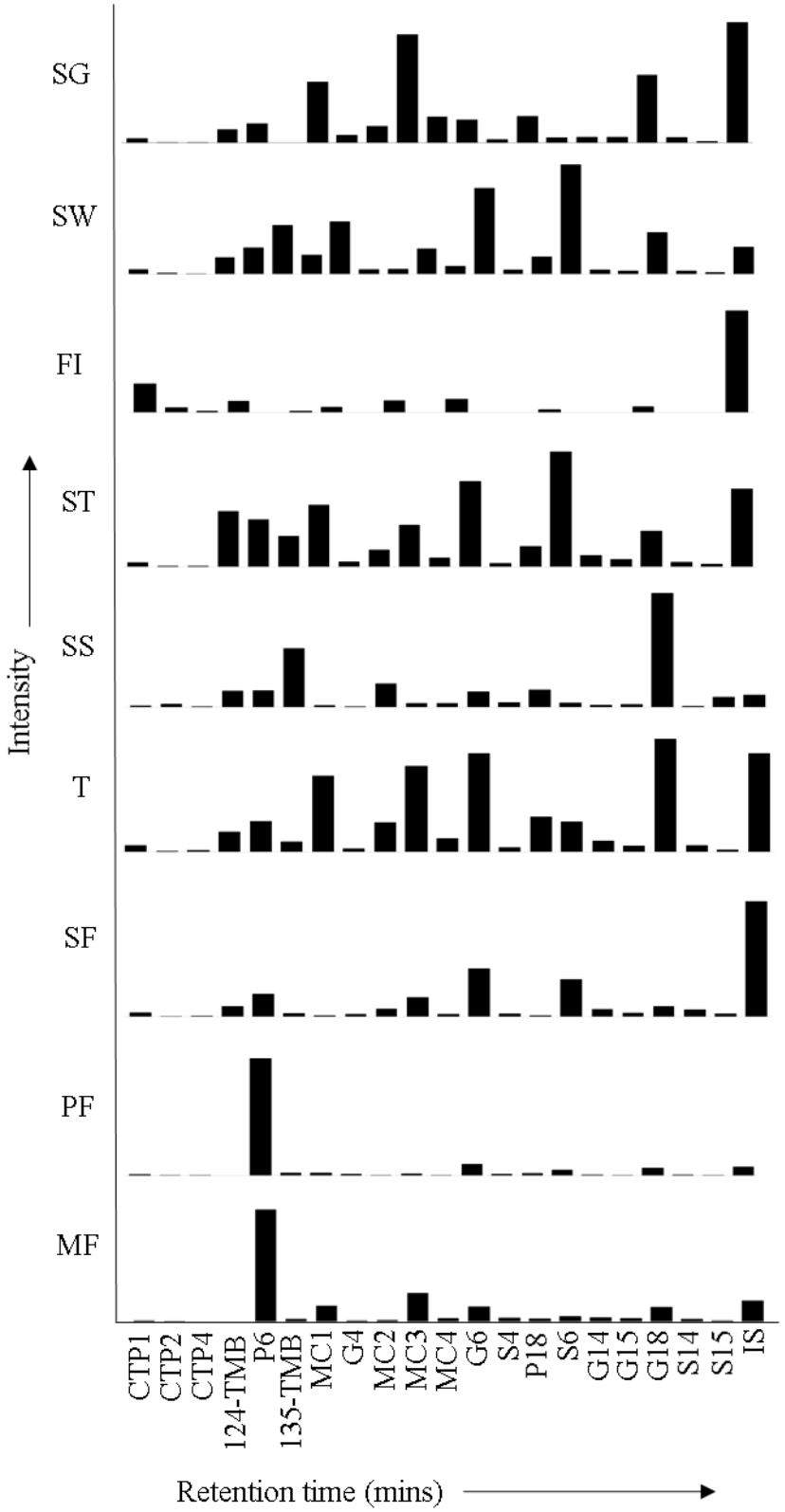


Figure 5.4 Simplified schematic of the total ion current (TIC) of thermochemolysis procuts from surface soils at each sub-environment across the transect (SG-MF).

5.3.3 Comparison of THM product proportions

The different percentages of the main lignin phenols (S4, S6, G4, G6, G18 and P18: Λ), gallic acid and 1,3,5-TMB, CTP's (1, 2 and 4) and MC's (MC1-4) are presented for wood litter (Figure 5.5(a)), fresh shoots / leaf litter (Figure 5.5(b)) and surface soils (Figure 5.5(c)) across the savannah-rainforest transect.

There are clear differences between the proportion of products in vegetation inputs of wood litter and fresh shoot / leaf litter and the surface soils. The vegetation inputs are composed mainly either of tannin (gallic acid) or lignin, and CTP's are also a relatively important product in wood litter of FI, SF PF and MF. Soils are predominantly either lignin (SS, SF and PF), MC's (SG and MF) or a similar proportion of both (T). Soils from SW and ST sub-environments have a predominant mix of the three latter product groups (tannin, methylated carbohydrates and lignin). Uniquely, FI has similarly predominant proportions of CTP's and lignin. Proportions of 1,2,4-TMB in both vegetation and soils are much smaller than lignin or tannin products, although are more significant in soils of SW, FI, ST and SF. 1,3,5-TMB has relatively small proportions in most samples, but is slightly more prominent in leaf litter (particularly sub-environment PF).

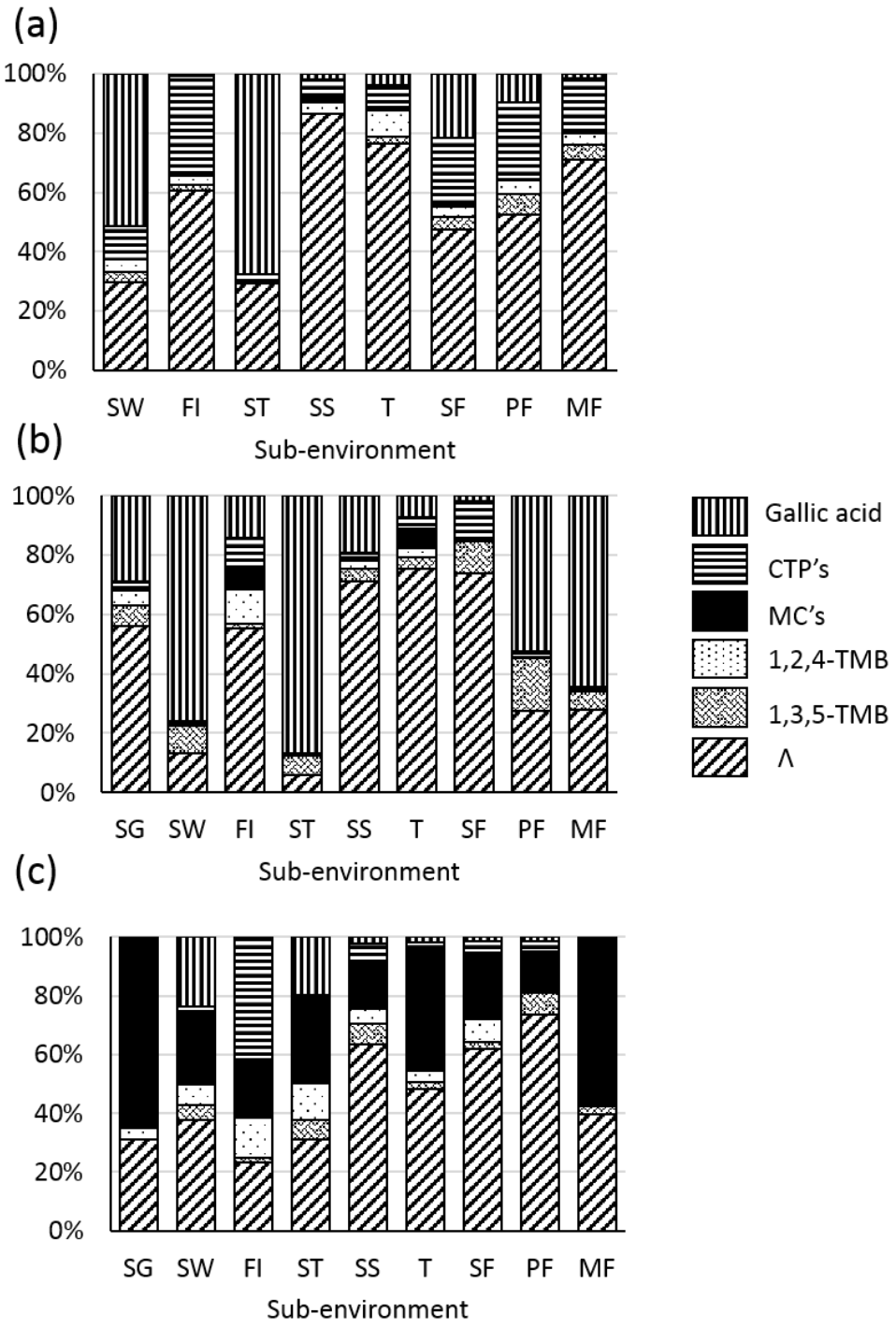


Figure 5.5 Relative proportions of lignin, non-lignin and carbohydrate yields in (a) wood litter, (b) fresh shoot / leaf litter and (c) surface soil in sub-environments across the transect.

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5.3.4 Carbohydrates

Figure 5.7 (a) shows THM products of group 3 (Table 5.1) with a base peak of *m/z* =129 and fragment ion of *m/z* = 101, which have been tentatively assigned as methylated carbohydrates according to the work of Fabbri et al. (1996) and Fabbri and Helleur (1999). This was initially done by Fabbri et al. (1996) from the NaOH extracted residue of humin and humic acids from an Italian agricultural soil (Carpi, near Bologna). Since then, further investigation has been undertaken by Fabbri and Helleur (1999) to verify the compounds in a series of monosaccharide hexose standards (specifically D-Glucose and D-mannose). These standards were labelled with TMAH and analysed using py-GCMS at 500 °C, resulting in the methylated carbohydrates with similar mass spectra. They were then tentatively assigned as tetra-*O*-methyl-3-deoxy hexanoic acids. The proposed formation of these methylated carbohydrates (also known as metasaccharinic acids) through TMAH thermochemolysis can be seen below in Figure 5.6.

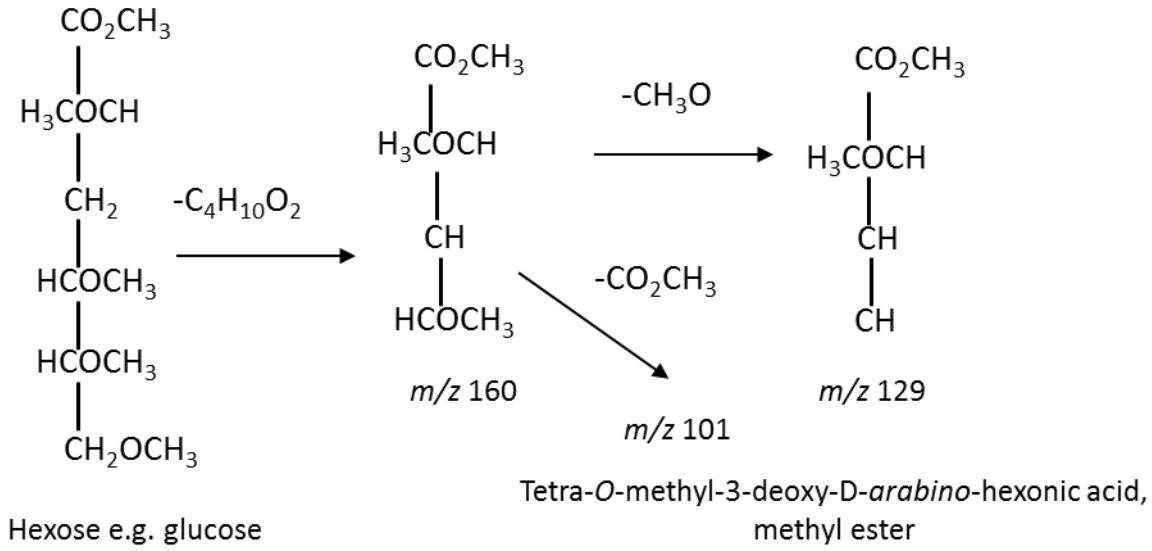

Figure 5.6 Proposed TMAH thermochemolysis formation of methylated carbohydrates from hexoses, adapted from Fabbri and Helleur, 1999.

As in Fabbri and Helleur (1999), the methylated carbohydrates detected here form two pairs: MC1 & 3 and MC2 & 4. The mass spectra of these compounds using both unlabelled and ^{13}C-labelled TMAH are shown in Figure 5.7 (a) and (b) respectively. From these spectra, it can be seen that the pair MC2 & 4 have fully methylated acidic oxygen functional groups from the increase in weight (+2, 3 or 4) upon ^{13}C-labelled TMAH thermochemolysis (thus giving an *m/z* 103 from *m/z*101, *m/z* 131 from *m/z* 129, *m/z* 164 from *m/z* 161 and *m/z* 195 from *m/z* 191 (Fabbri and Helleur, 1999). However, this is not seen in MC1 & 3, where only 2 or 3 functional groups have been methylated, instead of the respective 3 and 4 that are available. For example, *m/z* 147 gives *m/z* 149 and *m/z* 177 gives *m/*z 180, where ^{13}C-labelled TMAH should yield *m/z* 150 and *m/z* 182 respectively. This is apparent because of the difference in weight of 14 between *m/z* 147 and *m/z* 177 of MC1 & 3 compared to *m/z* 161 and *m/z* 191 of MC2 & 4: indicating an unmethylated oxygen functional group. This result has been presented previously in Schwazinger et al., 2002 and Abbott et al., (2013), and was suggested in the latter to need a greater amount of TMAH to fully methylate the compounds.

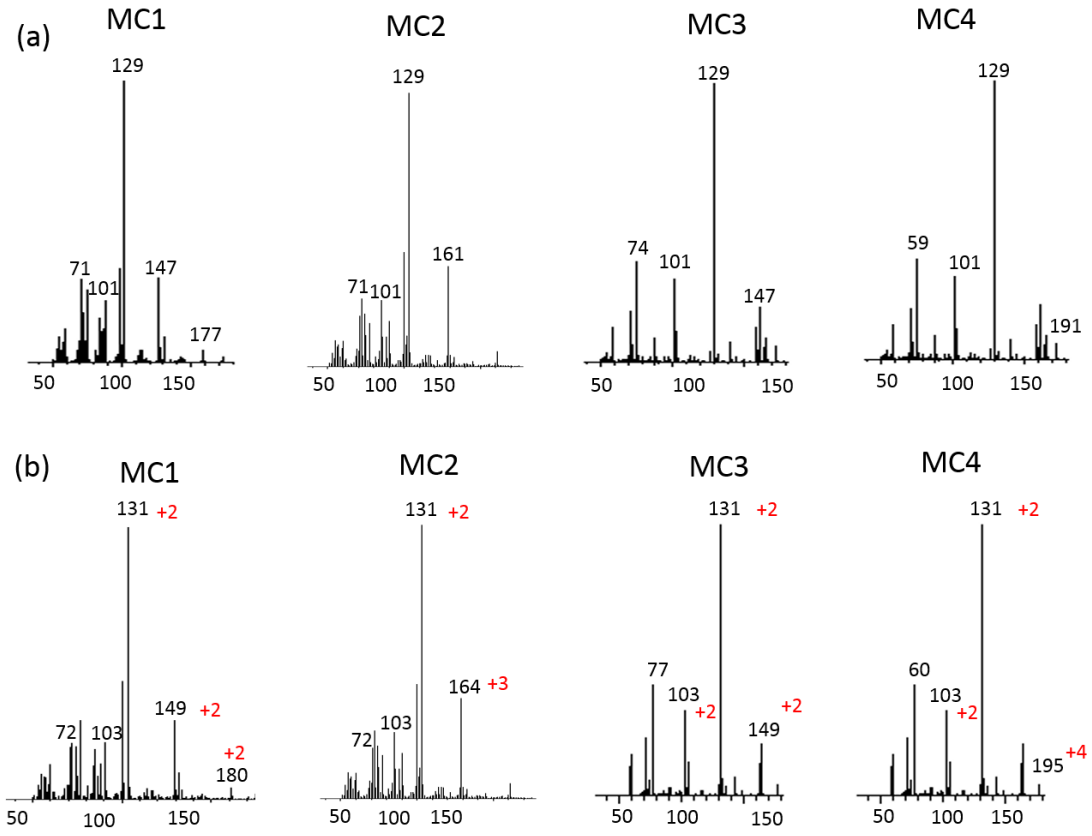

Figure 5.7 Mass spectra of methylated carbohydrates (MCs) 1-4 via (a) unlabelled TMAH and (b) ^{13}C-labelled TMAH. Red numbers indicate the number of extra hydroxyl groups methylated with ^{13}C-labelled TMAH as compared to unlabelled TMAH.

A standard of D-(+)-glucosamine hydrochloride was analysed using the same pyrolysis GC-MS procedure and internal standard (5α-androstane) as all other samples analysed in this study. Analysis of this glucosamine standard was undertaken in order to investigate the occurrence of methylated carbohydrates (MC's) in this compound to add evidence that these products are of microbial origin, as has been previously reported (Kandeler et al., 2000; Amelung et al., 2001; Glaser et al., 2004).

Figure 5.8 shows that the glucosamine standard analysed does produce all four MC products, in addition to 1,2,4-TMB. This therefore indicates that these products are likely to originate from microbial sources, in comparison to vegetation inputs. Literature on the origin of 1,2,4-TMB is currently uncertain, with reports of both carbohydrate (Fabbri and Helleur, 1999) and tannin sources (Nierop et al., 2005). The presence of 1,2,4-TMB also indicates that this product may be microbially derived, and may arise from the TMAH thermochemolysis of microbial carbohydrates. The origin of 1,2,4-TMB in the soils and vegetation analysed in this study is discussed further in Sections 5.3.10 and 5.4.3.

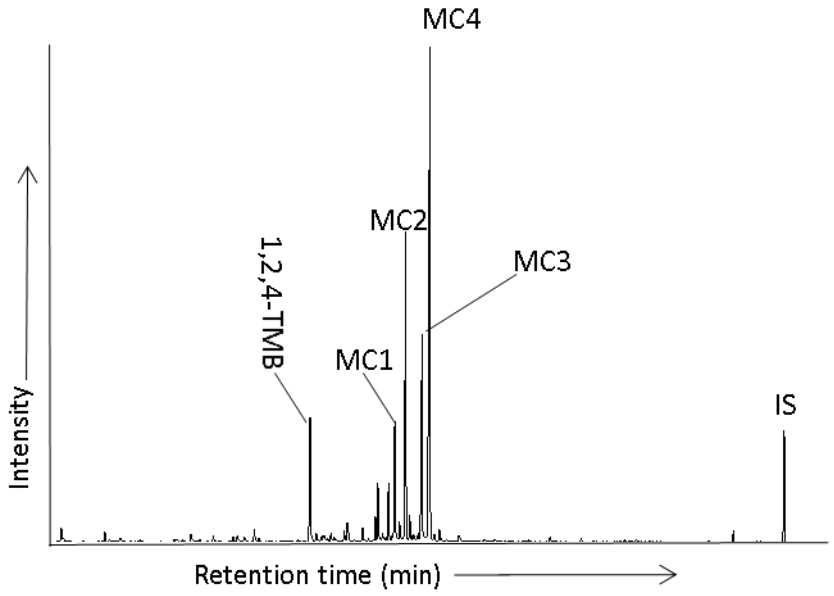

Figure 5.8 Partial chromatogram of D-(+)-glucosamine hydrochloride standard. IS: internal standard.

Figure 5.9 shows the amount of the methylated carbohydrates across the savannah-rainforest transect in wood litter (a), fresh shoot / leaf litter (b) and surface soils (c). Vegetation inputs of wood litter and fresh shoot / leaf litter are significantly lower than surface soil amounts, with the former ranging from 0 – 0.017 mg / 100 mg OC and surface soils from trace amounts of 0.05 mg / 100 mg OC to more significant amounts of 0.9 mg / 100 mg OC.

Further to this large difference in methylated carbohydrate amounts between vegetation and surface soils, the individual carbohydrates also differ in relative amounts within the sample set. For example, MC4 is predominant in both wood litter and fresh shoots / leaf litter (MC4 average = 0.004 mg / 100 mg OC, compared to an average of 0.001 – 0.003 mg / 100 mg OC for MC1, 2 & 3), whilst MC1 & 3 dominate in the majority of surface soil sub-environments (averages of 0.28 and 0.31 mg / 100 mg OC respectively compared to averages of 0.05 and 0.06 mg / 100 mg OC for MC2 & 4 respectively).

FI wood litter shows a significantly different MC composition, however: it has a dominant MC3 peak (0.014 mg / 100 mg OC), no detected MC1 and only traces of MC2 and 4 (0.0004 mg / 100 mg OC). FI leaf litter products included all MC's and are also dominated by MC3 (0.012 mg / 100 mg OC), with <0.005 mg / 100 mg OC of MC1 & 2 and trace amounts of MC4. FI soil also shows significant differences to all other soils analysed as only trace amounts of MC 1 & 3 were detected.

Sub-environment SG surface soil differs to the other soils studied with a predominance of MC2 (0.65 mg / 100 mg OC compared to 0.37, 0.1 and 0.16 mg / 100 mg OC for MC1, 3 and 4 respectively).

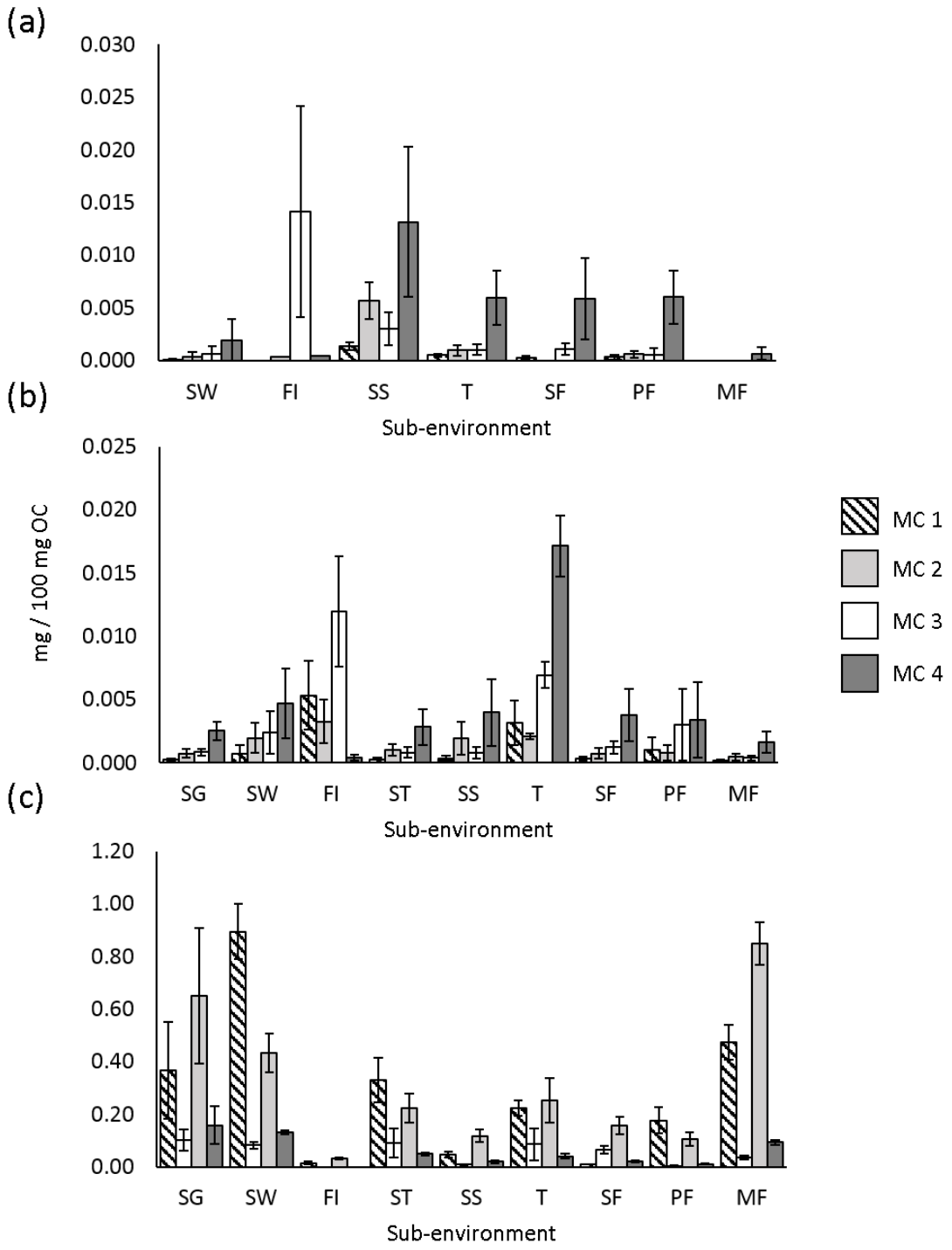

Figure 5.9 Methylated carbohydrates 1-4 in (a) wood litter, (b) fresh shoots / leaf litter and (c) surface soils across the transect. Standard error (S.E.) bars of 3 replicates shown.

A second set of carbohydrates were also identified (CTP1, 2 and 4) in traces of soil and vegetation samples across the savannah-rainforest transect. Figure 5.11 shows their mass spectra, with *m/z* 142, 156 and 154. These compounds result from the thermochemolysis of cellulose (Abbott et al., 2013) and have been given the numbering 1, 2 and 4 in Figure 5.2, Figure 5.3 and Figure 5.4; this numbering correlates to the compounds in Swain (2013) and Abbott et al., (2013). Figure 5.10 shows all four CTP's (labelled as 1, 2, 3 and 4) identified in the authentic cellulose standard analysed by Abbott et al. (2013). The third CTP (3) with an m/z of 156 (an isomer of CTP2) in Abbott et al., (2013) was not detected in these samples. These compounds have also previously been detected by Schwarzinger et al. (2002) and more recently by Bardy et al. (2011) in Amazonian podzols. Even more so than the MC's, these cellulose derivatives are still largely unknown compounds and as yet need further work to clarify their origin.

Figure 5.11 shows the alteration in methylation from unlabelled to ^{13}C-labelled TMAH for CTP1, 2 and 4. With ^{13}C-labelled TMAH analysis, all three CTP's show an additional weight of two, highlighting that there are two hydroxyl groups present on each of these products, resulting from the THM chemical reaction with TMAH.

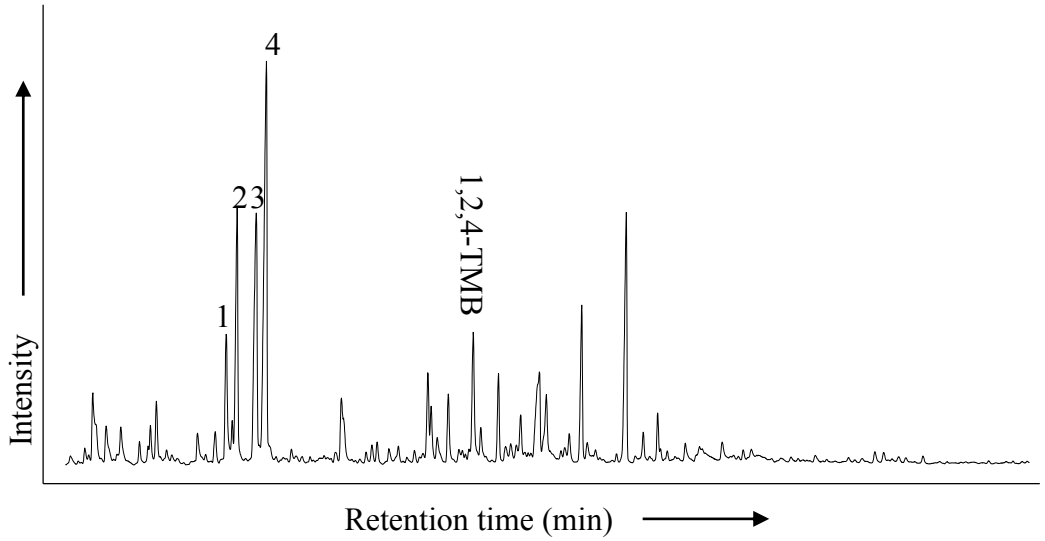

Figure 5.10 TIC of the authentic cellulose standard analysed in Abbott et al., 2013.

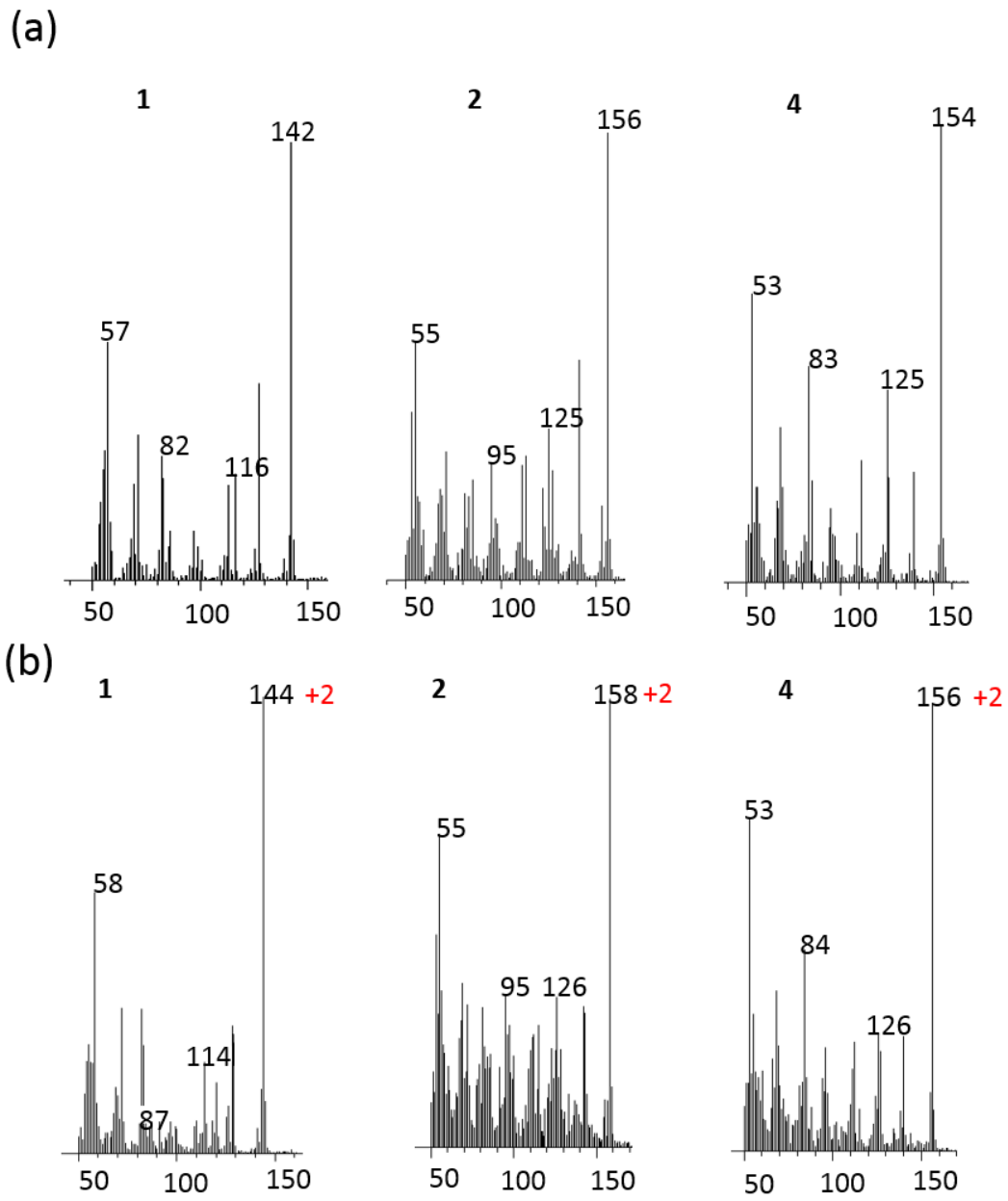

Figure 5.11 Mass spectra of the methylated carbohydrates using (a) unlabelled TMAH and (b) ^{13}C-labelled TMAH, which show an addition of two mass units (in red next to the main ion), as compared to those methylated with unlabelled TMAH.

Figure 5.12 shows how the amounts of the three detected CTP's (1, 2 and 4) change across the transect in vegetation inputs (a and b) and surface soils (c). CTP2 and 4 show similar low amounts throughout the transect for vegetation inputs and surface soils. CTP1 (*m/z* 142) was greater in vegetation inputs and soils than 2 and 4, except in ST fresh shoots / leaf litter which showed similar amounts, and SS soil which showed slightly lower amounts. All soils are degraded in comparison to total inputs, except for CTP1 (SG) and CTP2 (SS); these soils may indicate elevated microbial input or preservation. However, the elevated amount in soil compared to inputs is small: SG 0.02 mg / 100 mg OC and SS 0.002 mg / 100 mg OC. CTP1 fluctuates between soils, with SW, FI and PF having the greatest and SS the lowest amount. This may be due to vegetation inputs, which somewhat reflect this pattern.

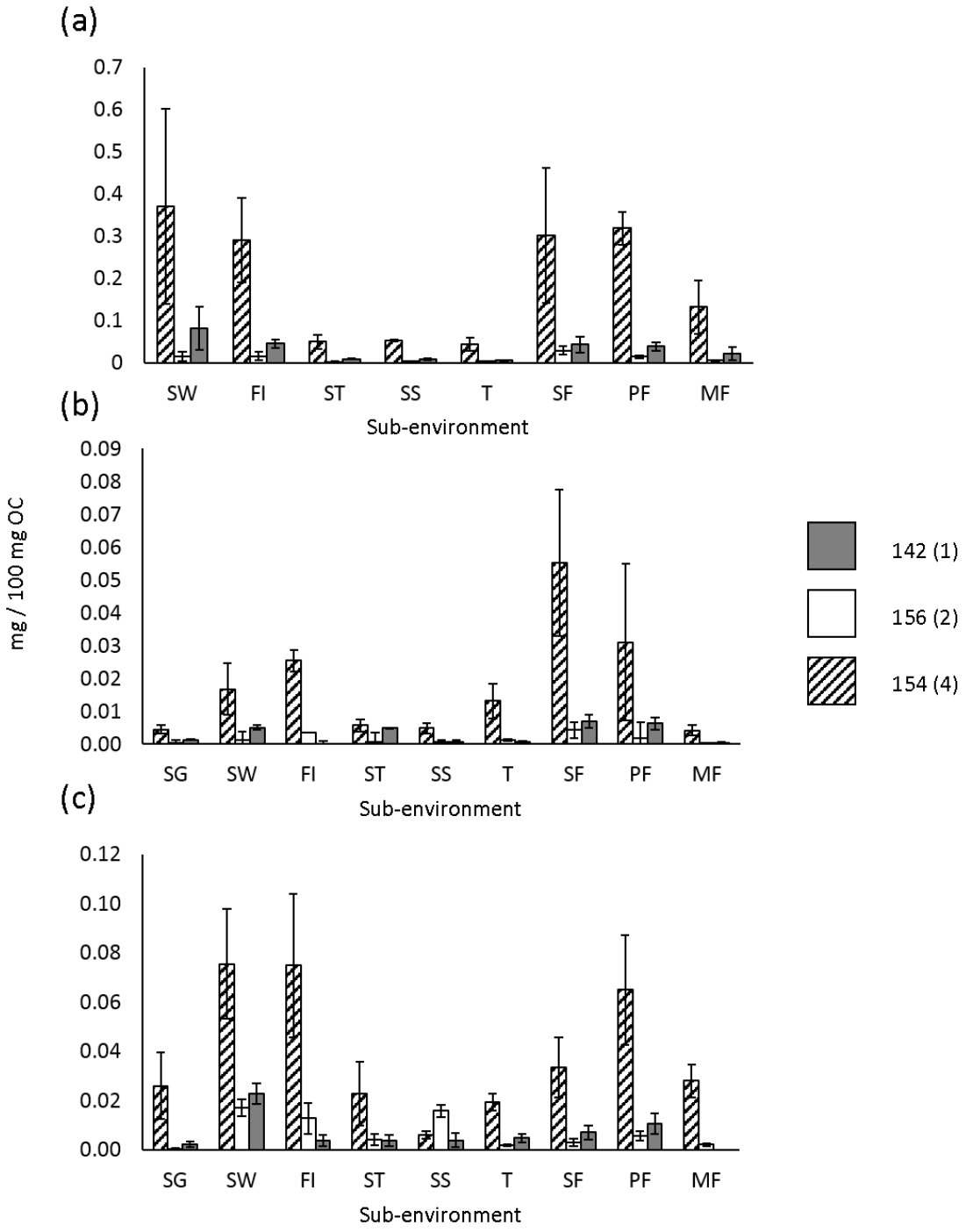

Figure 5.12 CTP1, 2 and 4 in (a) wood litter, (b) fresh shoots / leaf litter and (c) surface soils across the transect. Standard error (S.E.) bars of 3 replicates shown.

Figure 5.13 shows a comparison of the proportion of summed MC's and summed CTP's across the transect in wood litter (a), fresh shoot / leaf litter (b) and surface soils (c). The majority of surface soils show that the proportion of MC's are significantly greater than that of CTP's. FI soil is an exception here, which shows the reverse, with CTP's being present in greater amounts than MC's.

Wood litter shows that the CTP's are consistently greater in proportion to MC's. In fresh shoot / leaf litter the majority of sites also show a greater proportion of CTP's to MC's, except for SS and T. This shows compositional and potentially degradative differences at these sub-environments.

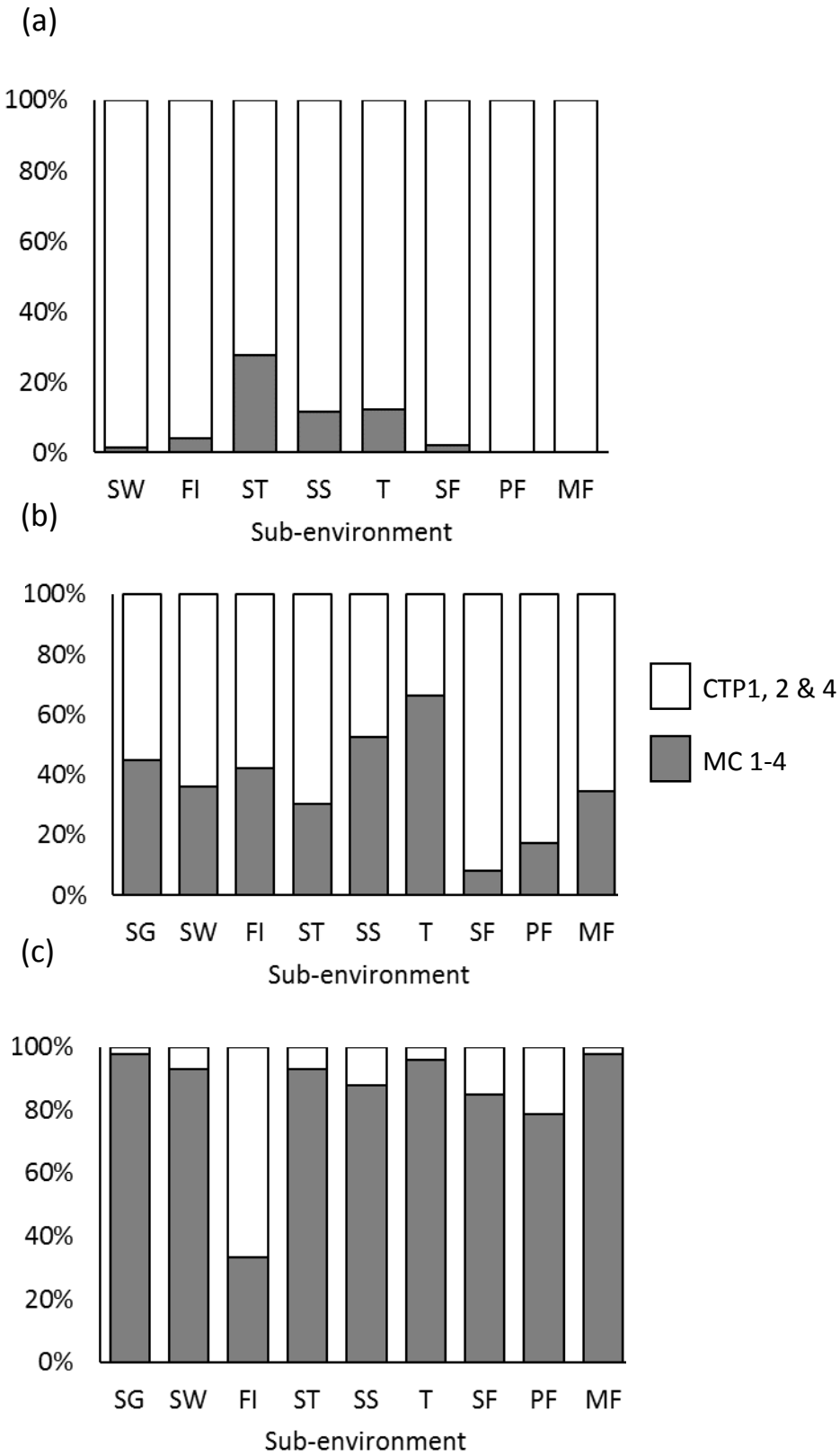

Figure 5.13 Comparison of MC and CTP yields in (a) wood litter, (b) fresh shoots / leaf litter and (c) surface soils across the transect.

5.3.5 Lignin

Lignin parameters were calculated to assess lignin phenol degradation in the surface soils and vegetation inputs across the savannah-rainforest boundary. As previously mentioned in Section 5.3.2, the phenols analysed here can potentially originate from other sources such as demethylated lignin (microbial oxidation) and tannins (Filley et al., 2006) and thus amounts calculated using un-labelled TMAH need to be corrected for these other sources with ^{13}C-labelled TMAH (Filley et al., 1999). The parameters are shown in Table 5.2 with corrected values indicated with a * and uncorrected values without. Individual lignin phenols were corrected via the calculation of the hydroxyl contents of intact lignin, altered demethylated lignin and non-lignin sources (see Appendix B for calculations).

Λ indicates the total of the main lignin phenols (G4, G6, G18, P18, S4 and S6). This parameter decreased significantly after correction; as little as 6% of the original uncorrected values were intact lignin phenols. Largely, this was due to alterations of G18, S6 and G6 after corrections. Ratios C/G and S/G are used as source indicators (non-woody and angiosperm / gymnosperm inputs respectively), and are calculated as (P18+G18)/(G4+G6) and (S4+S6)/(G4+G6) respectively (Filley et al., 2006). After correction C/G saw increases by an average of 19% for vegetation inputs and soils due to lower G6 amounts, although a few exceptions occurred where decreases were observed. S/G largely shows decreases after correction by an average of 50% due to lower S4 and S6 amounts, although a few increases did occur. Ratios $[Ad/Al]_G$ and $[Ad/Al]_S$ indicate oxidation extent for guaiacyl and syringyl phenols, respectively. They are calculated as G6/G4 and S6/S4, respectively (Filley et al., 2006). After correction of $[Ad/Al]_G$ there was an average decrease of 18% again due to lower G6 amounts, however, some soils did see a small (5%) increase. After correction of $[Ad/Al]_S$ there was an average decrease of 55% across all vegetation inputs and soils, again due to lower S6 amounts. Therefore, the largest decreases were observed with regards to the phenol S6, largely due to gallic acid. The proportion of this gallic acid relative to the other THM products analysed in vegetation inputs and surface soils can be seen in Section 5.3.1.

*Table 5.2 Lignin parameters in vegetation inputs and surface soils (Λ in mg / 100 mg OC). The corrected intact lignin values are displayed with a *, and the uncorrected values without. N/A = no wood litter was present at the savannah grassland sampling site, (continued overleaf)*

Site	Λ	Λ*	C/G	C/G*	S/G	S/G*	$[Ad/Al]_G$	$[Ad/Al]_G$*	$[Ad/Al]_S$	$[Ad/Al]_S$*
Savannah grassland										
Fresh shoots	0.35	0.19	3.78	10.19	1.85	0.64	7.15	1.89	14.82	0.73
Wood litter	0	0	0	0	0	0	0	0	0	0
0-6 cm soil	0.77	0.63	3.96	4.81	0.25	0.34	4.13	4.43	2.55	1.28
Savanah woodland										
Fresh shoots and leaf litter	2.51	0.54	3.92	7.75	23.53	1.22	1.64	0.75	45.79	0.35
Wood litter	3.76	1.24	0.13	0.17	5.02	1.68	0.81	0.53	4.84	0.31
0-6 cm soil	3.54	2.36	0.64	0.8	1.24	0.24	22.32	20.77	28.73	3.77
Forest island										
Leaf litter	0.23	0.16	3.05	4.10	2.04	0.57	2.95	2.25	6.31	0.74
Wood litter	0.77	0.66	0.23	0.25	0.76	0.83	1.19	1.02	1.12	0.96
0-6 cm soil	0.06	0.05	0.36	0.42	0.19	0.16	0	0	0	0
Savannah tree										
Fresh shoots and leaf litter	2.49	0.15	4.89	23.12	53.56	4.09	8.58	3.05	127.5	0.41
Wood litter	3.25	0.89	0.06	0.08	5.29	1.19	0.93	0.61	8.23	0.44
0-6 cm soil	1.42	0.74	0.61	0.79	1.3	0.31	19.2	17.35	35.68	5.52
Savannah swamp										
Fresh shoots and leaf litter	0.50	0.35	8.76	23.81	2.42	0.81	3.17	1.5	25.04	1.87
Wood litter	1.21	0.98	0.62	0.77	0.49	0.6	0.66	0.52	0.31	0.17
0-6 cm soil	0.86	0.75	1.87	2	0.53	0.29	20.89	24.77	6.14	2.72

Table 5.2 continued

Site	Λ	Λ*	C/G	C/G*	S/G	S/G*	$[Ad/Al]_G$	$[Ad/Al]_G$*	$[Ad/Al]_S$	$[Ad/Al]_S$*
Transition										
Leaf litter	0.65	0.32	4.2	7.89	0.64	0.88	8.01	8.63	5.44	1.81
Wood litter	0.71	0.54	0.27	0.15	0.68	0.71	0.87	0.75	0.77	0.5
0-6 cm soil	0.84	0.70	1.44	1.59	0.34	0.25	36.24	35.7	7.35	4.8
Swamp forest										
Leaf litter	0.66	0.40	0.45	0.64	2.13	1.58	3.13	2.42	13.59	4.79
Wood litter	1.45	0.78	0.33	0.21	1.38	0.88	1.23	0.92	3.06	0.75
0-6 cm soil	0.82	0.68	3.98	0.25	0.71	0.71	23.28	24.47	16.02	14.1
Petroplinthite hill forest										
Leaf litter	2.03	0.62	2.06	3.24	5.23	0.6	1.13	0.62	23.63	0.44
Wood litter	1.10	0.75	0.22	0.29	0.96	0.91	1.03	0.71	1.64	0.72
0-6 cm soil	1.88	1.58	0.65	0.7	0.55	0.55	10.12	9.9	4.53	3.87
Mixed tree spp. forest										
Leaf litter	0.43	0.12	1.59	2.69	5.69	0.81	3.96	2.58	41.54	2.31
Wood litter	0.87	0.62	0.18	0.28	0.48	0.72	1.44	0.85	0.6	0.44
0-6 cm soil	1.24	1.03	2.05	2.42	1.98	2.18	23.69	22.57	2.77	2.24

5.3.6 Gallic acid (tannin) and demethylated lignin sources

Figure 5.15 shows the amounts of intact lignin, demethylated lignin and gallic acid in syringyl phenol S6 in wood litter (a), fresh shoots / leaf litter (b) and surface soils (c) across the sampling transect. This syringyl phenol showed the most alteration after correction with ^{13}C-labelled TMAH.

The % aromatic hydroxyl contents of S6 (1% OH, 2% OH and 3% OH) were determined using ^{13}C-labelled TMAH. 1% OH refers to intact lignin, 2% OH to microbially demethylated lignin (syringic acid) and 3% OH to gallic acid (tannin derived). Filley et al., (2006) showed how multiple sources such as intact lignin and microbially altered lignin (specifically their methoxyl functionalities) can lead to the formation of identical products (Figure 5.14). Using previously established equations (Filley et al., 2006), (see Appendix B) these hydroxyl contents were calculated and then converted into mg / 100 mg OC. The unlabelled TMAH thermochemolysis of these samples allowed the identification of S6 and thus the baseline essential for accurately calculating the ^{13}C percentage addition.

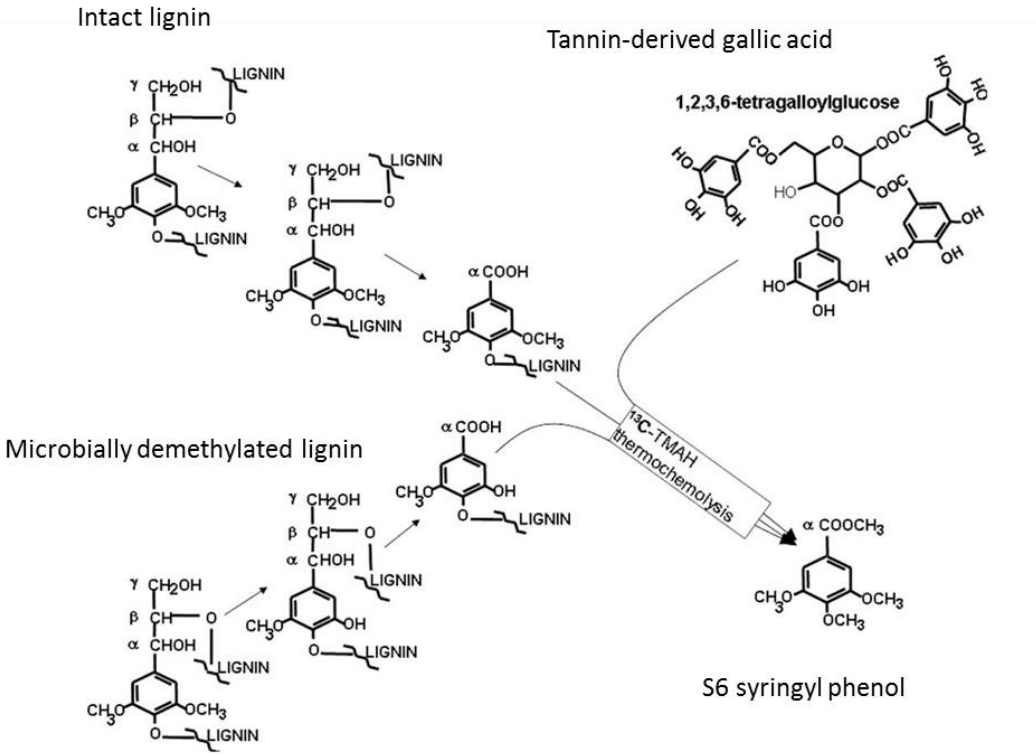

Figure 5.14 Diagram to show how multiple sources can result in the same methylated product (S6) upon TMAH thermochemolysis, adapted from Filley et al., 2006.

It is evident from Figure 5.15 a, b and c that gallic acid is most abundant in sub-environments SW and ST. In vegetation inputs there is an average amount of 2.6 mg / 100 mg OC (SW) and 2.16 mg / 100 mg OC (ST) and for surface soils an average of 1.48 mg / 100 mg OC (SW) and 0.47 mg / 100 mg OC (ST). All other sub-environments generally show much lower amounts (<0.27 mg / 100 mg OC), except for leaf litter in forest sub-environment PF (1.19 mg / 100 mg OC). Generally, both demethylated and intact lignin are much lower than gallic acid across vegetation inputs and surface soils. Demethylated lignin averages 0.05 mg / 100 mg OC in vegetation inputs and 0.04 mg / 100 mg OC in surface soils. Intact lignin averages 0.06 mg / 100 mg OC in vegetation inputs and 0.11 mg / 100 mg OC in surface soils.

Despite a low surface soil average, intact lignin is greater than gallic acid in all sites except SW and ST. This shows that these sub-environments have uniquely high amounts of gallic acid in surface soils along the savannah-rainforest transect. Wood litter and fresh shoot / leaf litter generally show greater amounts of gallic acid than demethylated or intact lignin across the transect.

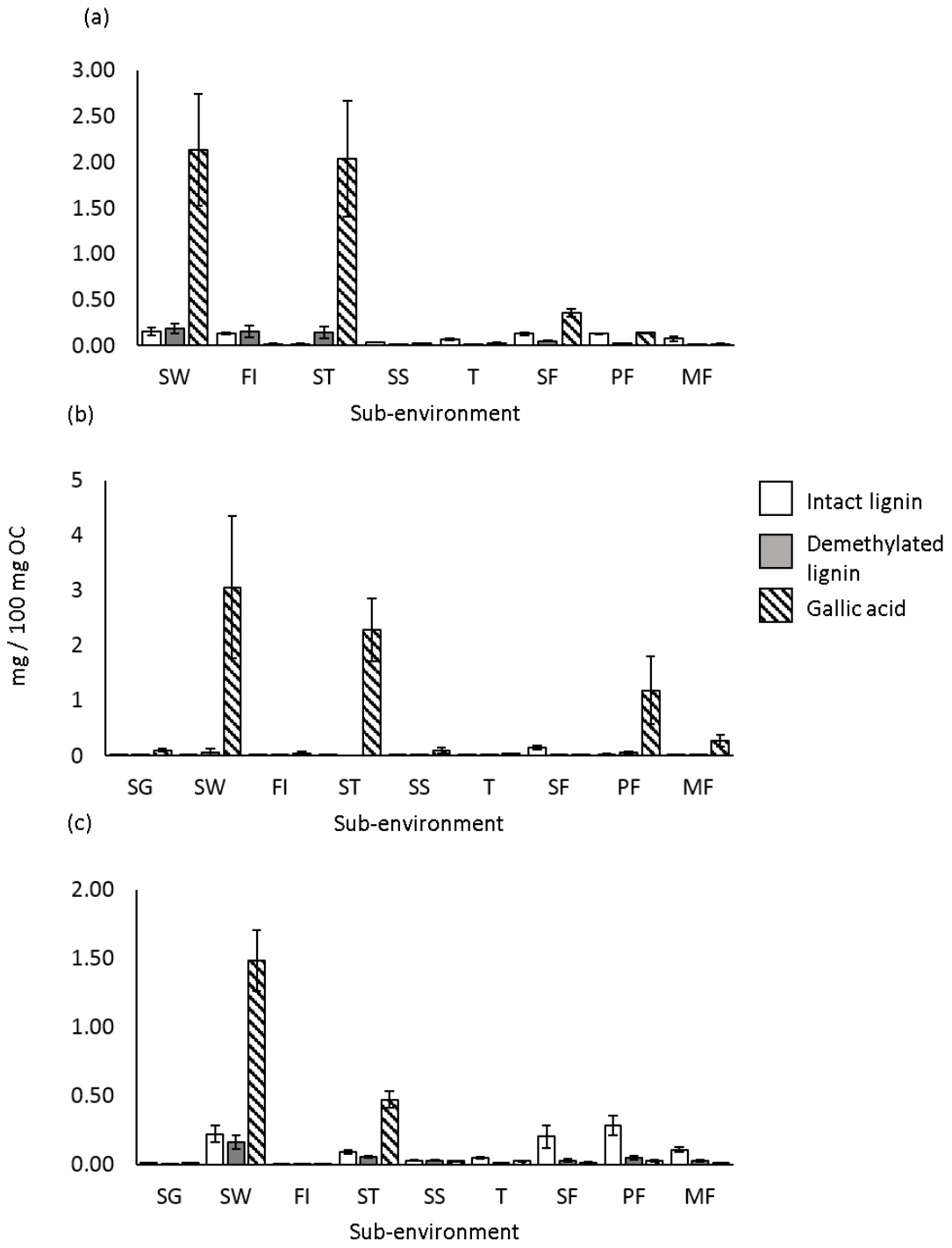

Figure 5.15 Intact lignin, demethylated lignin and gallic acid amounts across the sampling transect (SG-MF) in (a) wood litter, (b) fresh shoot / leaf litter and (c) surface soils. S.E. bars of 3 replicates shown.

5.3.7 Source Indicators - C/G and S/G

Table 5.2 shows the values for uncorrected and corrected (*) C/G and S/G parameters. These parameters indicate the non-woody input and angiosperm / gymnosperm input, respectively. After correction, the values of C/G for all fresh shoot / leaf litter and the majority of wood litter and surface soils increase. This highlights that G4 and G6 are more affected by microbially demethylated vanillic acid than G18 is by caffeic acid, resulting in a greater amount of intact G18. The exceptions are in the wood litter of T and both the wood litter and surface soils of SF which decrease, and therefore show the opposite effect of non-lignin sources. This may be due to differences in plant species and plant functional traits. Increases in C/G after correction for gallic acid and demethylation have also been identified in peat soils under an afforested conifer plantation by Swain (2013). However, other studies have shown no change in C/G after correction in sandy soils under oak woodland (Nierop and Filley, 2007).

Figure 5.16 (a) shows the values of C/G across the savannah-rainforest transect in fresh shoot / leaf litter, wood litter and surface soils. All wood litter across the transect show values <1, which indicates that G is dominant over C and thus there is a woody signature, which would be expected from these samples (Nierop and Filley, 2007). Conversely, most fresh shoot / leaf litter samples show a non-woody input, with values >1. Again, SF is an exception here, where the leaf litter gives a woody signal of guaiacyl predominance over cinnamyl. This may be due to plant species differences, which has been identified in previous studies (Swain et al., 2010), or preferential degradation of G18 relative to G4 and G6 in the leaf litter.

Across the savannah-rainforest transect, the sub-environment surface soils differ in woody / non-woody characterisation (Figure 5.16 (a)). Non-woody ratios are predominant in sites SG, FI, SS, T and MF. This would be expected at SG due to its wholly graminaceous input. In the latter four sites, selective degradation of guaiacyl over cinnamyl is apparent. However, in SW, ST, SF and PF the opposite is apparent in surface soils, highlighting selective cinnamyl degradation.

Table 5.2 shows that S/G values after correction largely decreased in fresh shoot / leaf litter, wood litter and surface soils. In some cases, this resulted in significant decreases indicating large tannin (gallic acid) inputs, as seen in Section 5.3.6. Exceptions to this

include wood litter at SS and T and leaf litter at T which all increased slightly. However, these increases are minimal: SS wood litter 0.49 to 0.6(*), T wood litter 0.69 to 0.71(*), T leaf litter 0.64 to 0.88(*). Additionally, FI wood litter and both SF and PF surface soils did not change from the original values (0.83, 0.71 and 0.55 respectively). This minimal change has also been detected in Nierop and Filley (2007).

Fresh shoot / leaf litter S/G of SW, ST and SF values were 1.2, 4.09 and 1.58, respectively, showing that an angiosperm signal dominates here. All other sub-environments had values <1, showing selective degradation of syringyl phenols of leaf litter. Wood litter at SW, FI and ST is predominantly angiosperm (>1), whilst the remaining sub-environments showed selective syringyl degradation. Figure 5.16 (b) shows the differences between vegetation inputs and surface soil S/G values. This highlights that surface soil S/G values are lower than vegetation values, thus syringyl phenols are degraded preferentially to guaiacyl. All surface values are <1 showing that angiosperms signals are lost. MF soil is >1, however the large error bars indicate a high degree of uncertainty.

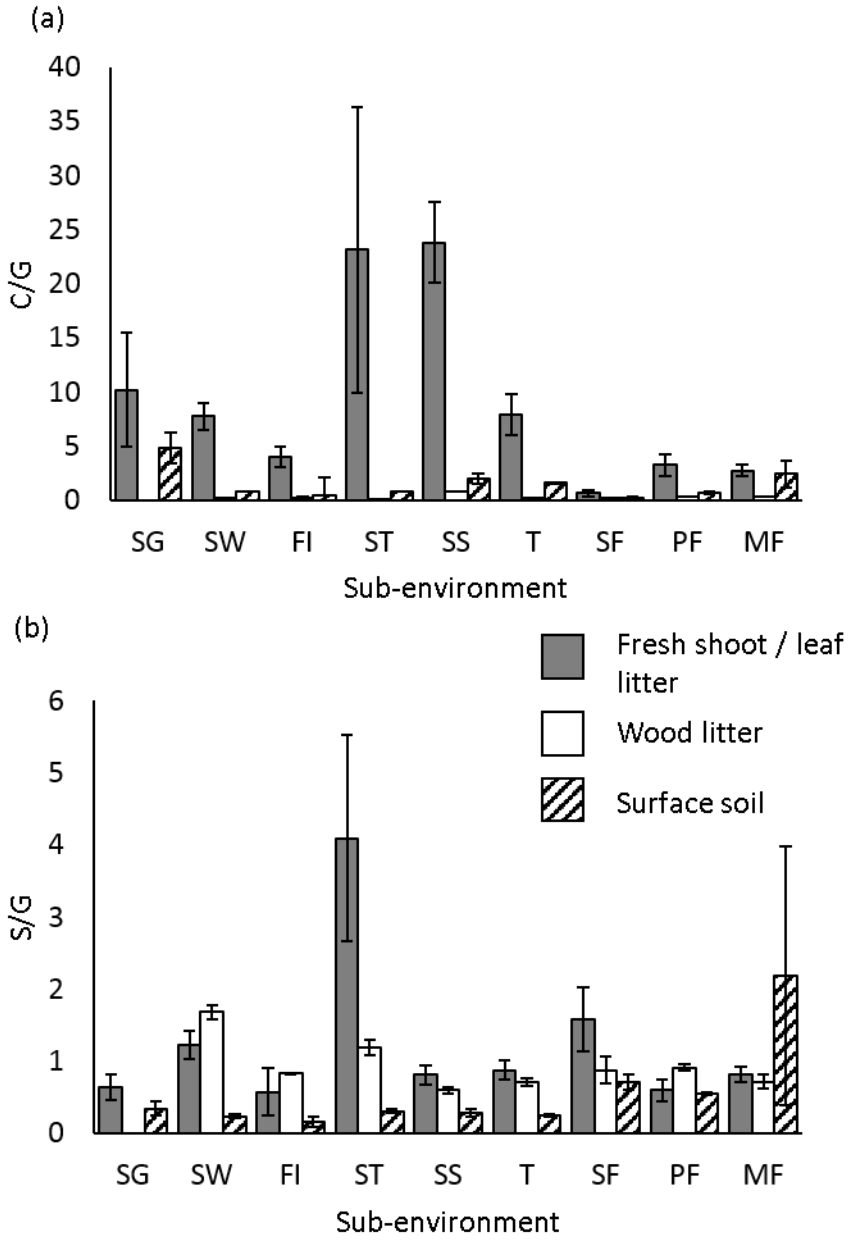

Figure 5.16 Lignin parameters (a) C/G and (b) S/G of surface soils and vegetation inputs across the transect. S.E. bars of 3 replicates shown.

5.3.8 Oxidation Extents - $[Ad/Al]_G$ and $[Ad/Al]_S$

Table 5.2 also shows the uncorrected and corrected (*) $[Ad/Al]_G$ and $[Ad/Al]_S$ values for fresh shoots / leaf litter, wood litter and surface soils across the savannah-rainforest transect. Both of these ratios generally decreased in vegetation and surface soils, as described by Nierop et al. (2005) and Swain et al. (2010). This is in general agreement with the above source indicator ratios (C/G and S/G). Oxidation ratios $[Ad/Al]_{G/S}$ could not be calculated for the surface soil of FI, as both G4 and S4 were not detected in the surface soils. This shows an advanced rate of degradation in this soil.

Increasing values indicate increasing oxidation, therefore, it is usually expected that values of these ratios are higher in soils than vegetation. Table 5.2 and Figure 5.17 shows that this is true of the majority of sites for both guaiacyl and syringyl. Wood litter generally has very low values for both $[Ad/Al]_G$: (0.52 – 0.92) and $[Ad/Al]_S$ (0.17 – 0.75), which are similar to those investigated in oak wood (Nierop and Filley, 2007). Figure 5.17 (a) and (b) show that all wood litter is less oxidised than surface soils.

Some surface soils do show similar oxidation levels to fresh shoot / leaf litter values, however. This is true of SG $[Ad/Al]_G$ which is likely due to the fresh shoot input; in the presence of polyphenols fresh vegetation can have values similar to those expected in soils (Nierop and Filley, 2007). The same is true of $[Ad/Al]_G$ MF surface soil and leaf litter, (2.31 and 2.24, respectively), however, unlike fresh shoots at SG, the leaf litter here may be exhibiting rapid degradation within this sub-environment. MF also shows similar $[Ad/Al]_S$ between soils and leaf litter (2.44 and 2.31 respectively), potentially for the same reason. T surface soil and leaf litter also shows this trend for $[Ad/Al]_S$, again suggesting that leaf litter is rapidly degraded in these sub-environments.

In surface soils, SG and PF have the lowest $[Ad/Al]_G$ ratios and thus lowest extents of oxidation. All other sub-environment surface soils have similar or near similar guaiacyl oxidation extents to each other. All other fresh shoot / leaf litter values are low, with SW and PF having the smallest guaiacyl oxidation extents.

MF has the lowest syringyl oxidation extent in surface soils (2.24), whilst SF has the highest syringyl oxidation extent in both surface soil and leaf litter (14.1 and 4.79, respectively). This may be due to a greater moisture content making microbial activity

more prolific than at other sites (Donnelly et al., 1990). All other sub-environments have similar surface soil syringyl oxidation extents.

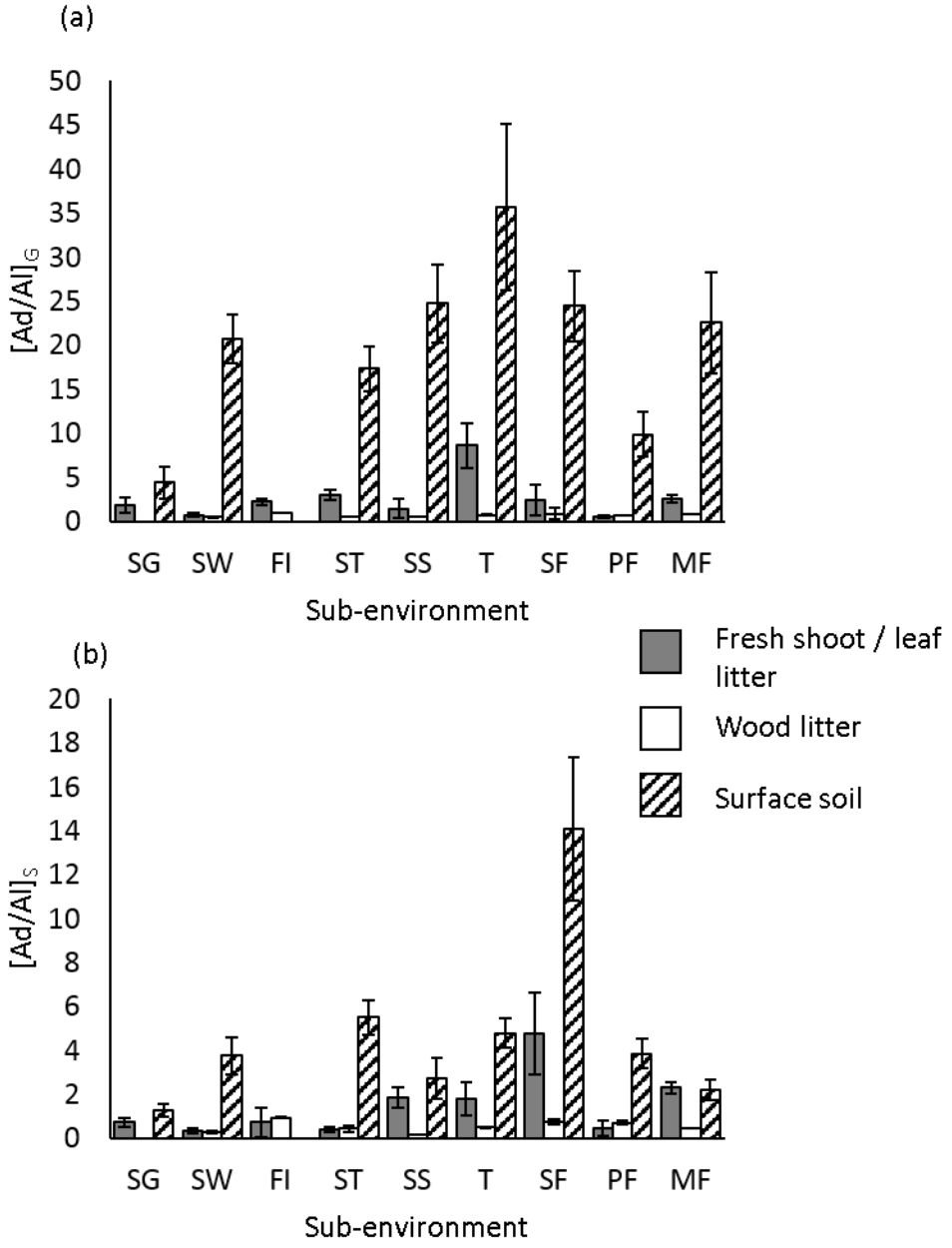

Figure 5.17 Oxidation extents (a) [Ad/Al$_G$ and (b) [Ad/Al]$_S$ of surface soils and vegetation inputs in sub-environments across the transect. S.E. bars of 3 replicates shown.

5.3.9 Lambda (Λ)

Table 5.2 shows uncorrected and corrected (*) Λ values in fresh shoot / leaf litter, wood litter and surface soils along the savannah-rainforest transect. Figure 5.18 represents the changes in corrected (*) Λ values in summed averages of fresh shoot / leaf litter and wood litter and averages of surface soils across the savannah-rainforest boundary. Summed vegetation and surface soil Λ show little variation between sub-environments (as seen above in degradation ratios). The most notable difference is in the surface soil of FI, which shows very low Λ (0.05 mg / 100 mg OC). This is because only lignin phenols G6, S6 and G18 were detected in the THM products, showing a highly degraded soil. The surface soil of SW and the vegetation of MF also show noticeable differences to the other sub-environments through greater Λ amounts, however, large error bars suggest the vegetation at MF needs further sampling.

Greatest Λ was seen in the surface soil of savannah sub-environment SW (2.36 mg / 100 mg OC). This is mainly due to phenols G6 and G18 (1.08 and 0.63 mg / 100 mg OC respectively). All other Λ values of surface soils across the transect were ≤1.6 mg / 100 mg OC, with the majority between 0.6 and 0.7 mg / 100 mg OC.

Degradation is apparent in the surface soils of FI, in which all phenols are heavily degraded, and G/S4 are absent from the THM products. ST, SS, SF and MF also show degraded Λ amounts compared to vegetation inputs, largely due to lower G4 and S4 degradation.

Although surface soil phenolic degradation is occurring, as apparent in Sections 5.3.7 and 5.3.8, the amounts of Λ in surface soils of SG, SW, T and PF are similar relative to their vegetation inputs, (apparent in the overlapping error bars between soils and their respective vegetation inputs). This is mainly due to amounts of phenols G6 and G18 in all of the latter soils. Further, SG surface soil has a slightly greater Λ than vegetation inputs (a difference of 0.44 mg / 100 mg OC), suggesting preservation of phenols. These phenols are specifically G18 and G6.

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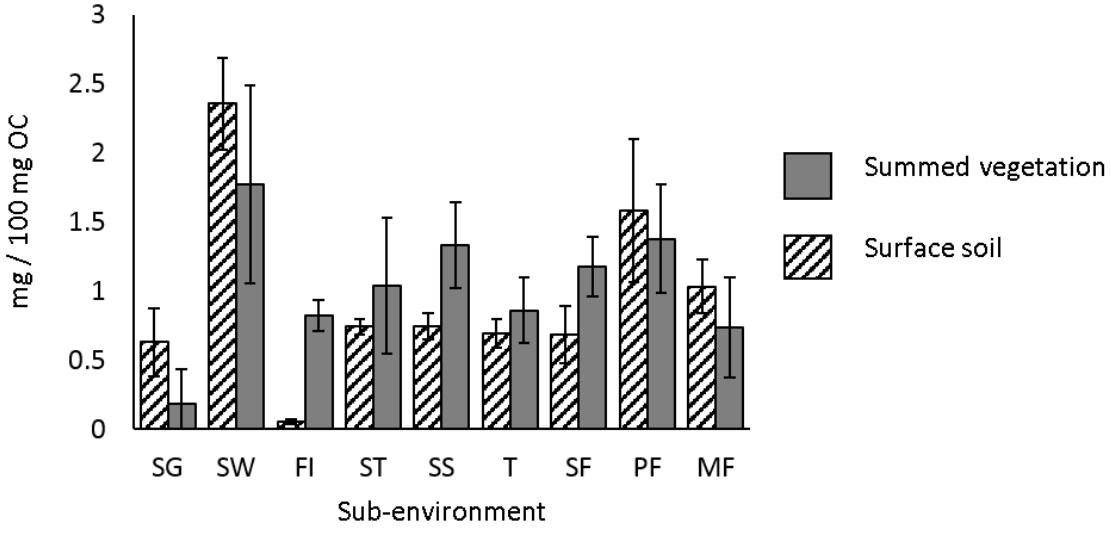

Figure 5.18 Lambda values (mg / 100 mg OC) for surface soils, and summed inputs = fresh shoot / leaf litter and wood litter across the transect. S.E. bars of 3 replicates shown.

5.3.10 Tannin input

As discussed in Section 5.3.3, tannin input can be a significant contributor to SOM parent materials, often in the form of gallic acid (Nierop and Filley, 2007). Their persistence in soils has mixed findings with both low amounts (Kuiters and Denneman, 1987; Schofield et al., 1998) and high amounts detected (Lorenz et al., 2000). Tannin phenols can be divided into two groups: hydrolysable (HT) and condensed (CT), (Harborne, 1997). As both of these groups are cleaved by the process of high temperature pyrolysis GC-MS and thus become amenable to analysis, they cannot be distinguished in order to find the relative input of gallic acid (Nierop and Filley, 2007). Alongside gallic acid, 1,3,5-TMB also detected within the samples analysed, has been assigned as a significant product of A-ring CTs, and thus is a useful marker for CT concentration in soil and vegetation samples (Nierop et al., 2005). Additionally, 1,2,4-TMB can also be derived from B-ring CTs, potentially providing another tannin marker (Nierop et al., 2005). However, this product can also be derived from polysaccharides, and to date the relative concentration from each source cannot be distinguished (Fabbri and Helleur, 1999).

Figure 5.19 shows the amount of 1,3,5-TMB, 1,2,4-TMB and gallic acid in (a) wood litter, (b) fresh shoots / leaf litter and (c) surface soil samples. All compound amounts are greatest at SW in surface soils but are much lower throughout the rest of the transect. The exception here is PF, which has similar amounts of 1,3,5-TMB to SW. Despite this, both 1,2,4-TMB and 1,3,5-TMB show suitability as markers for tannin due to the correspondence with gallic acid. The amount of 1,3,5,-TMB at PF is associated with relatively large standard error bars, thus, the amount would need to be confirmed with further analysis, which would further clarify 1,3,5-TMB as a potential tannin biopolymer. Leaf litter 1,3,5-TMB and gallic acid patterns also show a resemblance, with highest amounts in SW, ST and MF. A high amount is also seen in PF relative to other sites. Again this shows significant tannin input in these leaves. 1,2,4-TMB amounts are too low to indicate any resemblance to gallic acid. Wood litter 1,3,5-TMB, 1,2,4-TMB and gallic acid amounts show little similarity, however. This indicates that within wood litter these two products do not necessarily relate.

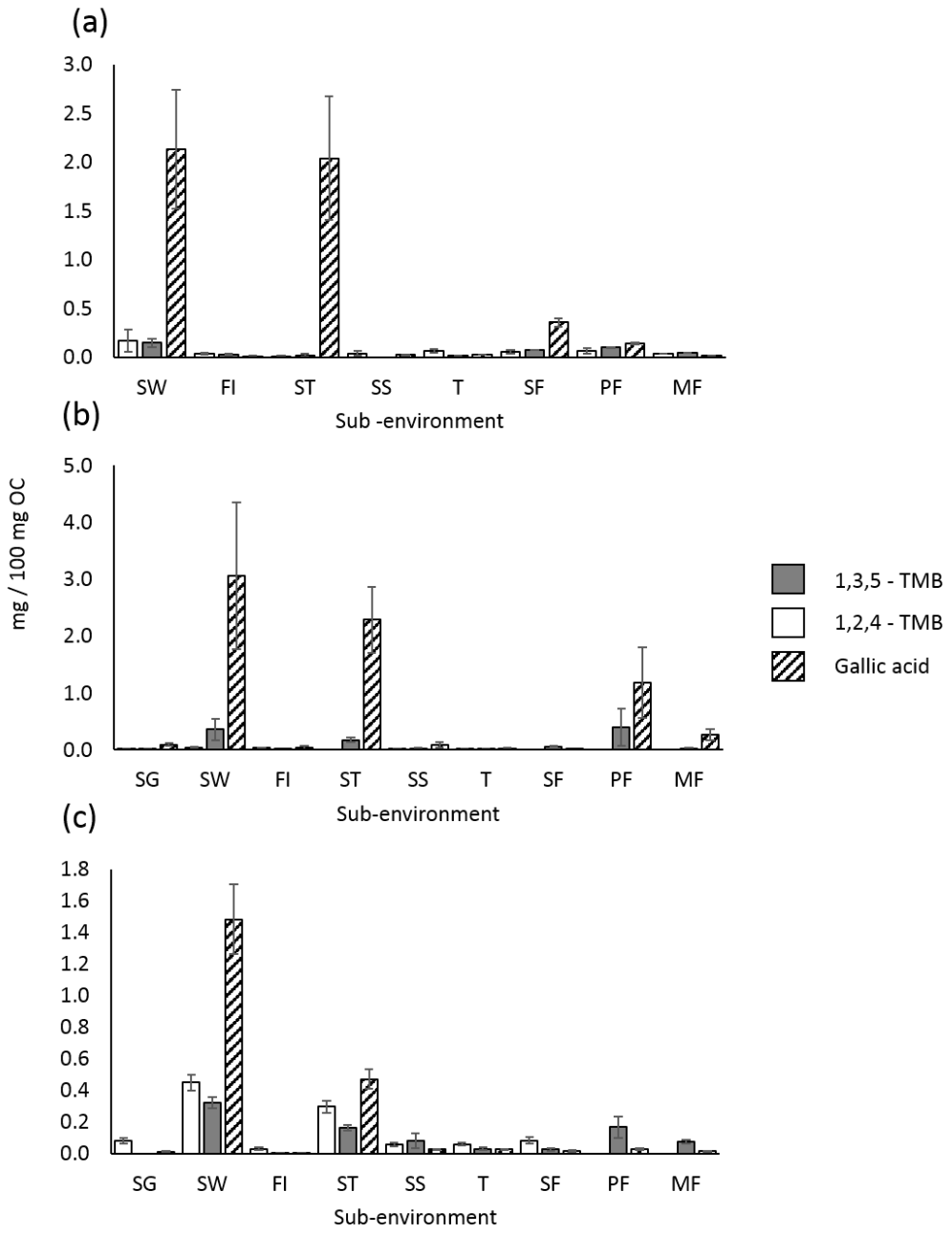

Figure 5.19 Comparison of the amounts of 1,3,5-TMB, 1,2,4-TMB and gallic acid inputs in (a) wood litter, (b) fresh shoots / leaf litter and (c) surface soils. S.E. bars of 3 replicates shown.

5.4 Discussion

5.4.1 THM product proportions and degradation of SOM components in soils

In Section 5.3.3 the proportions of each THM product were analysed. This analysis reveals differences between sub-environments, but also indicates information about the degradation state. For example, it is apparent that within the surface soils of sub-environments SG and MF the greatest proportion are the MC compounds, conversely in sub-environments SS, SF and PF, lignin amounts are greatest. This may indicate that less degradation is taking place at the latter sub-environments which have around ≥50% lignin of the total THM products analysed. Therefore, there may be differences in microbial communities or activity between the sub-environments. For example, the greater proportion of MC's in SG and MF than the other sites suggests that greater microbial activity may be occurring here, or that dead microbial biomass (necromass) is retained for longer in these soils.

In FI, SS and SF soils, where the lower proportion of MC's (18, 17 and 23% respectively) possibly indicates lower lignin degradation, it is possible that having wetter soils than sub-environments SG and MF (see Chapter 4, Section 4.6) contributes to this slower SOM turnover. Due to the higher volumetric water content of FI, SS and SF soils (67, 58 and 75% respectively) it is possible that limited oxygen availability is suppressing aerobic decomposition (Tate, 1979; Colberg, 1988). Despite this, results from Sections 5.3.7, 5.3.8 and 5.3.9 actually show that lignin in soils of FI, SS and SF is largely degraded. FI soil is heavily decomposed due to the absence of G4 and S4 phenols. It has been shown that annual variation in precipitation and short-scale rain events after and between dry periods may activate microbial populations (Austin et al., 2004; Abbott et al., 2013). These wet and dry cycles can cause the break-up of soil aggregates, thus releasing previously physically protected OM, allowing it to be more available to microbial attack. Drier soils after a wet period also result in an intensification of phenol oxidase release by microbes, previously suppressed by oxygen limitation, causing phenolic decomposition (Fenner and Freeman, 2011). Thus, the water retained in these soils from a rainfall event during the dry season may activate microbial decomposition of lignin. Sampling of sub-environments SF and SS was undertaken 2 and 3 days respectively after a heavy rainfall event (observation) in February (during the savannah dry season and early in the second dry season of the

rainforest). Despite these recent findings, no relationship was identified between any of the lignin parameters and volumetric water content (data not shown), thus, temporal investigations into lignin amounts between wet and dry cycles is needed. Further, stimulation of phenol oxidation through iron oxide has also been reported in humid tropical forest soils of Puerto Rico, where iron oxide acts as a reactive oxygen species for microbial respiration and an acidifier of OM (Hall and Silver, 2013). This decomposition is only active under aerobic conditions, when microbes are able to release phenol oxidase. Iron oxide originating from the plinthic parent material was observed in the soils investigated for this study (see Chapter 3 and Appendix E), and thus this is another likely decomposition mechanism here after water logging in the wet season. In FI, SS and SF soils, the lower proportion of MC's and more degraded lignin relative to SG and MF soils, indicate a high turnover of all SOM components. Therefore they present a situation in which the carbohydrate products resulting from microbial degradation of OM are degraded as well as the lignin, potentially also through the mechanisms described above.

The similarity in degraded SOM characteristics between the latter three soils (FI, SS and SF) may be further evidence to suggest that SS and FI were historically a part of the wider rainforest, as suggested in Chapter 4. In this area of Wowetta, savannah fires burn along the forest edge (noted in Chapter 3) which may have shaped the savannah encroachment and forest island (FI) apparent in the present day. FI surface soil is more degraded than SF which is apparent in the observed lack of G/S4 phenols; possibly indicating the effect of a more exposed position in the landscape. Being more exposed may induce greater losses of SOC, as soils are likely to dry out faster after wet periods allowing aerobic decomposition for a longer period within the year. Photo-degradation may also be greater in this soil compared to SF soil due to less dense surrounding vegetation cover. SS soil shows even lower SOC stocks than FI (Chapter 4) as well as degraded molecular characteristics. This may indicate a further progression of the rainforest – savannah transition, in which SOC stocks are becoming depleted, which may be an example of future alterations with climate change scenarios of warming and drying in this region (IPCC, 2013). Interestingly, SS shows less degraded lignin phenols than FI, which suggests different degradation mechanisms between the two sub-environment soils. For example, white-rot fungi may exist at FI but not SS due the

presence of trees, as well as lignin-degrading bacteria (Huang et al., 2013), therefore causing a greater extent of lignin degradation in FI soil.

Sub-environments SW, ST and PF have drier soils, (40 and 42%, volumetric water content, respectively, PF not measured but dryness observed), a significant proportion of plant inputs (gallic acid and lignin) relative to other THM products and greater amounts of lignin phenols (Sections 5.3.7, 5.3.8 and 5.3.9). It has been noted that plant phenols may have inhibitory effects on soil organisms in relation to SOM degradation (Hättenschwiler and Vitousek, 2000). For example, ferulic acid and gallic acid have been attributed to inhibiting spore germination and hyphal growth of saprotrophic fungi (Kuiters, 1990), whilst Schimel et al. (1998) also identified that condensed tannins from a species of alder tree reduced microbial decomposition and nitrogen mineralization. Therefore it could be tentatively speculated that the high proportions of phenols in these sub-environments may be causing microbial inhibition and thus lower proportions of MC's relative to the other THM products. This is especially prominent in SW and ST, where gallic acid proportions are large. Further, in near-by French Guiana leaf litter quality has been shown to control litter decomposition in tropical forests, suggesting that tree species may produce these phenols to reduce soil microbial activity (Hättenschwiler and Jørgensen, 2010). This may also be true of the rainforest tree species in the sub-environment PF. However, the role of these phenols and the microbial populations would need to be further investigated before the low proportion of MC's at these sites can be fully explained.

It has been suggested that in some environments, slower degradation of lignin exists as a result of adaptation to low nutrient contents (Mitchell et al., 1986), which may be causing phenolic stabilisation in the soils of SG, SW, T and PF, and lack of complete lignin degradation in FI, SS, SF and MF. All soils in this study are acidic: SW, ST, PF and MF pH 3-4 and SG, SS, T and SF pH 4.5-5 (see Chapter 3) and thus likely nutrient poor. However no correlation was detected between Λ and pH (data not shown). High vegetation input quantities may also result in the partial, but not complete, lignin degradation seen across the transect. In SG, low degradation in soil compared to input is likely due to the fresh shoots sampled, and the greater Λ in soil may be due to additional root inputs.

Lignin syringyl showed oxidation in all soils on the transect (Section 5.3.7 and 5.3.8). Savannah soils at SG, ST and SS are most exposed to sunlight due to low density vegetation cover (observation). Degradation of syringyl has been linked to mechanisms such as photo-oxidation, which may act alongside microbial degradation in these exposed soils, in particular (Austin and Vivanco, 2006). Another py-GC/MS study in semi-arid and arid biomes in the Fynbos of South Africa have also identified S over G preferential degradation (Carr et al., 2013). Syringyl phenols may also be preferentially degraded by microbial activity in transition and forest sites (Huang et al., 1998).

The $[Ad/Al]_{G/S}$ ratios in Section 5.3.8 show that sub-environments have different degrees of degradation: i.e. SF and MF soil have some of the greatest and lowest syringyl oxidation, respectively, whilst SG and PF have the lowest guaiacyl oxidation extents compared to other sub-environments studied. As G/S4 were not detected in the soil of FI, and Λ (Section 5.3.9) shows degradation compared to input amounts, this soil shows the greatest degradation. Differences in SOM composition between arid and semi-arid biomes within South Africa have also been identified, of which the decomposition extent was suggested to be the driving factor behind this compositional variability (Carr et al., 2013). The differing extents of oxidation described above also indicate a control over SOM composition, which is evident in the THM product proportions between sub-environments seen in Figure 5.5.

5.4.2 Source and occurrence of carbohydrates

The differences in both amount and composition of methylated carbohydrates between vegetation inputs and surface soils in Figure 5.5, Section 5.3.1, indicates that either there is preferential accumulation of MC1 & 3 in soils or that there are other sources of these methylated carbohydrates in the soil, such as microbes. A microbial origin of these MC's is suggested particularly by the small proportion of MC's in the fresh shoots of SG, which are less likely to have undergone degradation than the leaf litter in other sites. Thus, the carbohydrates MC1 & 3 may be from microbial biomass or necromass and form a significant contribution of the total extracted soil residue THM products in this study (Figure 5.5(c)). As described in Section 5.3.4, these MC's have been detected in the THM products of a series of hexose sugar standards (specifically D-Glucose and D-mannose) labelled with TMAH (Fabbri and Helleur, 1999) and in the THM products of glyceraldehyde labelled with TMAH (Schwarzinger, 2004). These hexoses in soils

have been used previously to indicate microbial biomarkers (Amelung, 2001; Kandeler et al., 2000), and it is also known that plants contribute few hexoses to soil in comparison to microbes (Amelung et al., 2008). On THM with TMAH analysis of the glucosamine standard in this study (Figure 5.8) all four MC's identified in the analysis of surface soils were detected. Glucosamine has previously been identified as a microbial marker for both fungi and bacteria (Amelung et al., 2001), further corroborating evidence for microbial origin of these MC's. Additionally, on analysis of the amino sugars galactose, glucosamine and muramic acid via gas chromatography, Glaser et al. (2004) suggested that these microbial biopolymers are best used for characterising soil microbial necromass, rather than living microbial biomass. This was also previously suggested by Guggenberger et al. (1999) and Amelung et al. (2001). Miltner et al. (2012) highlighted the importance of microbial necromass in SOM through incorporation of plant materials into living microbial biomass and ultimately dead microbial biomass (necromass). Further, Miltner et al. (2012) suggested that this is a much more important part of SOM than previously considered. Thus, it is likely that the MC's identified in this study, particularly MC1 & 3 due to their abundance in soils, represent microbial necromass. Where these MC's are in greatest proportion (sub-environments SG and MF), it is likely that more microbial activity is taking place. Thus, the MC's identified in this study, particularly in the soils of SG and MF, may indicate an important pool of stabilised SOM, accounting for 63% and 56% respectively of the total THM products analysed in these soil residues (Figure 5.5(c)). In experimental soils under C3 and C4 plants of northern France, Gleixner et al. (2002) also identified carbohydrates as a substantial part of SOM in relation to other major pyrolysis products. Further, Spaccini et al. (2000) showed that carbohydrates from plants are microbially transformed and stabilised within humic substances of soils. Kindler et al., (2006, 2009) and Miltner et al., (2009) also reported that after an incubation experiment, 40% of microbial biomass C residues remained in soils, and were thus a significant contribution to SOM.

Despite having a lower proportion than SG and MF soils, sub-environments SW - PF average 25% proportion of MC's in surface soil and thus highlight that these carbohydrates are a significant contribution to SOM across the transect. In order to further understand the relative proportions of the compounds studied here, and the use of MC's as microbial necromass biomarkers in these soils, further research needs to be

undertaken on the type and activity of microbial communities present e.g. bacterial and fungal. Analysis of extracted lipids may provide important information towards microbial biomass contributions.

Interestingly, sub-environment FI soil shows a large proportion of CTP's relative to other THM products (~36%), of which much lower proportions were detected in all other soils analysed (<10%), (Figure 5.5(c)). FI soil has a relatively smaller proportion of MC's, but a similar proportion of lignin products to CTP's. Only ~10% of FI leaf litter accounts for CTP's in the THM analysis, however, there are similar amounts in wood litter as in soils (almost 35%), (see Figure 5.12). As previously detailed in Section 5.3.4, these CTP's have not yet been properly assigned, and their origin is largely unknown. Figure 5.12 shows that the dominant CTP is the same in both vegetation and soil (142 *m/z*: CTP1) and, thus, suggest vegetation origin.

As may be expected, the vegetation THM product proportions at FI show similarity to those at SF with regard to CTP proportions, and T in regard to MC proportions (Figure 5.5(b)). FI wood litter characteristics are similar to that of SF, PF and MF in CTP's, lignin and 1,2,4-TMB proportions, (Figure 5.5(a)). However, CTP's and 1,2,4-TMB proportions in the soils of SF, PF and MF are much smaller than at FI. Assuming that the latter sub-environment is a remnant of the larger expanse of rainforest (as hypothesised in Chapter 4), the likeness to the transition (T) leaves may indicate that leaves at FI have adapted to the more exposed environment, as opposed to enclosed forest. Further, the differences in molecular soil characteristics compared to the other sub-environments show that a unique environment has developed here, where CTP's are a more important component of the SOM.

Section 5.3.4 and Figure 5.12 show that CTP's are degraded in surface soils from vegetation inputs across the transect. This may indicate that they are plant derived, and rapidly degraded upon entering the soil. Additionally, the glucosamine standard analysed did not show the CTP products, which may further suggest plant origin. CTP's have been correlated against lignin oxidation parameters $[Ad/Al]_{G/S}$ in addition to individual phenols G/S6 and G/S4, however, very weak relationships are observed (R^2 ≤0.4: data not shown). The weak relationship between CTP's and individual lignin phenols is positive (≤0.4), showing that when lignin phenols have a greater amount in soils, so do CTP's.This potentially points towards a plant origin of CTP's, as lignin is

purely of plant origin. The weak relationship between CTP's and oxidation parameters ($R^2 \leq 0.2$) is negative, further corroborating a plant origin. If CTP's are of microbial origin the latter relationship may otherwise be positive, which would indicate that CTP's are microbial products released after lignin oxidation. However, additional samples would be needed to verify these preliminary interpretations. Further, relationships between MC's and the above lignin phenols and oxidation parameters have been assessed. This revealed a set of very weak relationships ($R^2 \leq 0.1$, most are ≤ 0.06: data not shown), this lack of association between lignin and MC's may corroborate their microbial origin. It also suggests that microbes do not preferentially degrade lignin, as a negative relationship may in this case be observed. A positive relationship may have suggested either plant origin (as with CTP's). However, such inferences behind this lack of relationship are speculative.

Evidence from the occurrence of MC's in degraded FI, SS and SF soils shows that these CTP's may have a high turnover, even if microbially derived. Swain (2013) detected either decreases or insignificant changes between leaf litter and soil CTP amounts in Sitka spruce and moorland sites, with the moorland having a greater amount than the Sitka spruce sites. Aside from a tentative assignment, no other research has been carried out on these CTP's in soil and thus they require further analysis for verification of source and potential roles within SOM.

Figure 5.13 highlights clearly that vegetation inputs have a greater proportion of CTP's, and a lesser proportion of MC's than surface soils. The relative proportions observed in these soils have been previously attributed to preferential degradation of cellulose by white-rot fungi, which is known to be a principal energy source for heterotrophic microbial communities (Dijkstra et al., 1998; Huang et al., 1998). Huang et al. (1998) also reported Oh horizons rich in microbial metabolites (anhydrohexose and anyhdroglucosamine), which were attributed to fungal activity.

5.4.3 Source and occurrence of tannins

Section 5.3.10 analysed the role of tannin biopolymers in vegetation and surface soils. Both similarities and disparities between 1,3,5-TMB, 1,2,4-TMB and gallic acid occurrences were detected in Section 5.3.10. Most disparities were observed in wood litter samples. In peaty gley soils in the North East of the UK, Swain (2013) also

identified both similarities and disparities between gallic acid, 1,3,5-TMB and 1,2,4-TMB amounts, depending upon sub-environment (afforested conifer plantation and moorland). Mason et al. (2009) also observed similarities between gallic acid and 1,3,5-TMB in soil profiles between different sub-environments in the temperate zone. However, Nierop et al. (2005) identified that A-ring products such as 1,3,5-TMB were negatively correlated to gallic acid within a range of plant species from different environments in North America. These converse findings show that 1,3,5-TMB needs further assessment in its relationship to gallic acid. The correlation between 1,2,4-TMB and gallic acid, particularly in SW and ST surface soils, may indicate its source from tannin as opposed to polysaccharides, but warrants further investigation. Other studies comparing these compounds in soils and vegetation of the tropics have not yet been undertaken.

Gallic acid in the leaf litter of SW and ST samples may be due to the production of condensed tannins in order to prevent damage from herbivores, as these are known to inhibit digestion (Cooper & Owen-Smith, 1985). They can also be used as deterrents by producing an undesirable, astringent taste (Harborne, 1991; Bryant et al., 1992) and reduce protein and other nutrient availability (Robbins et al., 1987). Additionally, condensed tannins have been identified in rainforest plants, also associated with herbivore defence (Coq et al., 2009). Hättenschwiler et al. (2008) demonstrated that different plant species can have high variation in foliage and leaf litter chemistry within the same rainforest ecosystem, thus, the differences observed here between sub-environments may be expected.

5.4.4 Organic molecular chemistry compared to SOC stocks

SOC stocks from 0-6 cm depth of the surface soil were calculated for each sub-environment along the savannah-rainforest boundary transect in Chapter 4 (Table 4.1). These surface soil SOC stocks were correlated to the range of organic molecular products analysed at 0-6 cm surface soil in this chapter, including methylated carbohydrates, cellulose THM products, gallic acid, 1,3,5-TMB and lignin. No correlations were identified between any THM products and SOC stocks.

The greatest 0-6 cm SOC stocks are in FI and SF soils, however these showed some of the lowest amounts of lignin, MC and CTP characteristics across the savannah-

rainforest transect. The greatest amount of lignin, MC's and gallic acid are detected in the surface soils of SW1, which has one of the lowest 0-6 cm SOC stocks across the transect. This suggests that the turnover of molecular products may be faster than that of the bulk SOC, despite the large stocks likely accumulated over time. This indicates the importance of specific site conditions and vegetation inputs to both molecular characteristics and SOC stocks. It may also suggest that lignin may not be as important a component in soil carbon stocks as previously thought (Thevenot et al., 2010). Conversely, Carr et al. (2013) reported a positive correlation between lignin and TOC, and proposed that this represents an important role for less altered plant organic matter in SOC. Swain et al. (2010) also reported that, between two sites in the temperate zone, there were similarities between lignin amount and SOC stock in the surface horizons. However, the results from sub-environment SW indicate that high amounts of less altered plant-derived SOM, such as lignin, do not necessarily result in high SOC stocks.

Comparisons between molecular composition and SOC stocks have not been widely made in tropical soils, however, further research into this is recommended to establish how this varies within different sub-environments and ecosystems and how changes in climate may affect SOM and, thus, SOC stocks in the future.

5.4.5 Evidence of fire in SOM characteristics

Within the surface soils characterised, no evidence of fire was observed in the THM products. This may have been in the form of condensed aromatic structures and the evolution of O-alkyl C to furan-like structures (Baldock and Smernik, 2002; Almendros et al., 2003). However, other studies have also found decadal cycling of fire-affected SOM, indicating that fast turnover times of this material can occur (Alexis et al., 2012), as is indicated in this study.

5.5 Conclusions

This chapter summaries the analysis of SOM components via THM with unlabelled and ^{13}C-labelled TMAH across a savannah-rainforest boundary in central Guyana. Clear differences in molecular composition are apparent between the sub-environment soils. Average amounts of pyrolysis products show that SOM in SW and ST contain the greatest amounts of lignin and gallic acid and thus less-altered plant-derived OM. However, $[Ad/Al]_{G/S}$ ratios show that oxidation extents of lignin phenols are similar to the other soils studied here. This indicates that SW and ST have a higher input of lignin than other sites, despite similar degradation. In addition to the SG and MF soils, SW soil also has greater amounts of MC's compared to all other soils analysed, which suggests that both OM input and turnover is rapid in this soil. Greater gallic acid and lignin amounts than carbohydrates may indicate that microorganisms are preferentially degrading plant materials other than lignin and tannins, such as the CTP's or other more easily degraded sugars. Sub-environments SG and MF have a greater proportion of MC's than lignin or gallic acid, which indicates a greater alteration of plant-derived OM. MC's are likely to be microbially derived due to their more significant presence in surface soils than vegetation inputs. Their chemical structure (hexoses) also indicates microbial formation (Fabbri and Helleur, 1999; Schwarzinger et al., 2002). The poor correlations with lignin phenols may also suggest microbial origin, although this initial conclusion needs further investigation. The importance of MC's as potential microbial necromass in these soils is apparent from their proportion compared to other pyrolysis products, and their indicated stabilisation needs further investigation. Plant-derived OM shows relatively high degradation extents in all soils, however, the amounts of MC's are lower in soils ST – PF in comparison to SG, SW and MF, potentially suggesting either less microbial input or a faster turnover of MC's in these soils.

Soils of FI and SF sub-environments at first appear unusual because of high OM degradation (highest syringyl and high guaiacyl oxidation extents, low Λ, gallic acid and MC amounts), despite having the highest SOC and volumetric water contents, (see Chapter 4), which may otherwise suggest OM stabilisation. However, the likely reasons for high turnover are site specific and linked to volumetric water content, wet-dry cycles and the presence of iron oxide stimulating phenol and carbohydrate decomposition. The disconnection between SOM degradation and bulk SOC highlights that vegetation

inputs and microbial carbohydrates turnover faster than the bulk SOC, which calls for temporal scale studies to monitor fluctuations between SOM and SOC throughout the year. Investigation into the extracted lipid fraction of the soils studied here may provide useful information about the composition of the bulk SOC.

Due to the lack of correlation between the OM characteristics and measured environmental variables such as pH and volumetric water content, this also emphasises the need to study other variables in greater detail, such as temperature, iron oxides and photo-degradation. These abiotic factors all influence soil microbial communities and in turn, the fate of the carbohydrates they produce. However, leaf and wood quantities are also likely to affect SOM characteristics and microbial activity, and should be measured and compared across the transect in order to detect differences between sub-environments. Additionally, the significant amounts of MC's within the SOM of these sub-environments calls for the need to further investigate the types and activity of microbial communities.

Therefore, with predicted climate change of increasing temperature and alterations to precipitation patterns (IPCC, 2013), shifts in SOM characteristics are likely to be observed (Davidson and Janssens, 2006). In the rainforest studied here, where SOM turnover is high, a potentially warmer and drier climate (Bovolo et a., 2012; IPCC, 2013) may initially mean forest die-back, causing an influx of nutrients and fast carbon turnover, providing soil moisture is sufficient. However, as these nutrients are rapidly consumed by microbial populations and savannah vegetation takes precedence, inputs will decline, microbial communities are likely to change and the greater stock of SOC under forest such as FI and SF will be mineralised to CO_2 and CH_4, causing further atmospheric warming. Uncertainty in climate model predictions of precipitation (IPCC, 2013; IPCC, 2014) could indicate greater rainfall, potentially allowing the expansion of swamp forest into savannah swamp increasing SOC stocks and altering microbial communities. Savannah grasslands and woodlands which may convert to rainforest under this scenario may not see significantly increased SOC stocks (see Chapter 4; Section 4.3.3), however, changes in molecular SOM dynamics may alter, with lower amounts of tannins in inputs and soils. Thus, understanding SOM characteristics and the factors that affect them is key to understanding how future climate change and locally affected weather patterns will alter these SOC stocks.

Chapter 6. **Conclusions and Future work**

6.1 Conclusions

This study presents an assessment of the sub-environments, soil types, and their organic carbon characteristics investigated at two areas, Wowetta and Surama, on the savannah-rainforest boundary in Central Guyana. Specifically, major soil groups and sub-groups, bulk SOC and molecular organic carbon characteristics of the nine sub-environments were assessed. SOC was analysed using bulk density, horizon thickness and TOC measurements in full soil profiles. Surface soils and vegetation samples of the Wowetta sub-environments were analysed using thermally assisted hydrolysis and methylation (THM) in the presence of ^{13}C-labelled and unlabelled tetramethylammonium hydroxide (TMAH) for both phenolic (lignin and tannin) and non-phenolic (carbohydrate) products.

The lignin, tannin and carbohydrate biopolymers assessed reveal important site specific differences in SOM characteristics, and the processes influencing them, between the sub-environments analysed.

6.1.1 Savannah-rainforest boundary sub-environments and soil types

The heterogeneity of the savannah-rainforest boundary was revealed through the initial reconnaissance survey which highlighted nine sub-environments in Wowetta and Surama. These included: savannah grassland (SG1 & 2), savannah woodland (SW1 & 2), savannah tree (ST1 & 2), pisoplinthic savannah grassland (PSG), savannah swamp (SS), transitional zone (T1 & 2), swamp forest (SF1 & 2), mixed tree spp. forest (MF1 & 2), pisoplinthic forest (PF), kokret palm forest (KPF) and mora forest (MRF).

Within these sub-environments two major soil groups have been identified: plinthosols and gleysols. The soil type depend upon the position of the sub-environment in the landscape, which affects the influence of hydrology and parent material on soils. Several sub-groups of these two major soil types were identified, which in some cases depends upon both abiotic factors (such as hydrology) and biotic factors (vegetation input quantity), e.g. swamp forest humic gleysols. The ‘humic’ prefix reflects the high quantity of vegetation inputs from the forest and the main soil group ‘gleysol’ reflects the hydrological condition of the soil. Other soil subgroups had no significant or visible vegetation influence, e.g. haplic plinthosols, and were determined entirely upon parent material characteristics (the dominant presence of plinthic material within the soil

profile). Differences in the texture of soil profiles also determines the soil sub-groups, i.e. a rapid increase in clay in the profile of soil under mixed tree spp. forest gives an 'acric' plinthosol sub-group, and the sandy eluviated horizon of the soil profiles beneath savannah vegetation gives an 'albic' plinthosol sub-group. Both plinthosols and gleysols occur in savannah and rainforest environments, thus, vegetation is not a reliable indicator of soil type. From these results it is suggested that within sub-environments on the savannah-rainforest boundary, morphology, geology and hydrology are the best indicators of soil type.

Analysis into the mineralogy of a sub-set of surface soil samples via XRD revealed a primarily quartz, kaolinite and strengite composition. Quartz, kaolinite and strengite suggested a low ability to complex with organic matter.

6.1.2 SOC stocks

As soil type is linked to parent material and hydrological conditions at each sub-environment, these factors are very influential upon SOC stock. Wettest soil profiles (gleysols) have the highest SOC stocks, particularly those under forest vegetation (SF1 & 2 and FI: total soil profiles 388, 321 and 258 t C ha^{-1}, respectively). Gleysol under savannah vegetation (SS: 160 t C ha^{-1}) has a substantial carbon stock but it is significantly lower than SF1 & 2 and FI. Plinthosols have significantly lower SOC stocks than gleysols, regardless of savannah or rainforest vegetation (56 – 201 t C ha^{-1}). Therefore, soil type is a more accurate indicator of SOC stock than vegetation type.

In comparison to previous data for these soil and sub-environment types, SOC stocks estimated in this study (56 - 258 t C ha^{-1}) largely fall within or below previous estimates. However, SF1 & 2 SOC stocks are greater than previous estimates by 98 t C ha^{-1} and 31 t C ha^{-1}, respectively. High variability between the same sub-environments also exists between the sampling areas of Wowetta and Surama, e.g. within the sub-environment MF1 & 2 the former has 71 t C ha^{-1} and the latter 171 t C ha^{-1}. The disparities between the datasets and sub-environments highlights the need for high resolution sampling.

Some soil profiles show an increase in SOC in sub-surface horizons; this highlights the importance of taking into consideration individual horizons, their thickness and bulk density (Grüneberg et al., 2010). The importance of sampling a full soil profile where

possible, as opposed to only 0-1 m, is also highlighted through the differences observed between the SOC of full profiles in this study, and previous studies only measuring 0-1 m depth. Both of these sampling techniques enable more accurate estimations of SOC stocks.

6.1.3 Molecular SOM characteristics

Both plant and microbially-derived biopolymers have been identified in the THM analysis products. Plant biopolymers include lignin and tannins, and microbially-derived biopolymers include methylated carbohydrates (MC's) which were identified from their chemical structure (ion fragmentation patterns) in previous literature (Fabbri and Helleur, 1999; Schwarzinger, 2004). The amounts of MC's are much greater in surface soils than vegetation inputs which also suggests microbial formation. A second set of carbohydrate THM products (CTP's) were also identified, of which the origin is uncertain, hence they are currently only tentatively assigned. Wood litter has a greater amount of CTP's than surface soils, however, fresh shoots / leaf litter show mostly lower or similar amounts to soils. This indicates that the main source of these CTP's is in wood litter as compared to leaves. More work is needed to properly assign these products and their origin, for example microbial CTP products may also be possible.

Analysis of the SOM characteristics in the surface soils of the Wowetta sub-environments reveals clear differences in molecular composition. SW has the greatest amount of lignin and tannin products and thus unaltered plant derived material. Additionally, it has one of the greatest amounts of MC's; indicating high microbial activity. This indicates that microorganisms may be selectively degrading other plant components, such as CTP's and other more easily degraded sugars. SG and MF soils have the greatest amounts of MC's, highlighting high microbial activity. This is corroborated by degraded lignin and tannin products in these soils. SG may also have low inputs from vegetation due to the sparse grass cover.

In surface soils the amounts of CTP's relative to MC's are lower. This has previously been attributed to rapid degradation of these products by white rot fungi. Within fresh shoots / leaf litter, CTP's are a greater proportion and amount than MC's. This may highlight slower degradation and less microbial activity in fresh shoots / leaf litter than soils, which may be expected.

FI surface soil has the most degraded lignin and a much greater amount of CTP's than all other soils analysed. This may indicate a different microbial community to other soils; in which lignin is selectively degraded relative to CTP's, possibly suggesting the presence of white-rot fungi.

Despite FI and SF soils having the high SOC stocks, these soils have some of the most degraded lignin and tannin amounts across the transect. However, some of the lowest amounts of MC's were measured in these soils, suggesting high turnover of both vegetation and microbial inputs. The disparity seen between the SOC stocks and degradation of molecular products in soils suggests that these products have a higher turnover rate than the bulk SOC.

6.1.4 Implications for future climate and local weather scenarios

The results presented in this study highlight the forest island and swamp forest as key sub-environments and soils in relation to SOC stocks. These sub-environments have the most significant SOC stocks on the savannah-rainforest boundaries studied, which are greater than those previously estimated (Jobbágy and Jackson, 2000). However, from analysing the molecular characteristics of these soils it is also evident that they have a fast turnover of OM. These soil characteristics are likely largely due to the hydrological conditions of the site, with wet-dry cycles influencing both molecular turnover and bulk SOC stocks. In relation to future predictions of a warming and drying scenario for this region of the Amazon (IPCC, 2013) it is evident that these soils and their carbon stocks may be vulnerable to drying, potentially causing SOC loss. It has been postulated that alterations in precipitation are of greatest concern for Amazonian climate change, particularly within the dry season (Malhi et al., 2008). Previous Amazon droughts, such as the 2005 and 2010 ENSO events, demonstrate the vulnerability of rainforest mortality (Doughty et al., 2015). Thus, the savannah-rainforest boundary studied here may be subjected to increased forest mortality and die-back if local weather patterns are influenced by warmer and drier conditions. This may result in mineralisation and subsequent decrease of the significant SOC stocks of FI and SF if exposed through die-back. Historically, deforestation rates in Guyana are low (Food and Agriculture Organisation of the United Nations, 2005), however, as the pressure for resources also increases with expanding populations (Nobre et al., 1991; Nepstad et al., 2008), drier conditions may mean fires from agricultural practices may spread along the boundary

into the areas studied, causing further exposure of deeper rainforest SOC stocks. As an isolated strip of forest, FI may be most vulnerable to drying, conversion to savannah and resulting SOC loss. Equally, amplified resource use of timber and space for cattle may prevent SOC stocks increasing even if precipitation increases do occur. However, ultimately, the current uncertainty in precipitation predictions (IPCC, 2013; IPCC, 2014) means that it is difficult to extrapolate how these stocks and their molecular dynamics may be altered in the future. The data collected in this study can be used to inform management policies and practices within the rainforest and savannah areas of Wowetta and Surama. For example, areas with significant SOC stocks such as the rainforest swamps and forest island should be monitored for change in the long term, as well as being protected from logging.

6.2 Future recommendations and work

The results from this study have highlighted areas for further work, some of which will be detailed in the following paragraphs.

In order to create the baseline data established in this study, a transect in Wowetta and a selection of pristine sites in Surama was implemented for sampling. In future work, these sampling sites could be scaled up, employing a sampling grid method, which would include a wider area along the savannah-rainforest boundary. This would aid in verification of the data presented and quantification of variability along the savannah-rainforest boundary in Wowetta and Surama. The increased number of sampling sites would also allow more complex statistical analysis, such as multivariate analyses.

The overall extent of each sub-environment should be mapped in order to assess the relative importance of the SOC stocks of these areas to the country of Guyana. Through this, the carbon stocks of these sub-environments could be better protected and managed for the future. This will be particularly important for the swamp forest and forest island soils which hold the greatest SOC stocks, and face the prospect of drying out through increasing temperatures and decreasing precipitation (IPCC, 2013). This may lead to a loss in SOC, as indicated by the drier soils with smaller SOC stocks.

In addition to more thorough spatial sampling, it is recommended that bulk density soil sampling and monitoring of the water table depth is undertaken throughout the year. This would allow a clearer understanding of the hydrology, which is especially important in forest swamp, forest island and savannah swamp soils, and may provide more evidence to suggest fluctuating microbial activity between wet and dry cycles. This would further aid in understanding bulk SOC stocks, especially in the aforementioned soils. Sampling further into the dry season may also allow bulk density sampling of horizons which were inundated with water and thus not sampled, if the water table has lowered. This would verify surrogate data used in this study where bulk density samples could not be obtained. Further soil and vegetation sampling throughout the year would also allow temporal molecular changes to be analysed, which may change with season. For example, less SOM degradation may be observed in the wet season when all soils are likely to be inundated with water.

Although a lack of correlation between SOM characteristics and the abiotic factors pH and volumetric water content was observed in this study, correlations may appear if measured on a longer timescale, i.e. throughout the year. Exploring both abiotic and biotic controls in greater detail may also reveal possible stabilisation and degradative mechanisms of SOC in these soils. Effects of anaerobic conditions could be measured via redox potential in order to better investigate the differences in aerobic and anaerobic conditions between the sub-environments. This would allow for a greater understanding of the potential microbial SOM decomposers present. Analysing the microbial species and communities present will provide further information on the decomposition of SOM. Techniques such as polymerase chain reaction (PCR), which amplifies specific DNA fragments, or 454 sequencing and ion semiconductor sequencing, which allow population characterisation of microbes without the need for amplifying DNA, could be employed.

Microbial inputs are likely present throughout the surface soils of all sub-environments analysed (in the form of methylated carbohydrates: MC's), and are of particular importance in the surface soils of SG and MF. These potential microbial inputs may be stabilised through transformation into more recalcitrant forms, such as alkyl C (Baldock et al., 1992), and are thus worth further investigation. ^{14}C-radiocarbon dating these microbial inputs would allow an evaluation of their age. The origin of the carbohydrate THM products (CTP's) analysed in this study (postulated to be plant-derived), also need further investigation in order to properly assign them.

Strong bonds can form between organic material and iron oxyhydroxides, stabilising organic matter on the surfaces of these minerals. These strong bonds have been found between the latter metal surfaces and acidic organic ligands, after the oxidative degradation of lignocellulose (Kaiser and Guggenberger, 2000). However, polysaccharides have weaker bonds to metals and thus are less likely to be stabilised than lignin (Kaiser and Guggenberger, 2000). Due to the presence of iron in these soils from the plinthic parent material, it is possible that SOM is stabilised through such bonds. However, this may be more important in sub-surface horizons, where iron oxides were mostly observed, rather than at the surface of the soil profile. Such stabilisation may lead to a greater amount of lignin than carbohydrates in sub-surface horizons and requires further investigation in these soils.

Monitoring of soil temperature and sunlight levels throughout the year will provide data to correlate against SOM characteristics. Analysing sunlight levels may highlight photo-oxidative degradation of the lignin phenol syringyl as a major SOM control in exposed savannah sites: Austin and Vivanco (2006) found that photo-degradation through ultraviolet-B and total radiation was a main control on SOM decomposition in a semi-arid ecosystem. Analysing soil temperature within the different sub-environments may highlight differences between them as well as seasonal variations within sub-environments, which may affect SOM decomposition. However, temperature itself may not be a direct influence upon SOM degradation: Pisani et al. (2014) found that along an increasing mean annual temperature gradient, temperature had no effect upon lignin or microbially-derived aliphatics, although a stronger relationship was found in conjunction with mean annual precipitation. Therefore, temperature may be an important abiotic factor to consider in relation to other variables. Temperature sensitivity of SOM is also influenced by microbial temperature optima, microbial substrate availability and physiochemical controls of SOM such as pH, water, oxygen and nutrient supply (von Lützow and Kögel-Knabner, 2009). Thus, the latter abiotic variables need holistic consideration in future work in further understanding of the controls on SOM characteristics of these soils.

In order to better understand the amount of plant derived carbon entering the soil in each sub-environment, assessment of the quantities of fresh shoot / leaf litter and wood litter should be undertaken. Root litter quantities should also be taken into consideration, as these are likely to be of importance, (Rasse et al., 2005). Surface soils in the sub-environments of Surama should also be assessed for molecular characteristics. This may highlight differences within similar sub-environments of Wowetta and Surama. Investigation into sub-surface horizons of all soils may also reveal important information about the fate of lignin, tannin and carbohydrates.

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Appendix A. Extraction Experiment

The preferred method of extracting soils analysed by Py-GCMS lignin analysis at Newcastle University has been Soxhlet extraction (Mason et al., 2009; Swain et al., 2010). However, this can be a time-consuming process as samples need to be extracted for 48 hours, in order to ensure efficient extraction. In addition to this, pre-extraction of thimbles and glass wool for 24 hours must be undertaken in order to ensure that they are thoroughly clean before use. This method also means that a large amount of solvent (DCM and MeOH) is used. Thus costs and environmental impact is high due to the amount of equipment needed when analysing a large quantity of samples. A comparison between Soxhlet and pressurized lipid extraction (PLE), also known as ASE, was therefore undertaken for extracting soils for pyrolysis GC-MS analysis of lignin derivative products. ASE has also been designed to be an equivalent extraction method to that of the Soxhlet but also with the advantage of being more efficient (Richter et al., 1996). ASE is run at a higher temperature than Soxhlet (100 ˚C vs. 80 ˚C) therefore both the standard 100 ˚C temperature and the lower 80 ˚C on ASE were included in the experiment in order to investigate any differences this could possibly cause in this particular extraction technique.

A. 1. Aim and Objectives

The aim of the extraction experiment was to evaluate whether ASE could be used as a more efficient extraction technique in preference to Soxhlet, specifically for investigating soil organic carbon compounds.

The work program to address the aim was:

- Use two different temperature settings on ASE: conventional 100 ˚C and lower 80 ˚C (same temperature as used in Soxhlet extraction).
- Analyse results by running extracted soils on pyrolysis GC-MS and semi-quantitatively calculate amounts of lignin compounds present.
- Compare the amounts of each compound to look for differences in efficiency between the extraction methods (via standard error).

- Evaluate and recognise which method is best to use considering extraction efficiency, time and economic value.

A. 2. Methods

Extraction method potential

Table A.1 demonstrates the potential of each extraction method, considering the full number of samples that can be extracted in one run, the time taken to run this number of samples, and the cost involved. ASE extracts the greatest amount of samples in one extraction run, for a comparatively shorter length of time than the Soxhlet, it also extracts faster than this, and with greater ease. The expense of the extraction (e.g. additional equipment) is also greatly reduced when using the ASE. Costs are factored in for the analysis of 200 samples, which is the number to be extracted in this project.

Table A.1 Time and expense comparisons between Soxhlet and ASE.

	Soxhlet	ASE
No. of samples possible to extract at one time (incl. Blank)	6	12
Time	48 hours	8 hours
Minimum expense (£)	600	390

Samples

A single soil sample (0-5 cm from surface) was collected in a 125 ml DCM cleaned amber glass jar. This was then freeze dried and homogenised with an agate pestle and mortar, passed through a 355 µm sieve to remove plant material, and subsequently split into 4 parts for the respective extraction experiments (Soxhlet, ASE 100 °C and ASE 80 °C). The 4 samples were split again into triplicates prior to extraction. Sub-samples were analysed in triplicate.

Extractions

The conditions used for each extraction method are summarized in Table A.2. Soxhlet extraction was performed using a Scientific Laboratory Supplies Thermo Scientific

Soxhlet extractor. ASE extraction was implemented using a Dionex 200 model ASE. All samples were extracted using a 93:7 dichloromethane/methanol solvent ratio. Blanks were run with all extractions to check for possible contamination. All equipment parts in all extraction methods were cleaned prior to use with dichloromethane in order to remove all possible organic contaminants. Thimbles were extracted by Soxhlet for 24 hours and subsequently dried before use. Pre-extracted silica wool was used to prevent loss of soil from thimbles during extraction in both of these methods. 200 ml of solvent was used per sample by Soxhlet extraction. Extraction on the Soxhlet was undertaken for 48 hours.

ASE sample cells were sonicated in DCM for 30 minutes before rinsing with DCM prior to use to ensure thoroughly clean. 11 mL sample cells were used for which 65 mL solvent was used per cell. Solvent was heated for 5 minutes, static for 5 minutes and then flushed at 100 % volume. Cells were purged for 120 seconds for 3 cycles. The pressure used was 1500 psi, and two sets of samples were extracted at 100 $^{\circ}$C and 80 $^{\circ}$C. Samples were mixed with a roughly equal amount of extra pure sea sand in order to ensure minimum aggregation of the samples which may otherwise have prevented efficient extraction. The extra pure sea sand was also extracted in order to check for contamination. ASE extractions were run for 2.6 hours (40 minutes per cell).

Table A.2 Conditions used to compare Soxhlet and ASE extraction techniques.

	Soxhlet	ASE 100 ˚C	ASE 80 ˚C
Extraction solvent	93:7 DCM/MeOH	93:7 DCM/MeOH	93:7 DCM/MeOH
Solvent volume per sample (mL)	200	65	65
Pressure (psi)	Ambient	1500	1500
Temperature (˚C)	80	100	80
Time	48 hours	2.6 hours	2.6 hours
No. samples run	3 + blank	3 + blank	3 + blank

THM analysis

Online THM in the presence of TMAH was performed using a pulsed open mode pyrolysis system, specifically a CDS 1000 pyroprobe unit (Chemical Data Systems, USA) fitted with a platinum coil and a CDS 1500 valved interface. Approximately 20 mg of sample was weighed into a quartz pyrolysis tube plugged with pre-extracted silica wool. 6µl of the internal standard 5α-androstane (concentration = 0.1 mg/mL) with DCM was inserted prior to pyrolysis. 5µl of an aqueous solution of TMAH (25% w/w) was also inserted immediately before pyrolysis in order to derivatise lignin compounds. The quartz tube was inserted into the platinum coil of the pyroprobe and then heated 610 ˚C for 10 s (20 ˚C/ms temperature ramp). The platinum resistance-heated coil was interfaced with an HP5890 gas chromatograph with an open split and thermochemolysis products were separated on a 60 m HP5-MS column (0.25 mm internal diameter, 0.25 µm film thickness). Helium was used as a carrier gas due to its inert properties, at a flow rate of 1 mL/min. A solvent delay of 10 minutes was used. The GC oven was programmed from 50 to 220 ˚C at a rate of 1.5 ˚C/min, then isothermally held for 1 minute and finally raised to 320 ˚C at a 15 ˚C/min rate and held for 16 minutes. Compound detection was done using a HP 5973 mass selective detector in full scan mode (*m/z* 50-700).

The pyroprobe unit was calibrated prior to use. This was done using the salts lead chloride, lithium chloride, caesium chloride, potassium chloride and potassium iodide of which the melting points were known (ranging from 501 – 770 ˚C), (Bashir, 1999). From the observed temperature of the salt melting points in a quartz tube a calibration curve was created (Figure A.1) and the actual temperature within the quartz tube in the pyroprobe unit calculated. The temperature within the quartz tube was found to be different to that actually being used, roughly 150 ˚C hotter, and so the programmed temperature was corrected for this.

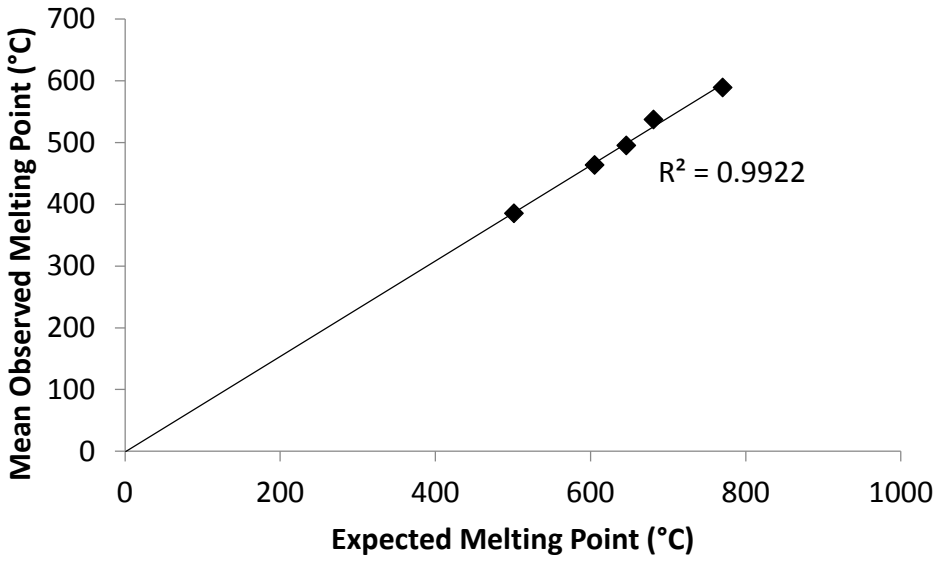


Figure A.1 Calibration curve for pyroprobe unit

Compound identification was based upon the NIST98 spectral library of that >95% and known retention times and ion fragmentation (Hatcher et al., 1995; del Rio et al., 1996; Filley et al., 2006). Compound weights (mg/ml) within each sample were calculated using spectral abundance against the internal standard. Means and standard errors (SE) of the triplicates for total soil residue yield and THM compounds were calculated and have been compared for significant differences between the methods below.

A. 3. Results

Sample weight after extraction

The weight of sample after extraction (soil residue yield) was calculated by subtracting the weight of extract from the weight of sample before extraction. ASE 80 ˚C had the lowest degree of variability between replicates (Figure A.2 and Table A.3). This shows that by bulk sample it is the most reliable method. However, as lignin phenols are an important part of the study, guaiacyl, syringyl and cinnamyl phenols have also been analysed in the extracted soil (see 'Lignin phenol amounts', below).

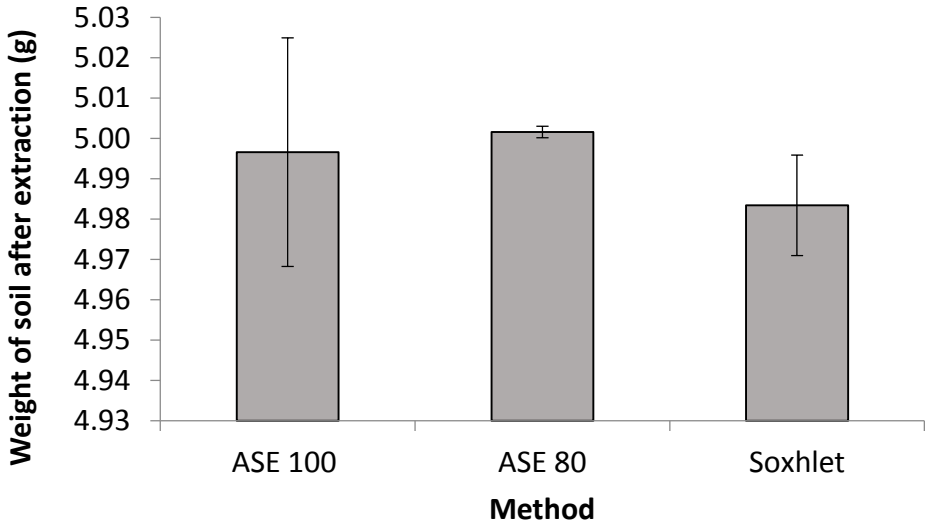


Figure A.2 Soil residue yield comparison of the three methods ASE 100 °C, ASE 80 °C and Soxhlet, with SE bars shown.

Table A.3 Descriptive statistics of soil residue yield from the three extraction methods ASE 100 °C, ASE 80 °C and Soxhlet.

Method	Mean	SE
ASE 100	5.00	0.03
ASE 80	5.00	0.00
Soxhlet	4.98	0.01

TMAH thermochemolysis products

THM in the presence of TMAH gave the methylated phenols guaiacyl (G), syringyl (S) and *p*-hydroxyphenyl cinnamyl (C) in all extraction methods.

Lignin phenol amounts

Following extraction the main lignin phenols guaiacyl, syringyl and cinnamyl (G, S and C) were assessed for analytical quantitative reproducibility during THM in TMAH using triplicate samples (Section 2.9). G phenols consist of G4+G6, S units consist of S4 + S6, and C units consist of G18 + P18. SE bars show that ASE 80 °C has a greater yield of G, S and C units than ASE 100 °C. However, it only shows a greater amount for G units than Soxhlet. Soxhlet showed greater yields than ASE 100 °C of all lignin units except for G. ASE 80 °C also recovered the greatest yield of total lignin products (including compounds other than the above mentioned) of all the extraction methods tested (Table A.4). Therefore ASE 80 °C is the most efficient at yielding lignin products.

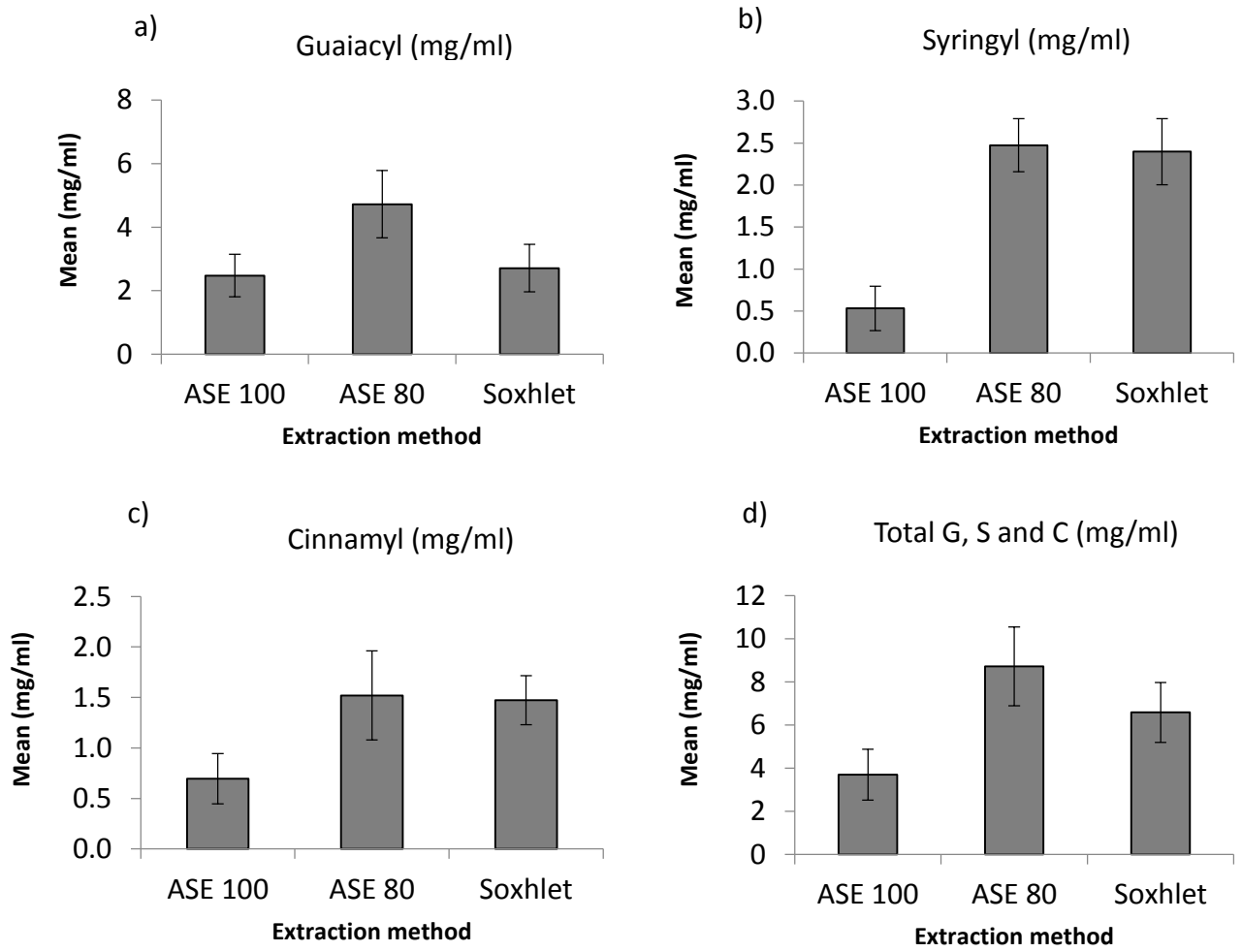


Figure A.3 Reproducibility of mean amounts of lignin phenols in ASE 100 °C, 80 °C and Soxhlet (a) guaiacyl units (b) syringyl units (c) cinnamyl units (d) sum of latter three lignin units

Table A.4 Phenolics recovered in samples in each extraction method and totals (mg/ml).

Method	G1	P6	G4	G6	S4	P18	S6	G18	Total
ASE 100 ˚C	3.55	1.46	1.00	1.48	0.06	0.24	0.47	0.45	8.72
ASE 80 ˚C	3.87	2.74	1.57	2.42	0.77	0.61	1.70	0.91	14.59
Soxhlet	2.34	2.61	1.50	2.71	0.00	0.66	2.40	0.81	13.04

Peak quality of TICs

Following extraction (ASE 80 °C) the macromolecular insoluble residues were depolymerised using THM in TMAH and these produced the cleanest peaks with less co-elution than with either Soxhlet extraction or the higher ASE extraction temperature. This also increased confidence in the identification and assignments of the thermochemolysis products.

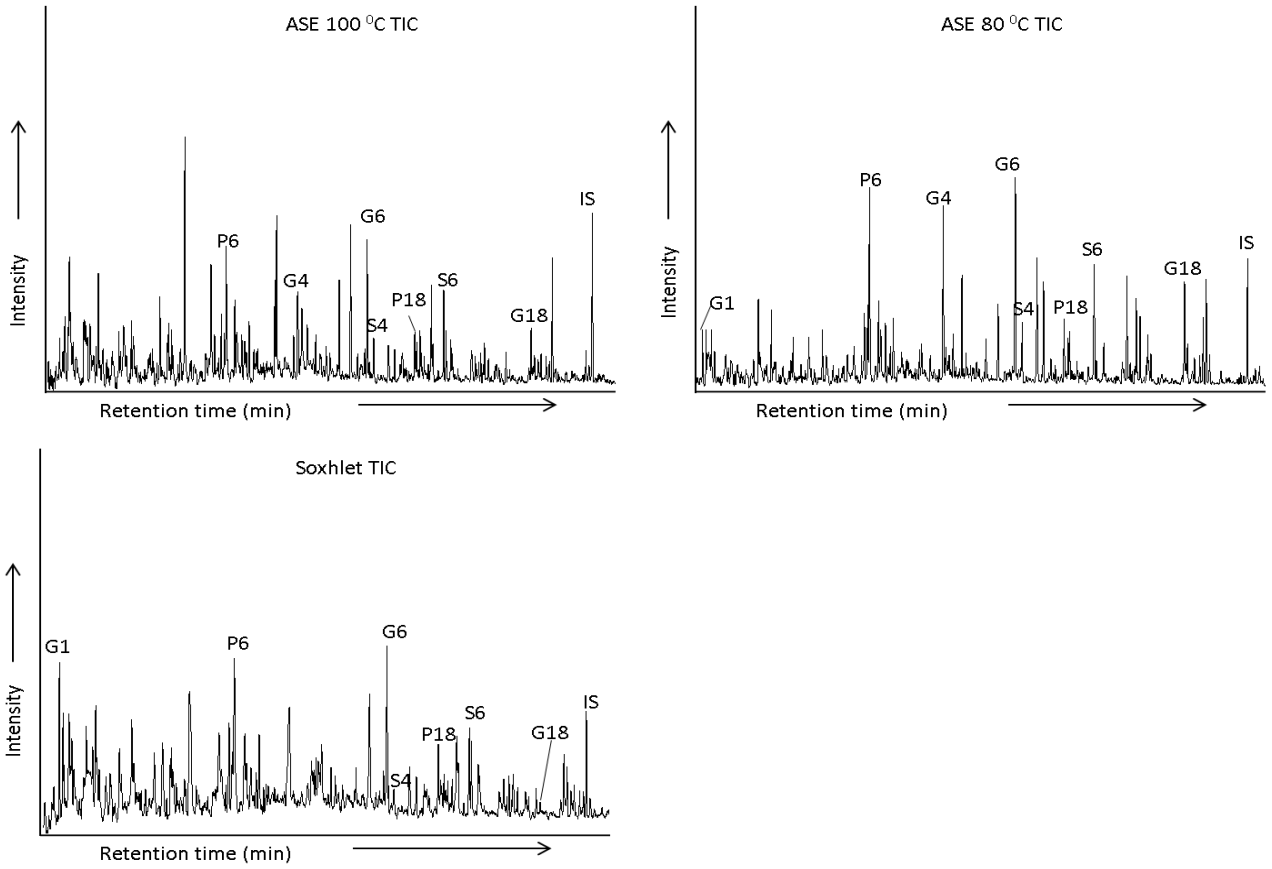


Figure A.4 TICs of ASE 100 ˚C, ASE 80 ˚C and Soxhlet labelled with main lignin compounds and internal standard (IS).

A. 4. Conclusion

This extraction experiment has revealed that when run at a temperature of 80 ˚C (the same as that of the Soxhlet) the ASE has a greater recovery of total insoluble residue yield with greater reproducibility than both ASE 100 ˚C and Soxhlet. It also has the greatest yield of total lignin phenols, despite not having greater G, S and C components. The ASE is also more efficient in terms of time and greatly reduces the cost of the extraction process.

Appendix B. Equations for determining the demethylated lignin and tannin content of lignin phenols

The following equations have been employed to determine the original aromatic methoxyl/hydroxyl content of 3,4-dimethoxy (guaiacyl, 1 and 2) and 3,4,5-trimethoxy (syringyl, 3 and 4) compounds, taken from (Filley et al., 2006).

Equation B.1 % Hydroxyl (G6, G5 and G18)

$$= 100\ x\ \left[\frac{M_{L+1} - (M_{L+1})_{calc})}{M_L + M_{L+1} - (M_{L+1})_{calc}}\right]$$

Where

$$(M_{L+1})_{calc} = M_L\ x\ \left(\frac{M_{UL+1}}{M_{UL}}\right)\ x\ \left(\frac{N_L}{N_{UL}}\right)$$

Equation B.2 % Hydroxyl (G4)

$$= 100\ x\ \left[\frac{(M_{L+2})_{calc}}{(M_{L)_{calc}} + (M_{L+2})_{calc}}\right]$$

Where

$$(M_L)_{calc} = \left(M_{L-1}\ x\ \left(\frac{M_{UL}}{M_{UL-1}}\right)\ x\ \left(\frac{N_L}{N_{UL}}\right)\right)$$

Where

$$(M_{L+1})_{calc} = \left(M_L - \left(M_{L-1}\ x\ \frac{M_{UL+1}}{M_{UL}}\right)\ x\ \left(\frac{N_L}{N_{UL}}\right)\right)$$

$$- \left(\left(M_{L+1} - \left(M_{L+2}\ x\ \frac{M_{UL+1}}{M_{UL}}\right)\ x\ \left(\frac{M_{UL-1}}{M_{UL}}\right)\right)\ x\ \left(\frac{M_{UL-1}}{M_{UL}}\right)\right)\left(\frac{N_{UL+1}}{N_{UL}}\right)\left(\frac{M_{UL+1}}{M_{UL}}\right)$$

Where

$$(M_{L+2})_{calc} = M_{L+1} - \left(M_{L+2}\ x\ \frac{M_{UL-1}}{M_{UL}}\right)\ x\ \left(\frac{N_{L+1}}{N_{UL}}\right) - (M_{L+1})_{calc}$$

Equation B.3 % Hydroxyl (S6 and S5)

% 1 Hydroxyl

$$= 100\ x\ \left[\frac{(M_L)}{M_L\ +\ [M_{L+1} -\ (M_{L+1}\ )_{calc}] +\ [M_{L+2} -\ (M_{L+2})_{calc}]}\right]$$

% 2 Hydroxyl

$$= 100\ x\ \left[\frac{M_{L+1} -\ (M_{L+1})_{calc}}{M_L\ +\ [M_{L+1} -\ (M_{L+1})_{calc}] +\ [M_{L+2} -\ (M_{L+2})_{calc}]}\right]$$

% 3 Hydroxyl

$$= 100\ x\ \left[\frac{M_{L+2} -\ (M_{L+2})_{calc}}{M_L\ +\ [M_{L+1} -\ (M_{L+1})_{calc}] +\ [M_{L+2} -\ (M_{L+2})_{calc}]}\right]$$

Where

$$(M_{L+1})_{calc} = \left(M_L\ x\ \left(\frac{M_{UL+1}}{M_{UL}}\right)\ x\ \left(\frac{N_L}{N_{UL}}\right)\right)$$

Where

$$(M_{L+2})_{calc} = \left([M_{L+1} -\ (M_{L+1})_{calc}]\ x\ \left(\frac{M_{UL+1}}{M_{UL}}\right)\ x\ \left(\frac{N_L}{N_{UL}}\right)\right)$$

Equation B.4 % Hydroxyl (S4)

%1 Hydroxyl

$$= 100\ x\ \left[\frac{(M_{L-1})}{M_{L-1}\ +\ [(M_{L-1}) +\ (M_{L+1}\ )_{calc}] +\ [M_{L+2} -\ (M_{L+2})_{calc}]}\right]$$

%2 Hydroxyl

$$= 100\ x\ \left[\frac{(M_{L+1})_{calc}}{(M_{L-1}) +\ [(M_{L+1})_{calc}] +\ [(M_{L+2})_{calc}]}\right]$$

%3 Hydroxyl

$$= 100\ x\ \left[\frac{(M_{L+2})_{calc}}{(M_{L-1}) + [(M_{L+1})_{calc}] + [(M_{L+2})_{calc}]}\right]$$

Where

$$(M_{L+1})_{calc} = M_{L+1}$$

$$-\left[\left(M_L - M_{L-1}\ x\ \left(\frac{M_{UL}}{M_{UL-1}}\right)\right)\ x\ \left(\frac{M_{UL-1}}{M_{UL}}\right)\ x\left(\frac{N_L}{N_{UL}}\right)\right.$$
$$\left. + \left(M_{L+2} - M_{L+3}\ x\ \frac{M_{UL+1}}{M_{UL}}\right)\ x\ \left(\frac{M_{UL+1}}{M_{UL}}\right)\ x\ \left(\frac{N_{L+1}}{N_{UL}}\right)\right]$$

Where

$$(M_{L+2})_{calc} = M_{L+2}$$
$$- \left[M_{L+1}\right.$$
$$\left. - \left(M_{L+2} - M_{L+3}\ x\ \frac{M_{UL+1}}{M_{UL}}\right)\ x\ \left(\frac{M_{UL+1}}{M_{UL}}\right)\ x\left(\frac{N_{L+3}}{N_{UL}}\right)\ x\ \left(\frac{M_{UL}}{M_{UL-1}}\right)\right]$$

Appendix C. Photographs of field sites taken from each sample pit location.

Wowetta sub-environments

Figure C.1 The first sample site on the Wowetta transect: savannah grassland (SG1) with scattered trees, savannah woodland and rainforest backed by the Iwokrama Mountains to the north east. The transect extends south east from this site. GPS coordinate: N 4.020778 W 59.047431. Altitude: 91 m a.s.l

Figure C.2 The second sample site: savannah woodland (SW1) facing north east. GPS coordinate: N 4.019415 W 59.046491. Altitude: 91 m a.s.l.

Figure C.3 Fruiting flowers on the branches of savannah woodland trees which show these species are angiosperms. This is important to note for molecular lignin investigations (see Chapter 5).

Figure C.4 Forest island (FI) between areas of savannah (sample sites SG1 and SW1 to the north west, sample sites ST1 and SS to the south east), before the savannah-rainforest boundary proper. GPS coordinate: N 4.017538 W 59.045352. Altitude: 99 m a.s.l.

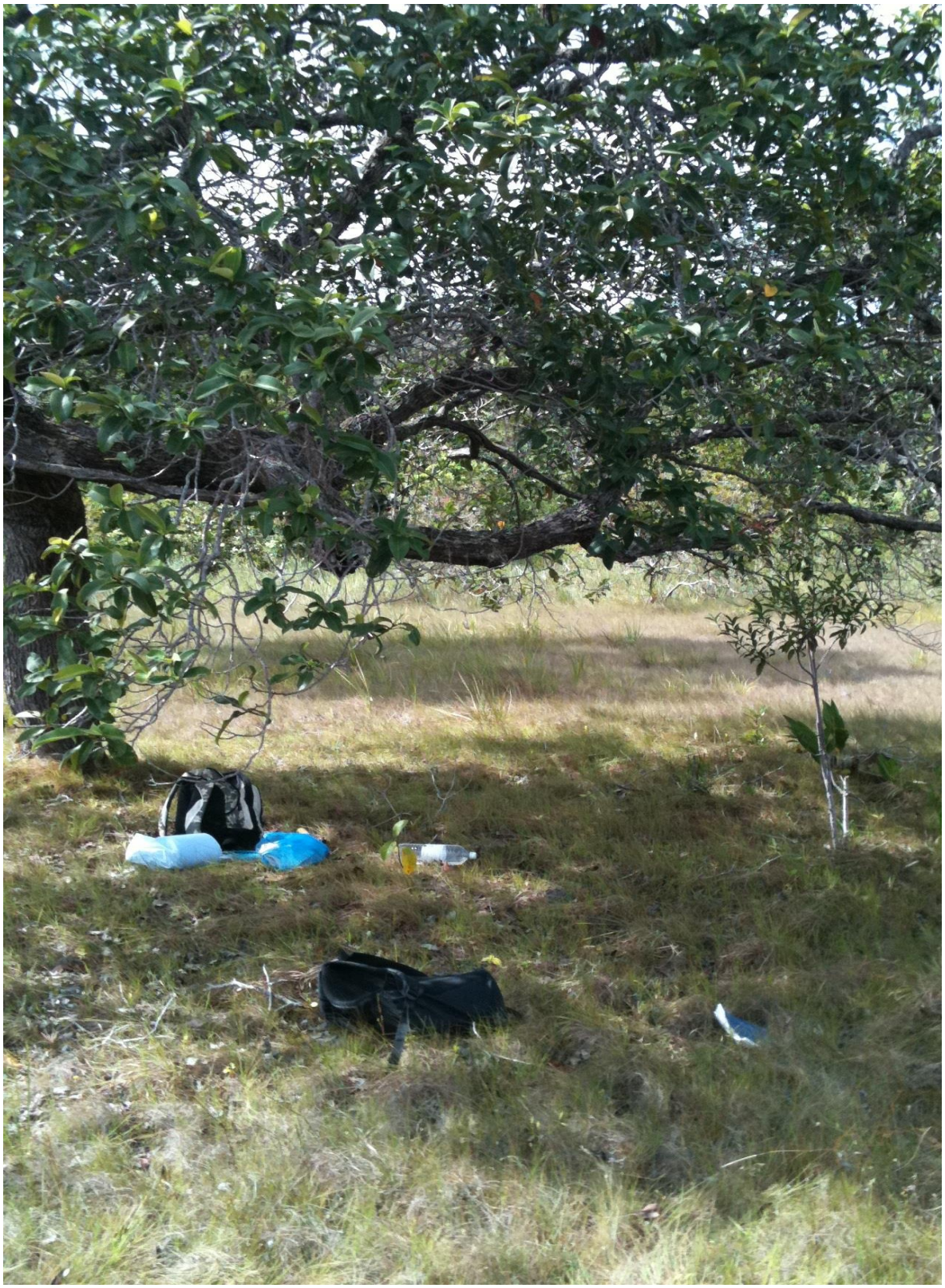


Figure C.5 Savannah tree (ST1) with forest island (FI) behind to the north west. GPS coordinates: N 4.015541, W 59.043929. 97 m a.s.l.

Figure C.6 Savannah swamp (SS) with scattered palm trees. Rainforest and mountains in the background to the north east. GPS coordinates N 4.013315 W 59.042. 90 m a.s.l.

Figure C.7 Sixth sample site on the transect: dense muri scrub vegetation of the transition zone (T1) between savannah to the north west and rainforest to the south east. GPS coordinates: N 4.005092 W -59.036534. 90 m a.s.l.

Figure C.8 Facing north east at the seventh sample site: Swamp forest (SF1). Located in a depression before a pisoplinthic mound to the south east (PF - next sample site), with a closed high canopy with abundant shrub layer vegetation. GPS coordinates: N 4.00198 W 59.029819. 107 m a.s.l.

Figure C.9 Facing north east on the eighth sample site: mixed tree spp. forest on a pisoplinthic mound (hardened laterite nodules), (PF). This forest has a closed high canopy with abundant shrub vegetation. GPS coordinates: N 4.001714 W 59.027946. 154 m a.s.l.

Figure C.10 Facing north east, the ninth and final sample site on the Wowetta transect: mixed tree spp. forest (MF1). Located on even ground, below and to the south east of the pisoplinthic forest (PF) sample site. Again, it has a closed high canopy and abundant shrub layer. GPS coordinates: N 4.000836 W 59.024487. 119 m a.s.l.

Surama sub-environments – photographs taken from the sample pit location

Figure C.11 Facing north west, savannah grassland (SG2) with rainforest behind. GPS coordinates: N 4.153051 W 59.060868. 78m a.s.l.

Figure C.12 Facing north west, savannah grassland on a pisoplinthic hill (PSG). GPS coordinates: N 4.155779 W59.06157. 79 m a.s.l.

Figure C.13 Facing north towards the rainforest, savannah woodland (SW2). GPS coordinates: N 4.161674 W 59.061503. 79 m a.s.l

Figure C.14 Facing west towards the rainforest, savannah tree (ST2), noticeably smaller and likely younger than that sampled at Wowetta. GPS coordinates: N 4.150107 W 59.069757. 84 m a.s.l.

Figure C.15 Facing north, with rainforest behind transition zone (T2) between savannah and rainforest. Vegetation at this sample site is very different to muri scrub at Wowetta due to a greater occurrence of grass, smaller shrubs / young trees and open canopy. GPS coordinates: N 4.16419 W 59.057214. 85 m a.s.l.

Figure C.16 Facing north into the rainforest, kokret palm forest (KPF). Primarily composed of kokret palm, with some smaller shrubs beneath. GPS coordinates: N 4.162185 W 59.080134. 82 m a.sl.

Figure C.17 Facing north, swamp forest (SF2), noticeably drier ground conditions than at Wowetta. High closed canopy with less dense shrub layer than SF1 at Wowetta. GPS coordinates: N 4.153726, W 59.07649. 77 m a.s.l.

Figure C.18 Facing north, mora species dominated forest (MRF) which borders on the Iwokrama rainforest reserve. Closed high to emergent level canopy with dense shrub layer vegetation. GPS coordinates N 4.181601 W 59.061086. 43 m a.s.l.

Figure C.19 Mixed tree spp. forest (MF2). Closed high level canopy with dense shrub layer vegetation. GPS coordinates N 4.171157 W 59.082382 110 m a.s.l.

Appendix D. Soil profile field and laboratory data

Table D.1 Field and average laboratory measurements for soil profiles at Wowetta. n.d = not detected

Boundaries (cm)		Texture	pH	TOC (%)	Colour
Wowetta					
SG - Albic plinthosol					
A	52	sand	4.49	0.70	10YR 4/2 - dark greysih brown
Eag	103	loamy sand	4.53	0.03	10YR 8/1 white
B	120	loamy sand	4.65	0.02	
Bv	137	clay loam	4.61	0.03	10YR 6/8 reddish yelow 10YR 8/1 white
SW - Albic plinthosol					
A	9	sandy loam	3.90	1.61	7.5YR 3/2 - dark brown
Eag	39	loamy sand	4.29	0.20	7.5YR 6/2 - pinkish grey and 5/2 - brown
Bg	64	sandy loam	4.55	0.52	10YR 4/4 and 4/6 - dark yellowish brown; mottled
Bv	200	clay	5.25	0.04	10YR 7/8 5YR 5/8 - yellowish red and 5Y 8/1 white; mottled
FI - Humic gleysol					
Ah	27	humified	5.00	6.50	7.5YR 2.5/1 - black
Eag	220	clay	5.13	0.10	10YR 5/1 - grey
Bv	280	clay	5.86	0.03	7.5YR 6/1 - grey and 7.5YR 8/1 - white and 10YR 7/8 - yellow; mottled

Boundaries (cm)		Texture	pH	TOC (%)	Colour
ST - Albic plinthosol					
Ah	10	sand with semi-fibrous material	3.93	2.86	2.5YR 3/1 - very dark grey
Eag	98	sand	5.66	1.00	2.5YR 7/2 - light grey
Bvg	125	sandy loam	6.11	0.06	10YR 6/8, 2.5Y 8/1, 2.5YR 3/6 - dark red and 2.5Y 7/4 - pale yellow; mottled
Bv	200	clay			10YR 7/8, 5YR 5/8 - yellowish red and 5Y 8/1 - white; mottled
SS - Plinthic gleysol					
Ahg	54	sandy loam	4.83	2.22	10YR 3/2 - very dark greyish brown
Bvg	105	sandy loam	5.77	0.05	Gley 1 8/1 white and 10YR 7/8, 8/8 - yellow; mottled
B2	200	sand	7.33	0.06	7.5YR 7/1 - light grey
T - Gleyic plinthosol					
A	26	sandy clay loam	4.84	2.10	10YR 3/3 - dark brown
Bv	264	clay	4.44	0.08	10YR 7/1 - light grey, 10YR 6/8 - brownish yellow and 2.5YR 4/8, 5/8 - red mottled
SF - Humic gleysol					
Oh	27	humified	5.04	12.49	7.5YR 2.5/1 - black
Eag	265	sandy clay	4.4	0.30	10YR 5/1 - grey
Bv	333	clay	4.45	0.10	7.5YR 6/1 grey, 7.5YR 8/1 white and 10YR 7/8 yellow - mottled
PF - Pisoplinthic plinthosol					
Apx	56	sand	4.52	2.71	10YR 3/4 - dark yellowish brown
Bpx	70	sand	4.61	1.97	2.5Y 5/4 - light olive brown
MF - Acric plinthosol					
A	23	silt loam	4.33	1.14	10YR 3/2 - dark brown
E	42	sandy loam	4.55	0.10	7.5YR 5/2 - brown
Bv	195	clay	4.46	0.09	2.5Y 8/1 and 7/2 - white and pale red, 5YR 5/8 and 4/6 - yellowish red; mottled

	Water table depth (cm)	Plinthic depth (cm)
Wowetta		
SG - Albic plinthosol		
A	110	144
Eag		
B		
Bv		
SW - Albic plinthosol		
A	100	200
Eag		
Bg		
Bv		
FI - Humic gleysol		
Ah	25	300
Eag		
Bv		
ST - Albic plinthosol		
Ah	100	200
Eag		
Bvg		
Bv		
SS - Plinthic gleysol		
Ahg		
Bvg		
B2		
	25	95
T - Gleyic plinthosol		
A		
Bv		
SF - Humic gleysol		
Oh	50	166
Eag		
Bv		
PF - Pisoplinthic plinthosol		
Apx		
Bpx	n.d	0
MF - Acric plinthosol		
A		
E		
Bv	80	282

Table D.2 Field and average laboratory measurements at Surama. n.d = not detected

	Boundaries (cm)	**Texture**	**pH**	**TOC (%)**	**Colour**
Surama					
SG - Haplic plinthosol					
A	54	loamy sand	5.09	0.78	2.5Y 4/3 - olive brown
B	207	sandy loam	5.51	0.29	10YR 5/8 - yellowish brown and 2.5YR 4/6 - red; mottled
PSG - Pisoplinthic plinthosol					
Apx	10	loamy sand	5.25	1.68	10YR 3/6 - dark yellowish brown
Bpx	40	loamy sand	5.5	1.06	2.5Y 5/6 - light olive brown
SW - Haplic plinthosol					
Av	50	sand	5.37	1.05	10YR 2/1 - black and 3/2 - very dark greyish brown
Bv	203	sandy loam	5.4	0.1	2.5YR 4/6 - red and 10YR 4/2 - dark greyish brown; mottled
Bv2	300	loamy sand	5.52	0.1	2.5Y 6/1 - grey
ST - Haplic plinthosol					
A	30	sandy loam	5.44	0.82	2.5Y 4/2 - dark greyish brown
B	200	loamy sand	5.41	0.31	2.5Y 5/6 - light olive brown
Bv	300	loamy sand	5.44	0.1	5YR 5/8 - yellowish red and 2.5Y 6/3 - light yellowish brown; mottled

	Boundaries (cm)	Texture	pH	TOC (%)	Colour
T - Haplic plinthosol					
Av	36	silt loam	5.11	0.47	2.5Y 4/4 - olive brown
Bv	88	silt loam	5.28	0.48	2.5Y 5/4 - light olive brown
SF - Plinthic gleysol					
Ea	6	sandy loam	5.52	5.45	10YR 3/3 - dark brown
Bv	260	sandy clay loam	5.51	0.71	2.5Y 6/1 - grey and 10YR 6/3 - brownish yellow; mottled.
PKF - Haplic plinthosol					
Av	103	loamy sand	5.81	0.63	10YR 3/3 - dark brown
Bv	303	sandy loam	5.59	0.1	10YR 5/4 and 6/8 - yellowish brown
MRF -Haplic plinthosol					
Av	56	clay loam	5.41	0.55	2.5Y 5/4 - light olive brown
Bv	300	clay loam	5.41	0.27	10YR 6/8 - brownish yellow
MF - Haplic plinthosol					
Av	60	loamy sand	6.04	0.56	10YR 3/3 - dark brown
Bv	196	sandy clay loam	5.92	0.38	10YR 6/8 - brownish yellow

	Water table depth (cm)	Plinthic depth (cm)
Surama		
SG - Haplic plinthosol A B	n.d	n.d
PSG - Pisoplinthic plinthosol Apx Bpx	n.d	0
SW - Haplic plinthosol Av Bv Bv2	n.d	n.d
ST - Haplic plinthosol A B Bv	300	n.d
T - Haplic plinthosol Av Bv	n.d	n.d
SF - Plinthic gleysol Ea Bv	270	n.d
PKF - Haplic plinthosol Av Bv	n.d	n.d
MRF -Haplic plinthosol Av Bv	n.d	n.d
MF - Haplic plinthosol Av Bv	n.d	n.d

Appendix E. Soil mineralogy – XRD results

Inventory

Sample A – SF1 surface soil

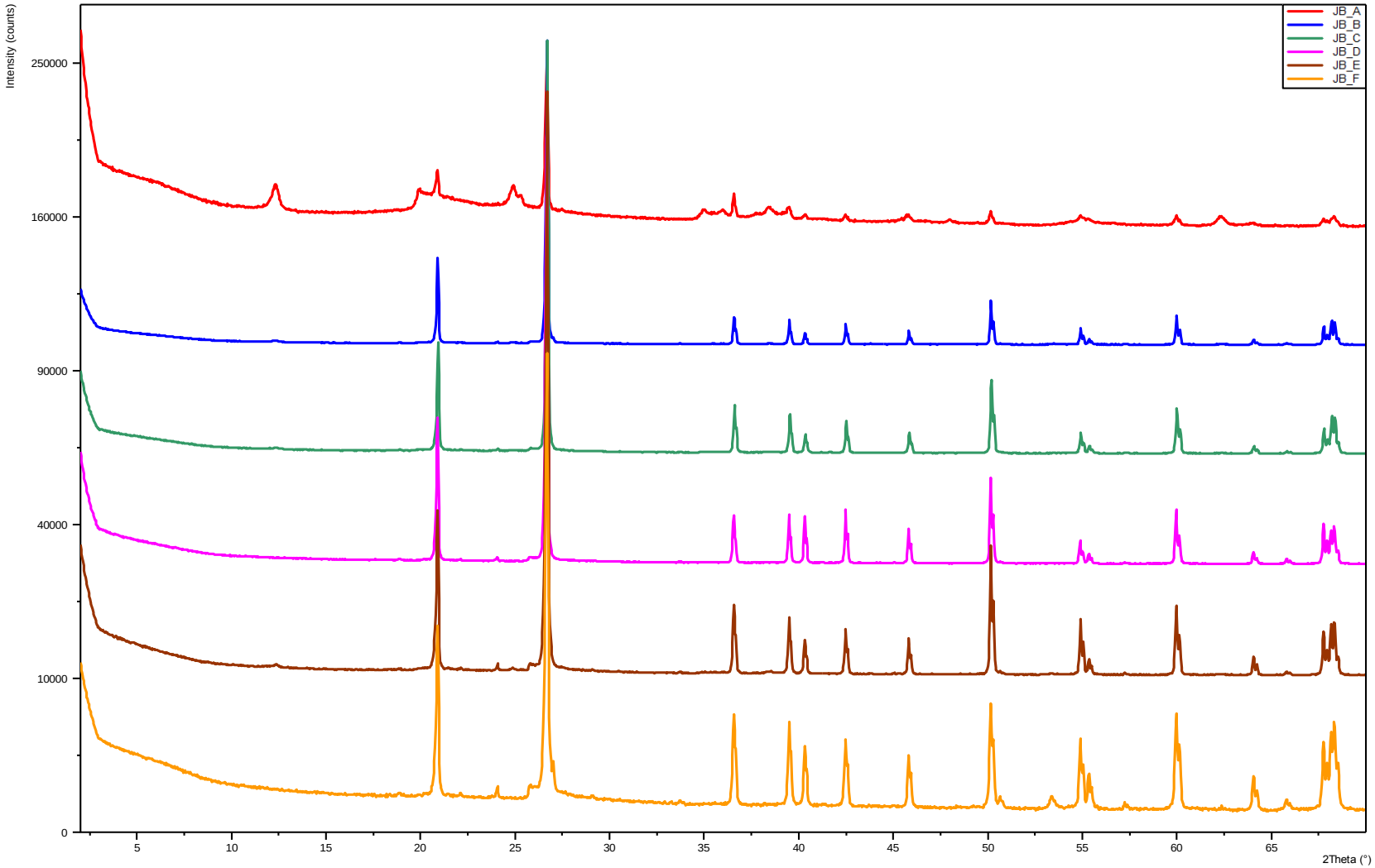
Sample B - MF1 surface soil

Sample C – T1 surface soil

Sample D – SW1 surface soil

Sample E – SS surface soil

Sample F – ST1 surface soil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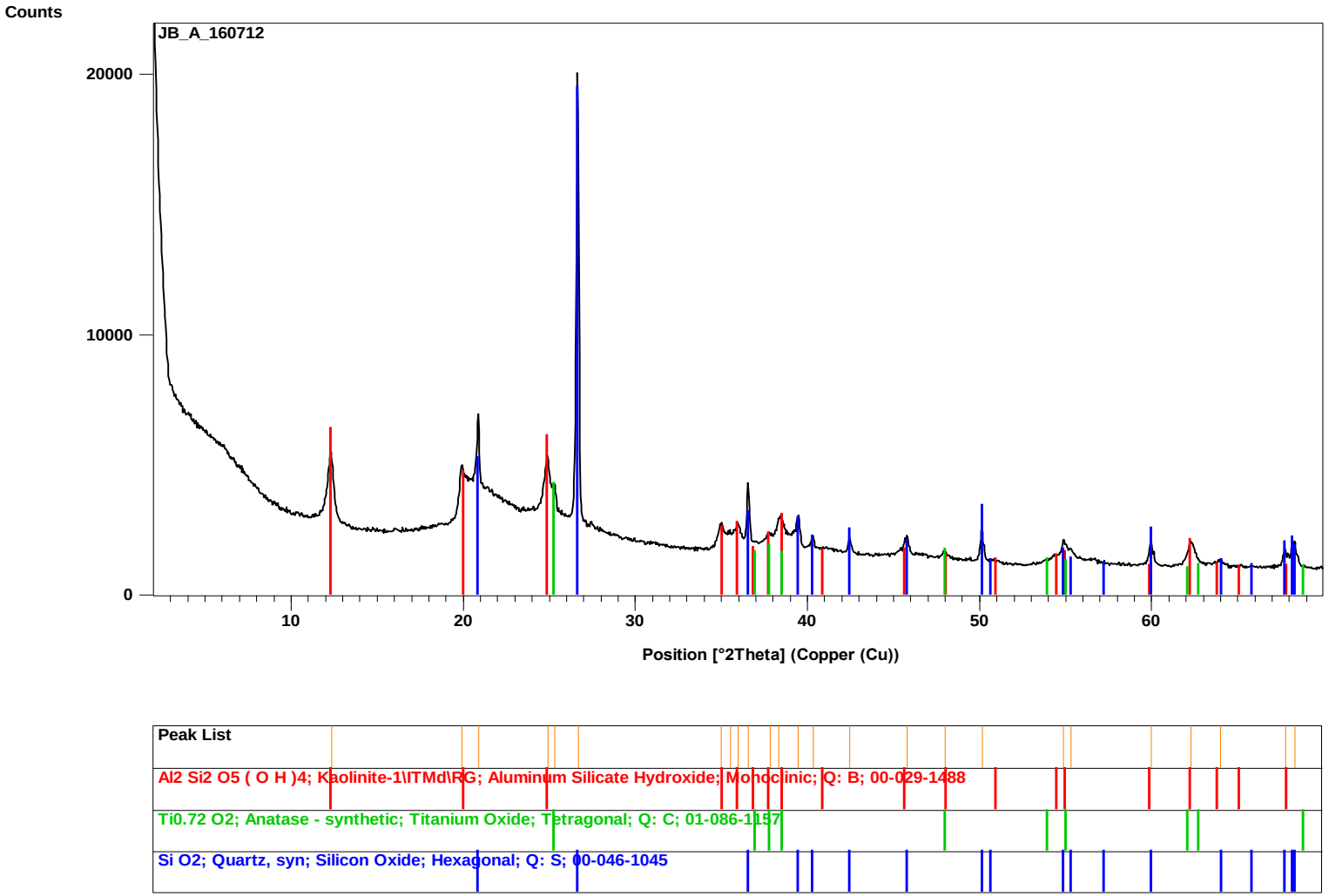
2Theta (°)
JB_A
JB_B
JB_C
JB_D
JB_E
JB_F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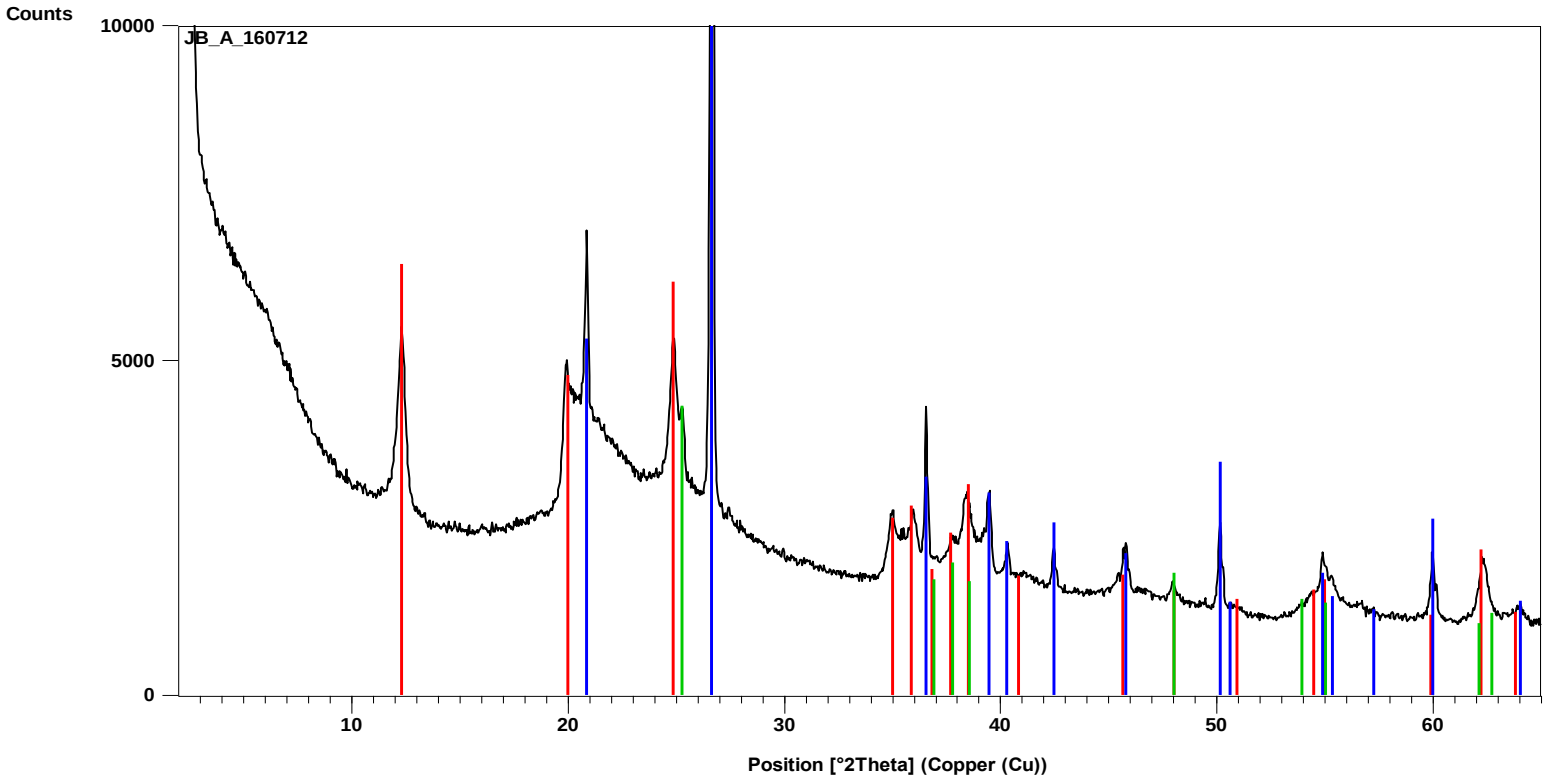
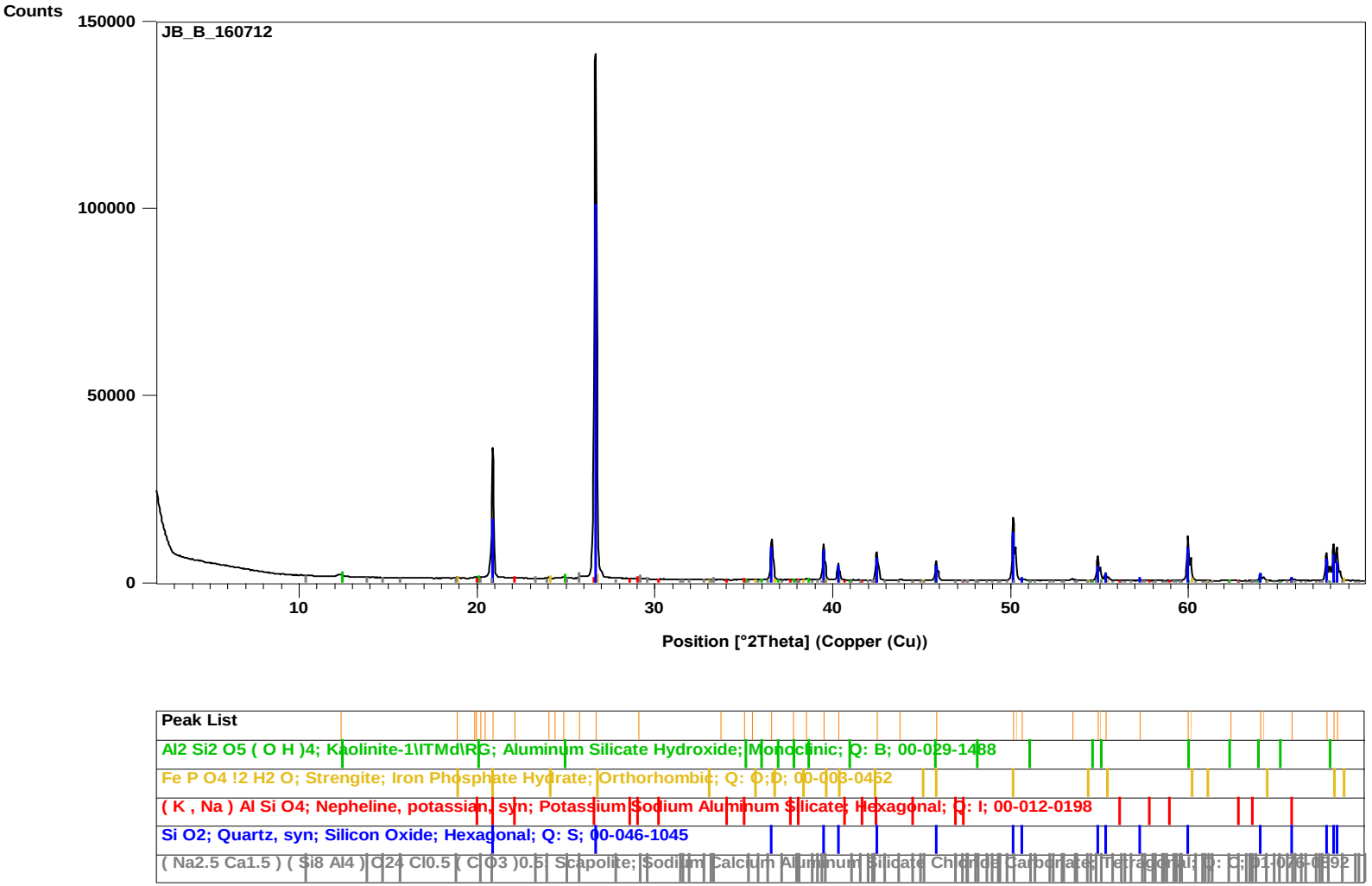
Ti0.72 O2; Anatase - synthetic; Titanium Oxide; Tetragonal; Q: C; 01-086-1157
Si O2; Quartz, syn; Silicon Oxide; Hexagonal; Q: S; 00-046-1045



Peak List
Al2 Si2 O5 (O H)4; Kaolinite-1\ITMd\RG; Aluminum Silicate Hydroxide; Monoclinic; Q: B; 00-029-1488
Ti0.72 O2; Anatase - synthetic; Titanium Oxide; Tetragonal; Q: C; 01-086-1157
Si O2; Quartz, syn; Silicon Oxide; Hexagonal; Q: S; 00-046-1045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40
50
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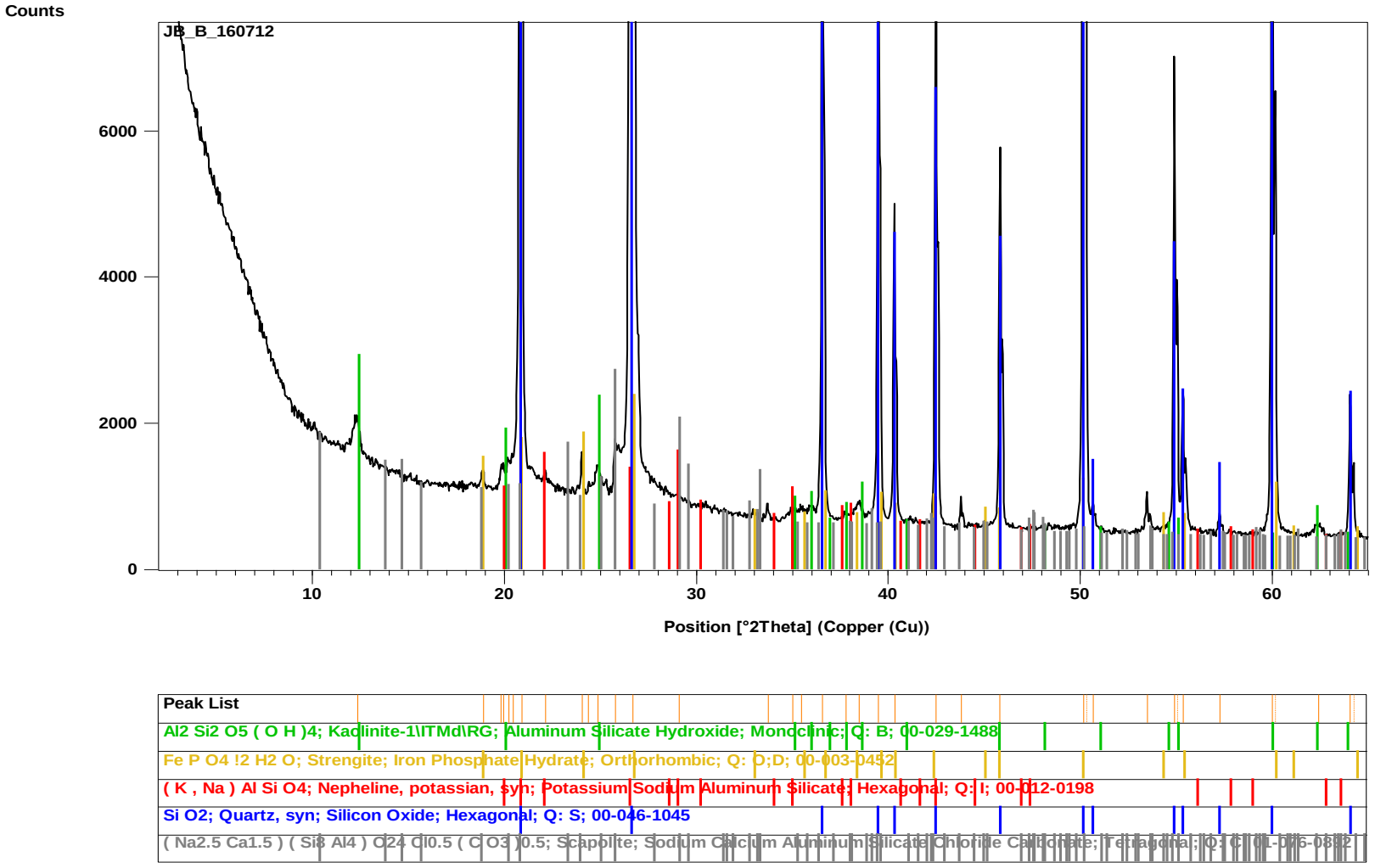
Position [°2Theta] (Copper (Cu))
Peak List
Al2 Si2 O5 (O H)4; Kaolinite-1\ITMd\RG; Aluminum Silicate Hydroxide; Monoclinic; Q: B; 00-029-1488
Fe P O4 !2 H2 O; Strengite; Iron Phosphate Hydrate; Orthorhombic; Q: D; D; 00-003-0452
(K , Na) Al Si O4; Nepheline, potassian, syn; Potassium Sodium Aluminum Silicate; Hexagonal; Q: I; 00-012-0198
Si O2; Quartz, syn; Silicon Oxide; Hexagonal; Q: S; 00-046-1045
(Na2.5 Ca1.5) (Si8 Al4) O24 Cl0.5 (C O3)0.5; Scapolite; Sodium Calcium Aluminum Silicate Chloride Carbonate; Tetragonal; Q: C; 01-078-0892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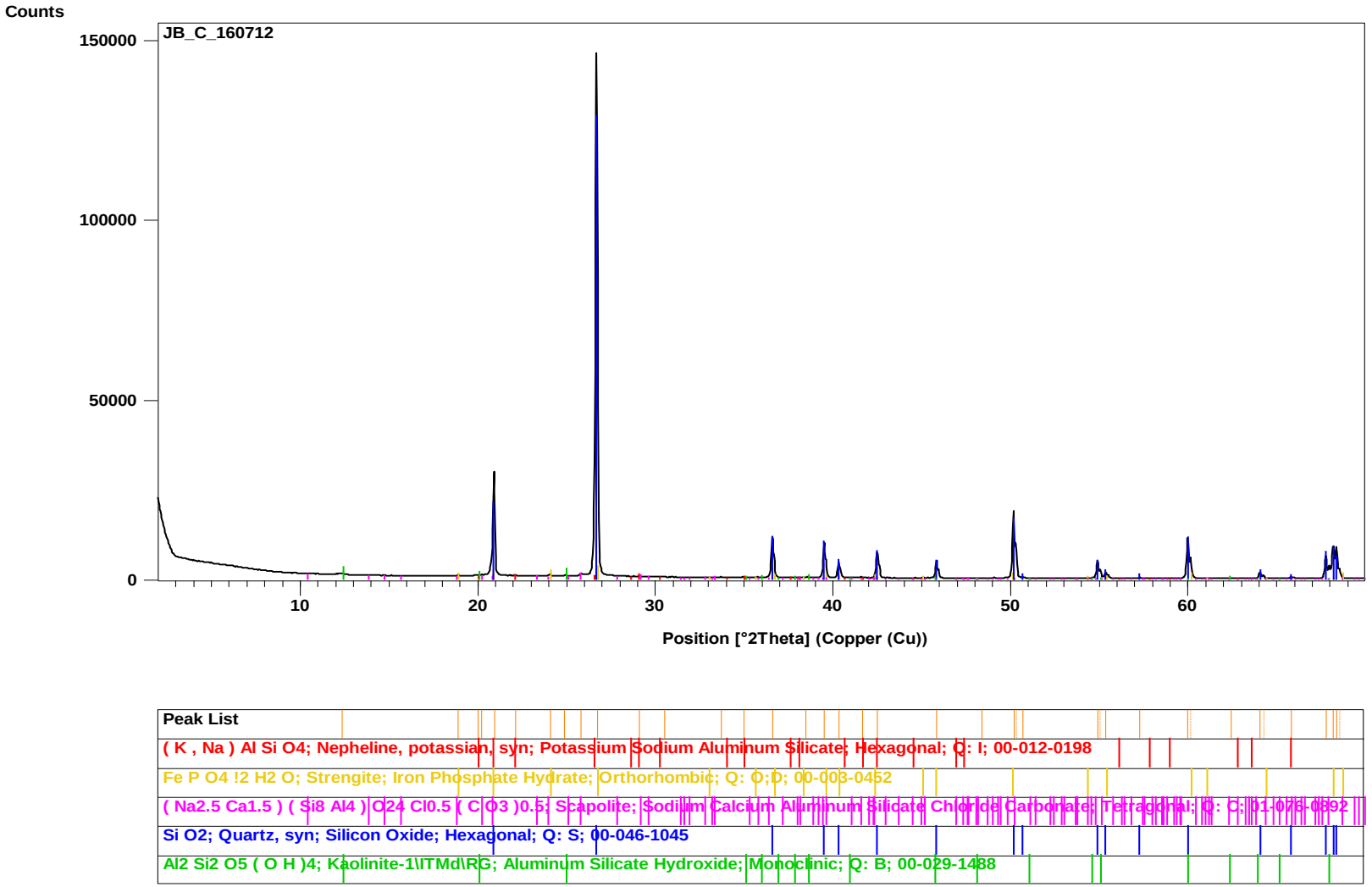
Fe P O4 !2 H2 O; Strengite; Iron Phosphate Hydrate; Orthorhombic; Q: D;D; 00-003-0452
(K , Na) Al Si O4; Nepheline, potassian, syn; Potassium Sodium Aluminum Silicate; Hexagonal; Q: I; 00-012-0198
Si O2; Quartz, syn; Silicon Oxide; Hexagonal; Q: S; 00-046-1045
(Na2.5 Ca1.5) (Si8 Al4) O24 Cl0.5 (C O3)0.5; Scapolite; Sodium Calcium Aluminum Silicate Chloride Carbonate; Tetragonal; Q: C; 01-076-0832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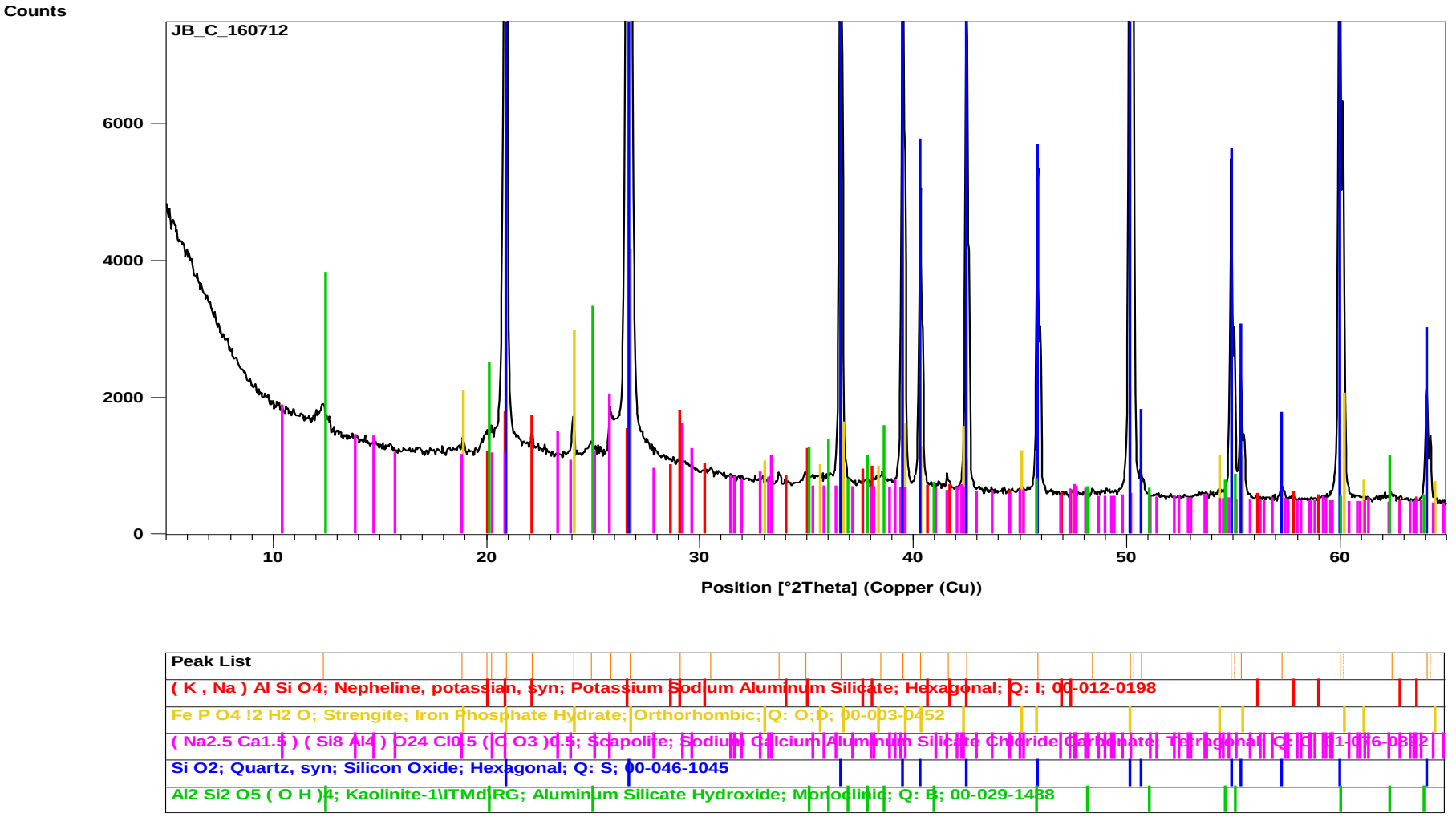
Fe P O4 !2 H2 O; Strengite; Iron Phosphate Hydrate; Orthorhombic; Q: D; D; 00-003-0452
(Na2.5 Ca1.5) (Si8 Al4) O24 Cl0.5 (C O3)0.5; Scapolite; Sodium Calcium Aluminum Silicate Chloride Carbonate; Tetragonal; Q: C; 01-078-0892
Si O2; Quartz, syn; Silicon Oxide; Hexagonal; Q: S; 00-046-1045
Al2 Si2 O5 (O H)4; Kaolinite-1\ITMd\RG; Aluminum Silicate Hydroxide; Monoclinic; Q: B; 00-029-1488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(K , Na) Al Si O4; Nepheline, potassian, syn; Potassium Sodium Aluminum Silicate; Hexagonal; Q: I; 00-012-0198
Fe P O4 !2 H2 O; Strengite; Iron Phosphate Hydrate; Orthorhombic; Q: O; D; 00-003-0452
(Na2.5 Ca1.5) (Si8 Al4) O24 Cl0.5 (C O3)0.5; Scapolite; Sodium Calcium Aluminum Silicate Chloride Carbonate; Tetragonal; Q: I; 01-076-0882
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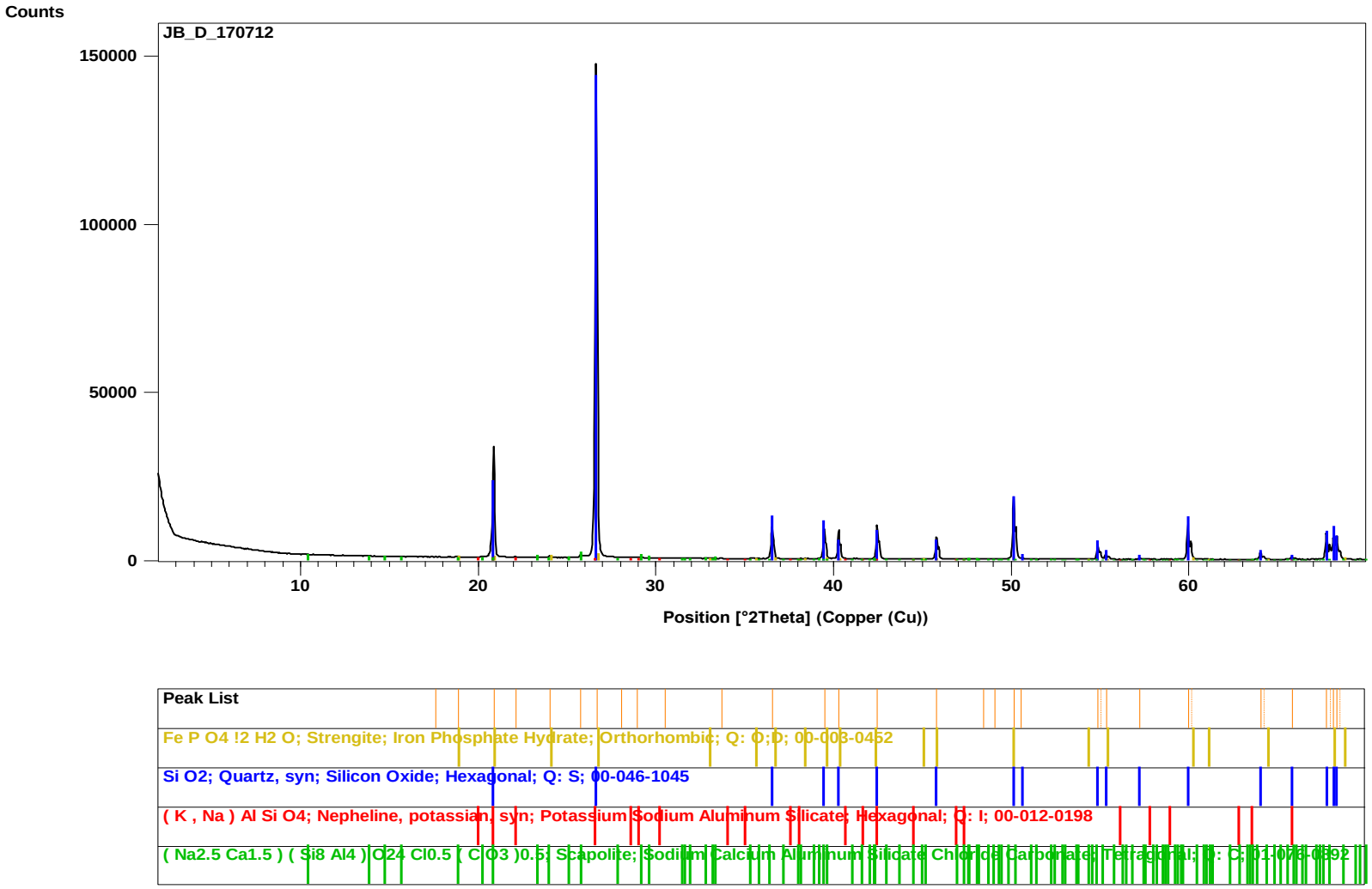
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Position [°2Theta] (Copper (Cu))
Peak List
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Si O2; Quartz, syn; Silicon Oxide; Hexagonal; Q: S; 00-046-1045
(K , Na) Al Si O4; Nepheline, potassian, syn; Potassium Sodium Aluminum Silicate; Hexagonal; Q: I; 00-012-0198
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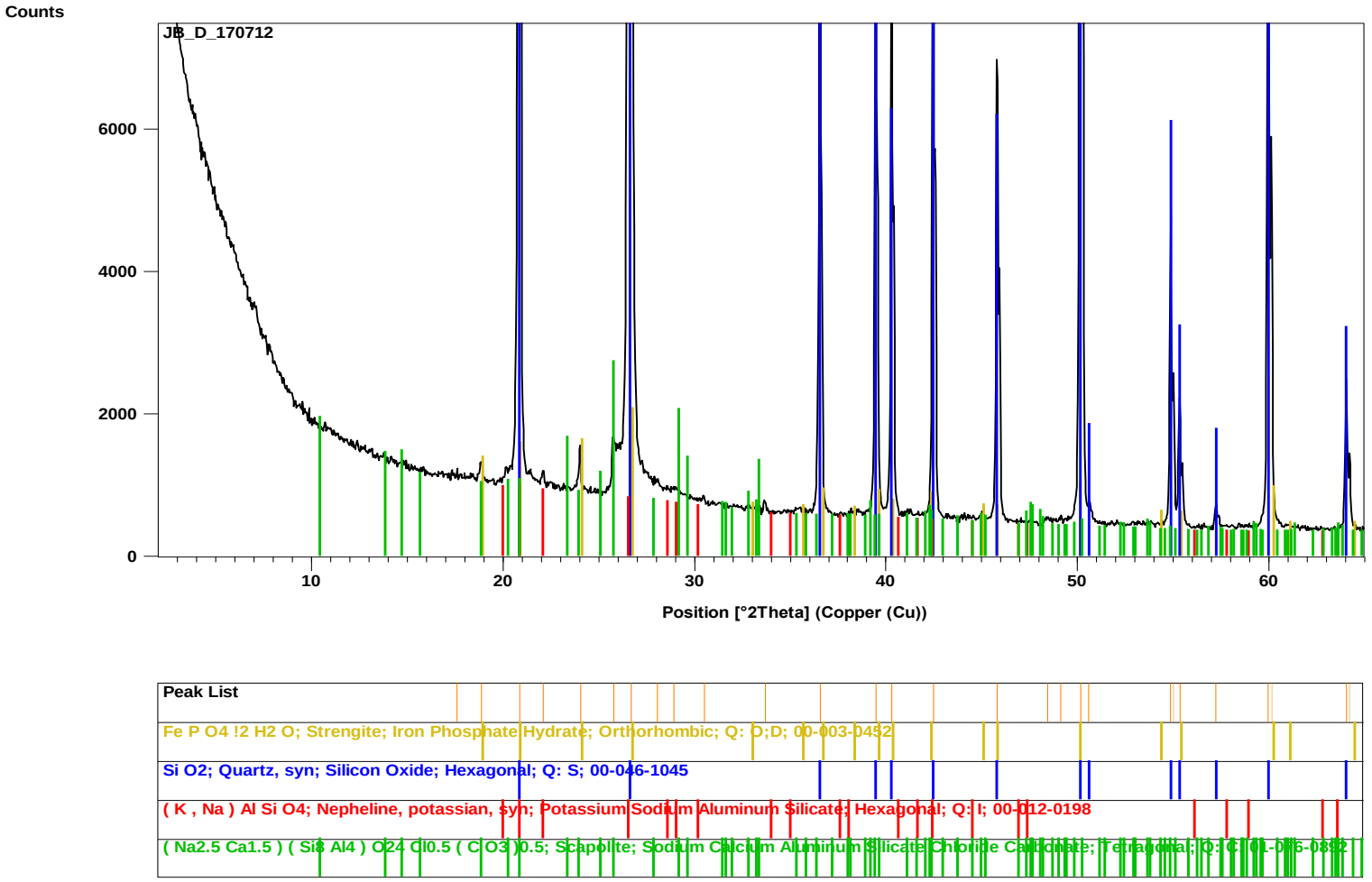
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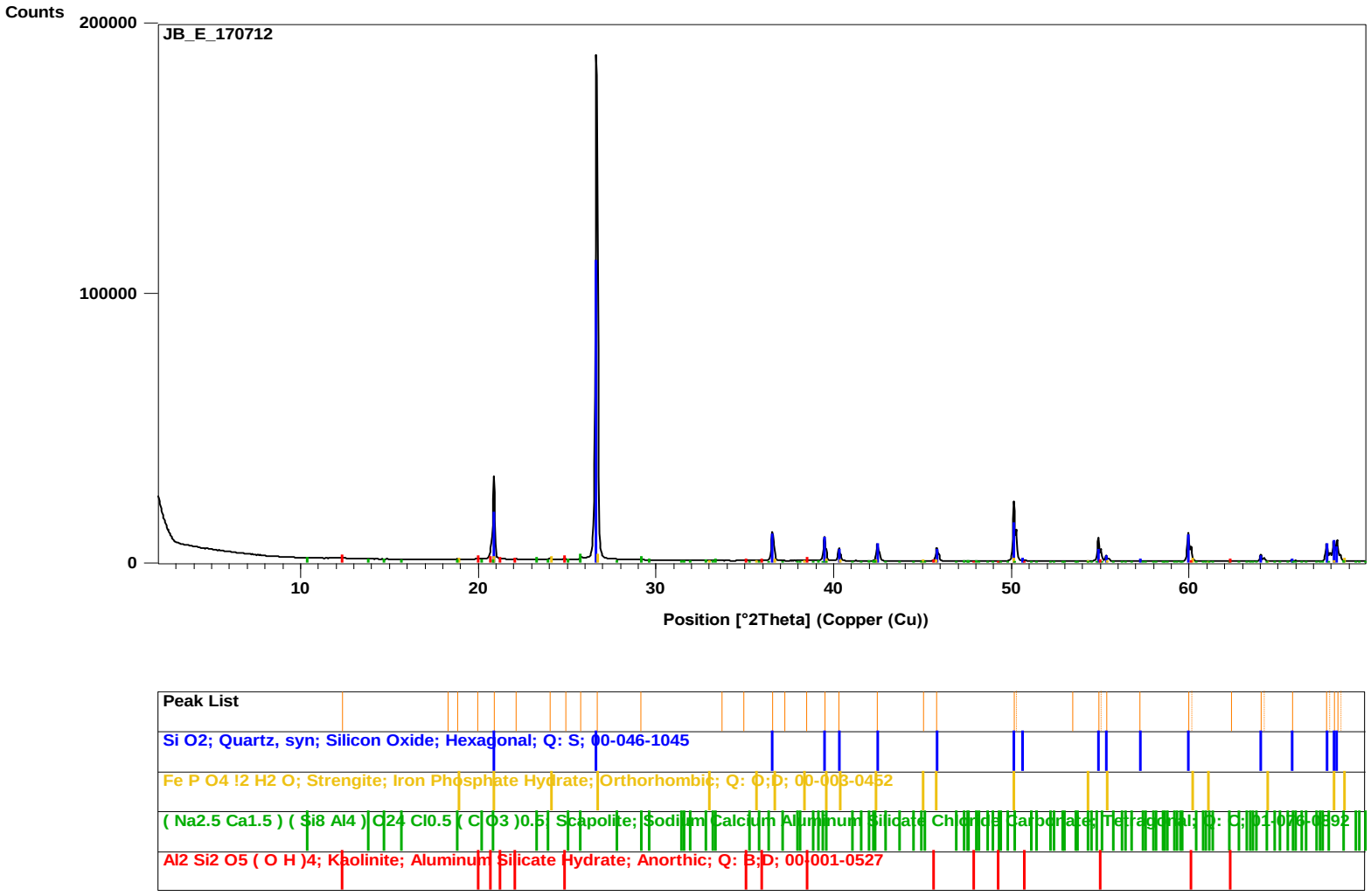
(K , Na) Al Si O4; Nepheline, potassian, syn; Potassium Sodium Aluminum Silicate; Hexagonal; Q: I; 00-012-0198
(Na2.5 Ca1.5) (Si8 Al4) O24 Cl0.5 (C O3)0.5; Scapolite; Sodium Calcium Aluminum Silicate Chloride Carbonate; Tetragonal; Q: C; 01-076-0882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60
Position [°2Theta] (Copper (Cu))
Peak List
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Fe P O4 !2 H2 O; Strengite; Iron Phosphate Hydrate; Orthorhombic; Q: D;D; 00-003-0452
(Na2.5 Ca1.5) (Si8 Al4) O24 Cl0.5 (C O3)0.5; Scapolite; Sodium Calcium Aluminum Silicate Chloride Carbonate; Tetragonal; Q: C;D; 01-074-0892
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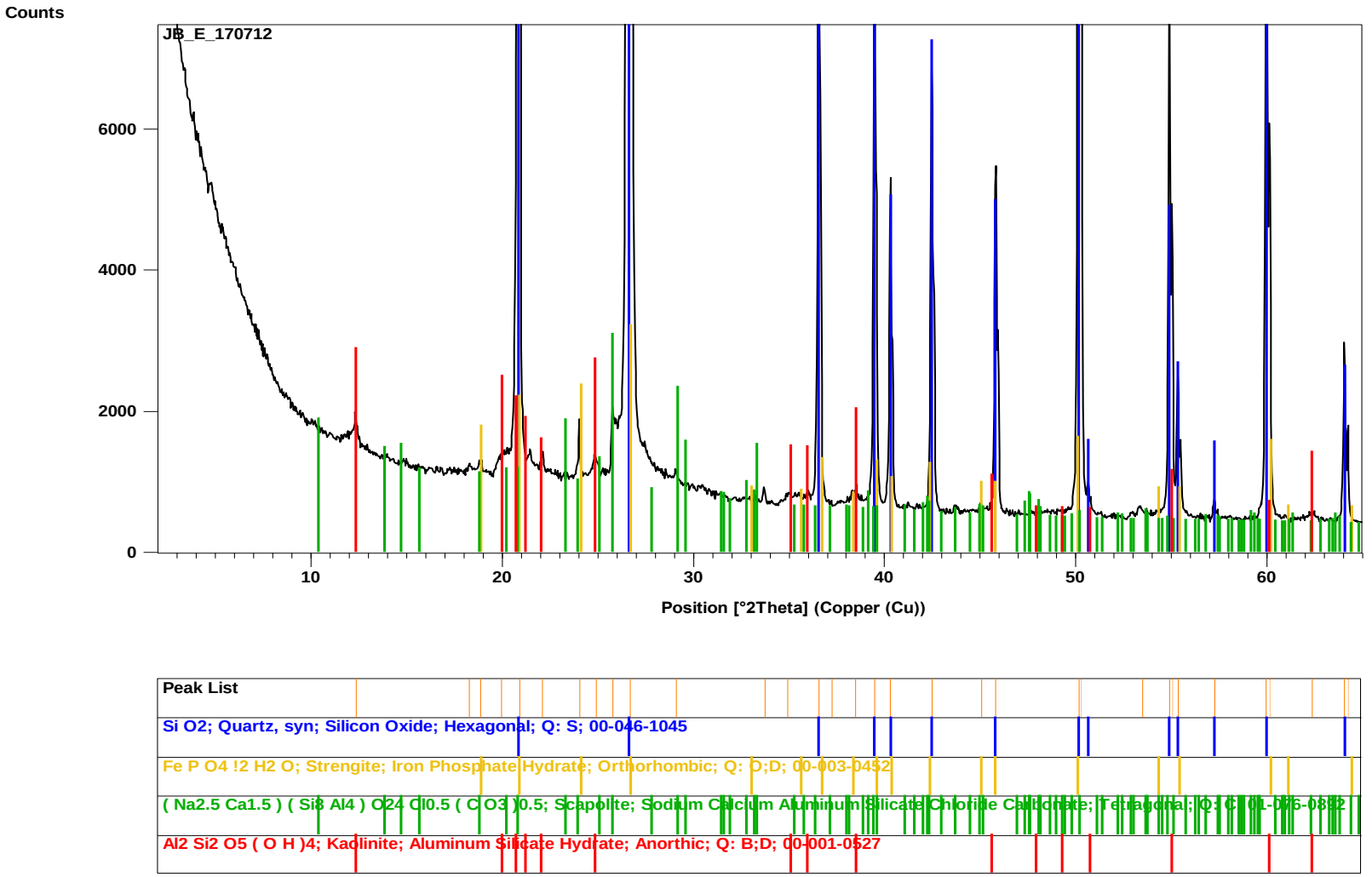
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Position [°2Theta] (Copper (Cu))
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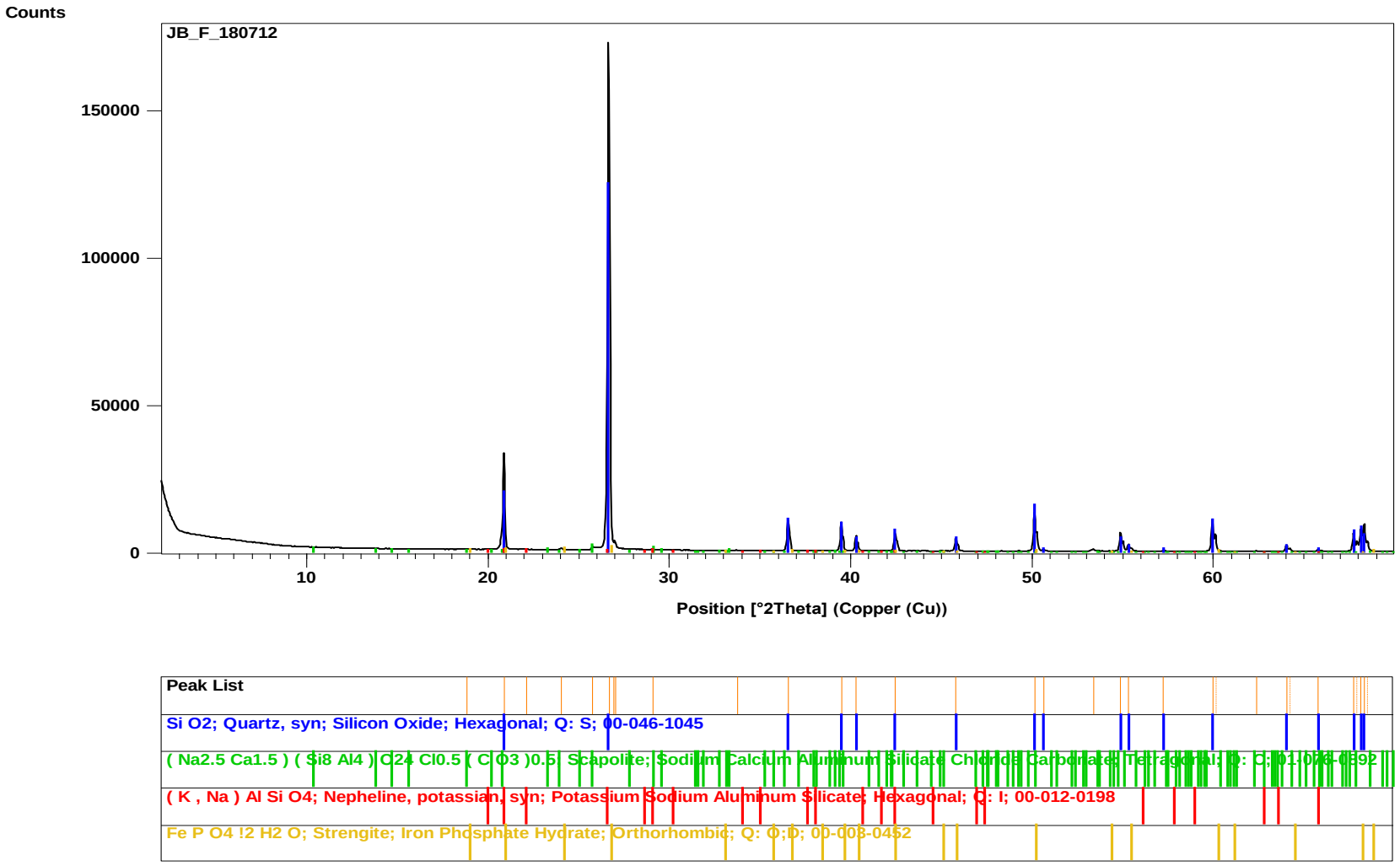
Fe P O4 !2 H2 O; Strengite; Iron Phosphate Hydrate; Orthorhombic; Q: O;D; 00-003-0452
(Na2.5 Ca1.5) (Si8 Al4) O24 Cl0.5 (C O3)0.5; Scapolite; Sodium Calcium Aluminum Silicate Chloride Carbonate; Tetragonal; Q: C; 01-076-0832
Al2 Si2 O5 (O H)4; Kaolinite; Aluminum Silicate Hydrate; Anorthic; Q: B;D; 00-001-0527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Position [°2Theta] (Copper (Cu))
Peak List
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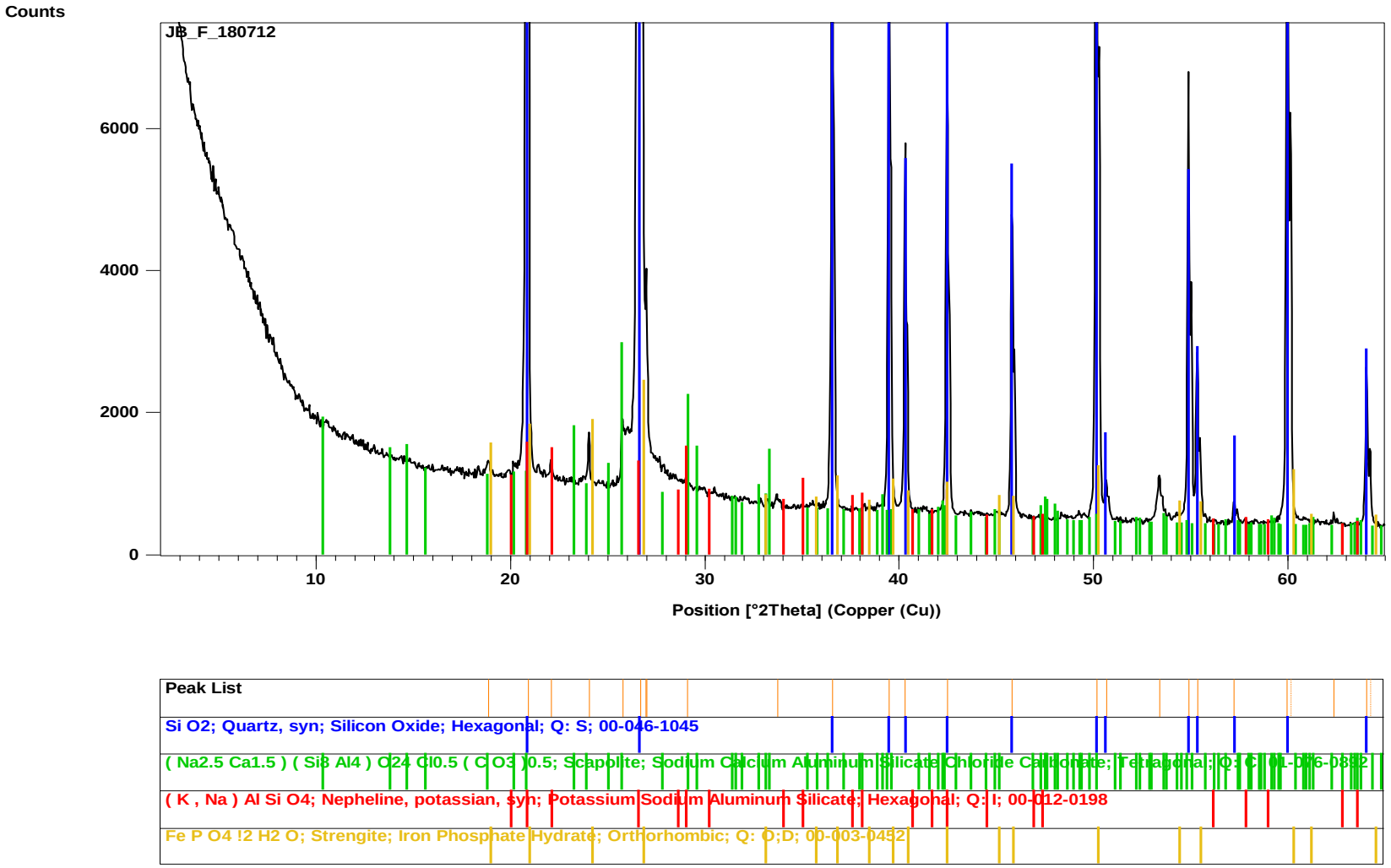
(Na2.5 Ca1.5) (Si8 Al4) O24 Cl0.5 (C O3)0.5; Scapolite; Sodium Calcium Aluminum Silicate Chloride Carbonate; Tetragonal; Q: C; 01-078-0892
(K , Na) Al Si O4; Nepheline, potassian, syn; Potassium Sodium Aluminum Silicate; Hexagonal; Q: I; 00-012-0198
Fe P O4 !2 H2 O; Strengite; Iron Phosphate Hydrate; Orthorhombic; Q: O; 00-008-0452


Counts
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4000
2000
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50
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Position [°2Theta] (Copper (Cu))
Peak List
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(Na2.5 Ca1.5) (Si3 Al4) O24 Cl0.5 (C O3)0.5; Scapolite; Sodium Calcium Aluminum Silicate Chloride Carbonate; Tetragonal; Q: C; 01-086-0832
(K , Na) Al Si O4; Nepheline, potassian, syn; Potassium Sodium Aluminum Silicate; Hexagonal; Q: I; 00-012-0198
Fe P O4 !2 H2 O; Strengite; Iron Phosphate Hydrate; Orthorhombic; Q: D;D; 00-003-0452

Appendix F. Partial chromatograms for the total ion current (TICs) of all sample sites in Wowetta

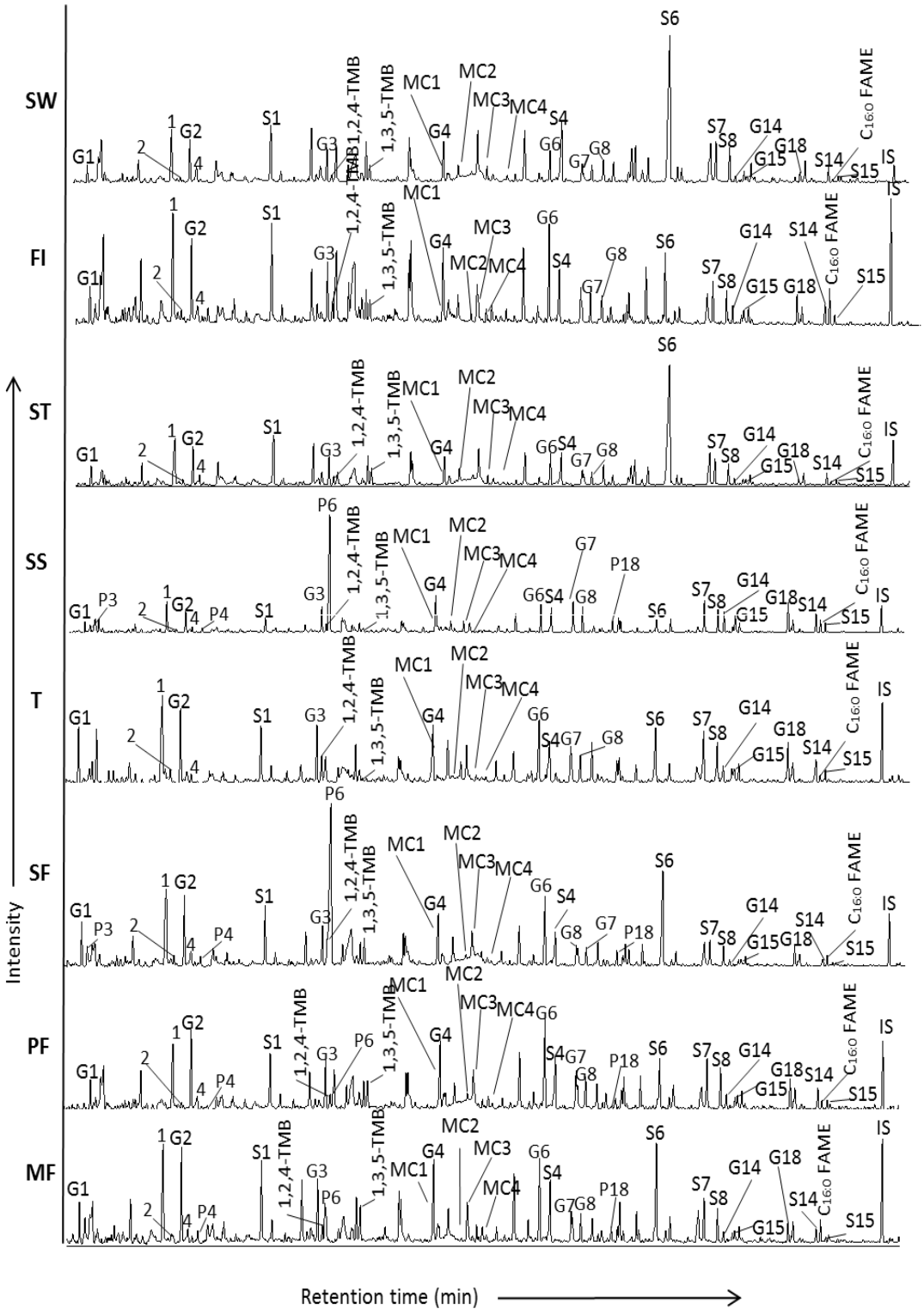

Figure F.1 Partial chromatogram for the total ion current (TIC) of thermochemolysis products from wood litter for the sub-environments SW – MF.

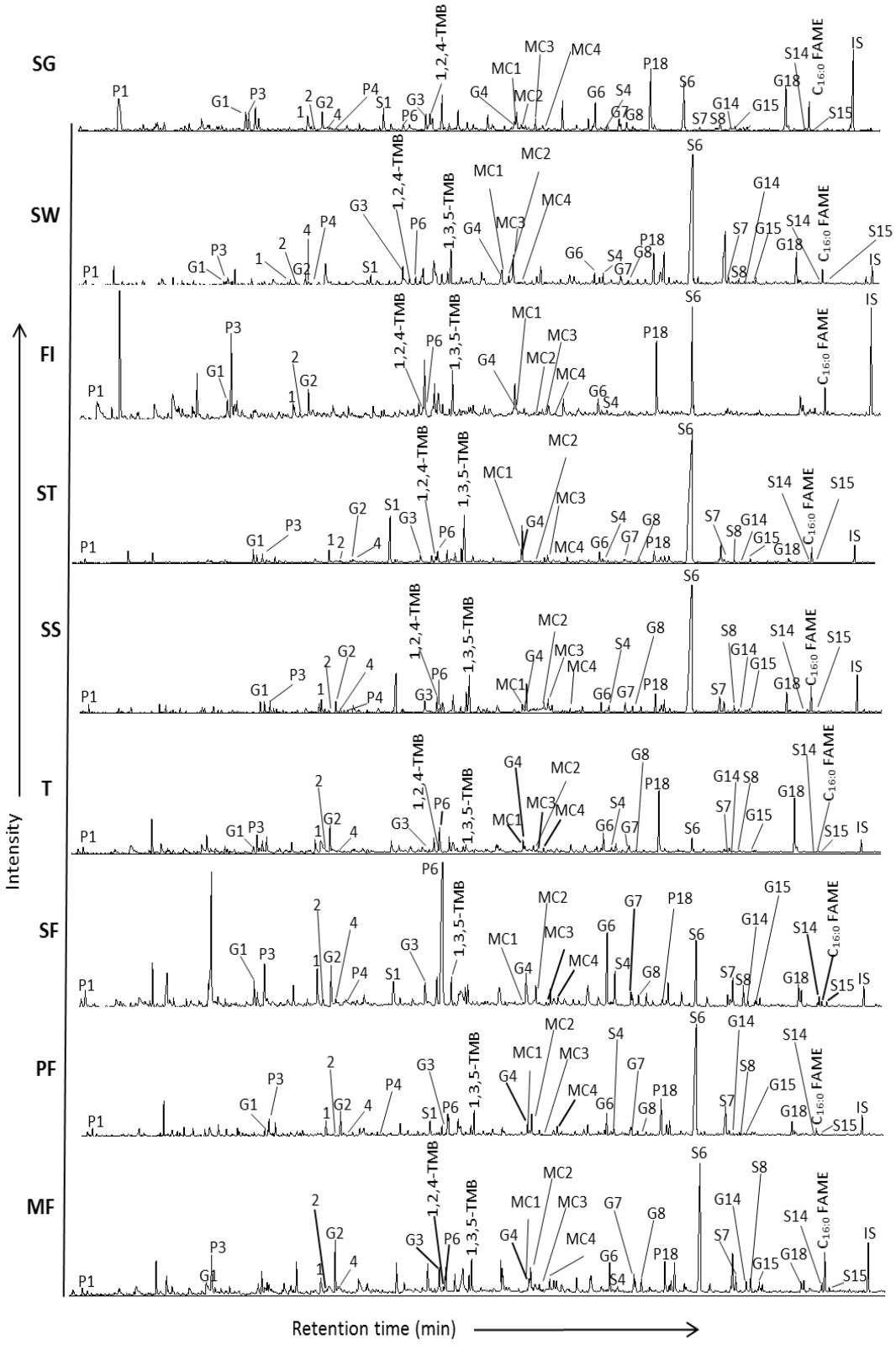

Figure F.2 Partial chromatogram for the total ion current (TIC) of thermochemolysis products from fresh shoot / leaf litter at each sub-environment across the transect (SG – MF).

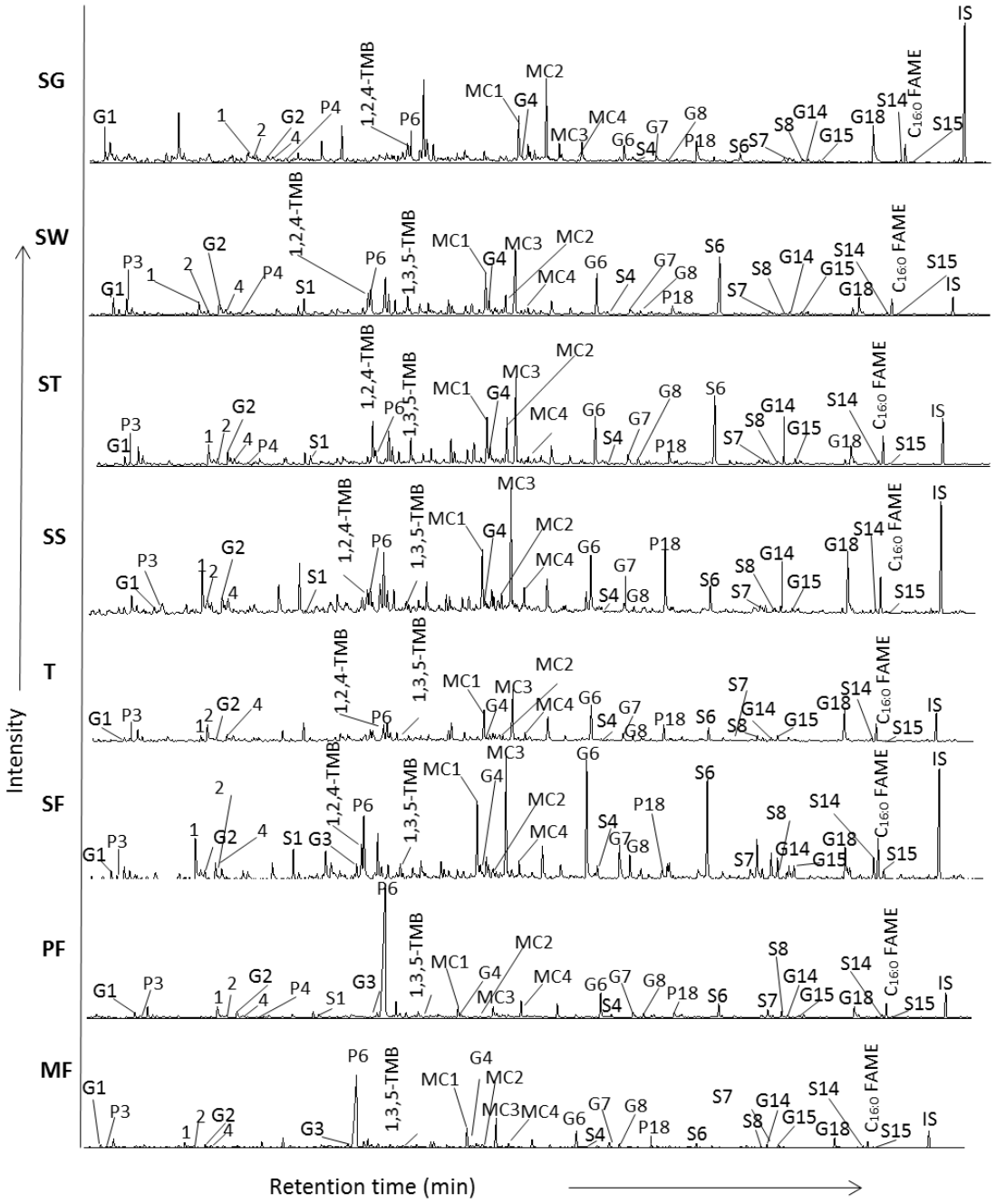

Figure F.3 Partial chromatogram for the total ion current (TIC) of thermochemolysis products from surface soils at each sub-environment across the transect (SG-MF).

Appendix G. Average values of the THM products

Table G.1 Averages values (of triplicates) in mg / 100 mg OC for THM products of wood litter, fresh shoot / leaf litter and surface soil samples

Site	G4		G6		G18		S4		S6	
Savannah grassland										
Wood litter	N/A		N/A		N/A		N/A		N/A	
Fresh shoots	0.020	(0.008)	0.014	(0.004)	0.054	(0.010)	0.011	(0.004)	0.005	(0.001)
0-6 cm soil	0.031	(0.011)	0.089	(0.033)	0.318	(0.143)	0.018	(0.008)	0.013	(0.003)
Savanah woodland										
Wood litter	0.293	(0.096)	0.133	(0.032)	0.073	(0.024)	0.581	(0.195)	0.156	(0.045)
Fresh shoots and leaf litter	0.019	(0.011)	0.018	(0.011)	0.120	(0.081)	0.036	(0.021)	0.009	(0.008)
0-6 cm soil	0.061	(0.016)	1.078	(0.121)	0.635	(0.093)	0.066	(0.014)	0.222	(0.060)
Forest Island										
Wood litter	0.158	(0.000)	0.161	(0.000)	0.062	(0.000)	0.135	(0.000)	0.129	(0.000)
Leaf litter	0.012	(0.004)	0.023	(0.005)	0.023	(0.005)	0.007	(0.001)	0.006	(0.005)
0-6 cm soil	0.000	(0.000)	0.031	(0.009)	0.017	(0.008)	0.000	(0.000)	0.005	(0.002)
Savannah tree										
Wood litter	0.238	(0.093)	0.132	(0.046)	0.039	(0.020)	0.331	(0.140)	0.146	(0.063)
Fresh shoots and leaf litter	0.004	(0.002)	0.009	(0.002)	0.025	(0.007)	0.021	(0.008)	0.010	(0.003)
0-6 cm soil	0.022	(0.005)	0.329	(0.020)	0.172	(0.019)	0.018	(0.003)	0.092	(0.012)
Savannah swamp										
Wood litter	0.270	(0.035)	0.143	(0.020)	0.230	(0.037)	0.208	(0.026)	0.035	(0.005)
Fresh shoots and leaf litter	0.002	(0.001)	0.011	(0.006)	0.094	(0.028)	0.004	(0.002)	0.008	(0.004)
0-6 cm soil	0.003	(0.002)	0.261	(0.068)	0.233	(0.025)	0.061	(0.036)	0.028	(0.003)

Site	G4		G6		G18		S4		S6	
Transition										
Wood litter	0.167	(0.022)	0.126	(0.020)	0.044	(0.004)	0.135	(0.012)	0.067	(0.008)
Leaf litter	0.006	(0.002)	0.025	(0.004)	0.011	(0.006)	0.012	(0.005)	0.016	(0.003)
0-6 cm soil	0.007	(0.001)	0.236	(0.027)	0.288	(0.048)	0.011	(0.002)	0.050	(0.005)
Swamp forest										
Wood litter	0.164	(0.030)	0.209	(0.033)	0.000	(0.000)	0.199	(0.052)	0.129	(0.015)
Leaf litter	0.038	(0.011)	0.101	(0.024)	0.042	(0.018)	0.052	(0.019)	0.141	(0.033)
0-6 cm soil	0.013	(0.006)	0.265	(0.088)	0.062	(0.021)	0.019	(0.008)	0.204	(0.080)
Petroplinthite hill forest										
Wood litter	0.202	(0.015)	0.141	(0.013)	0.064	(0.010)	0.183	(0.015)	0.127	(0.006)
Leaf litter	0.094	(0.053)	0.036	(0.014)	0.110	(0.044)	0.080	(0.052)	0.022	(0.011)
0-6 cm soil	0.080	(0.026)	0.592	(0.171)	0.398	(0.181)	0.097	(0.041)	0.283	(0.073)
Mixed tree spp. forest										
Wood litter	0.163	(0.043)	0.136	(0.033)	0.068	(0.024)	0.164	(0.057)	0.073	(0.025)
Leaf litter	0.009	(0.004)	0.020	(0.008)	0.011	(0.002)	0.007	(0.003)	0.016	(0.006)
0-6 cm soil	0.017	(0.005)	0.344	(0.094)	0.384	(0.080)	0.092	(0.047)	0.108	(0.018)

Site	P18		Gallic acid		CTP1		CTP2		CTP4	
Savannah grassland										
Wood litter	N/A		N/A		N/A		N/A		N/A	
Fresh shoots	0.084	(0.019)	0.097	(0.028)	0.004	(0.001)	0.0004	(0.0002)	0.001	(0.001)
0-6 cm soil	0.161	(0.059)	0.011	(0.004)	0.026	(0.014)	0.0003	(0.0003)	0.002	(0.001)
Savanah woodland										
Wood litter	0.000	(0.000)	2.133	(0.608)	0.370	(0.230)	0.014	(0.011)	0.082	(0.051)
Fresh shoots and leaf litter	0.233	(0.125)	3.058	(1.285)	0.017	(0.008)	0.001	(0.001)	0.005	(0.002)
0-6 cm soil	0.294	(0.058)	1.482	(0.222)	0.075	(0.022)	0.017	(0.003)	0.023	(0.004)
Forest Island										
Wood litter	0.018	(0.000)	0.011	(0.010)	0.291	(0.100)	0.016	(0.010)	0.046	(0.010)
Leaf litter	0.089	(0.011)	0.041	(0.033)	0.025	(0.003)	0.004	(0.001)	0.000	(0.000)
0-6 cm soil	0.000	(0.000)	0.002	(0.001)	0.075	(0.029)	0.013	(0.006)	0.004	(0.002)
Savannah tree										
Wood litter	0.000	(0.000)	2.039	(0.629)	0.050	(0.017)	0.003	(0.001)	0.009	(0.001)
Fresh shoots and leaf litter	0.083	(0.030)	2.288	(0.575)	0.006	(0.002)	0.001	(0.0002)	0.005	(0.003)
0-6 cm soil	0.109	(0.021)	0.470	(0.061)	0.023	(0.013)	0.004	(0.002)	0.004	(0.002)
Savannah swamp										
Wood litter	0.092	(0.016)	0.023	(0.003)	0.053	(0.002)	0.003	(0.000)	0.008	(0.001)
Fresh shoots and leaf litter	0.236	(0.115)	0.097	(0.049)	0.005	(0.002)	0.001	(0.0003)	0.001	(0.001)
0-6 cm soil	0.086	(0.052)	0.025	(0.003)	0.006	(0.001)	0.016	(0.002)	0.004	(0.003)

Site	P18		Gallic acid		CTP1		CTP2		CTP4	
Transition										
Wood litter	0.000	(0.000)	0.027	(0.003)	0.045	(0.016)	0.003	(0.001)	0.005	(0.001)
Leaf litter	0.251	(0.148)	0.032	(0.006)	0.013	(0.005)	0.001	(0.0003)	0.001	(0.000)
0-6 cm soil	0.103	(0.015)	0.026	(0.003)	0.019	(0.003)	0.002	(0.000)	0.005	(0.002)
Swamp forest										
Wood litter	0.047	(0.013)	0.357	(0.043)	0.302	(0.159)	0.029	(0.010)	0.043	(0.019)
Leaf litter	0.025	(0.008)	0.010	(0.002)	0.055	(0.022)	0.004	(0.002)	0.007	(0.002)
0-6 cm soil	0.008	(0.006)	0.016	(0.006)	0.033	(0.012)	0.003	(0.001)	0.007	(0.003)
Petroplinthite hill forest										
Wood litter	0.037	(0.005)	0.141	(0.006)	0.319	(0.039)	0.014	(0.004)	0.039	(0.010)
Leaf litter	0.281	(0.152)	1.187	(0.617)	0.031	(0.024)	0.002	(0.002)	0.006	(0.005)
0-6 cm soil	0.134	(0.047)	0.026	(0.007)	0.065	(0.022)	0.006	(0.002)	0.011	(0.004)
Mixed tree spp. forest										
Wood litter	0.015	(0.003)	0.015	(0.005)	0.132	(0.063)	0.0051	(0.002)	0.022	(0.015)
Leaf litter	0.052	(0.017)	0.268	(0.102)	0.004	(0.002)	0.0003	(0.0001)	0.0005	(0.0002)
0-6 cm soil	0.087	(0.016)	0.014	(0.002)	0.028	(0.007)	0.0022	(0.001)	0.000	(0.000)

Site	MC1		MC2		MC3		MC4	
Savannah grassland								
Wood litter	N/A		N/A		N/A		N/A	
Fresh shoots	0.0002	(0.0001)	0.0007	(0.0004)	0.0008	(0.0003)	0.003	(0.001)
0-6 cm soil	0.366	(0.183)	0.101	(0.040)	0.651	(0.258)	0.157	(0.072)
Savanah woodland								
Wood litter	0.0001	(0.0001)	0.0004	(0.0004)	0.0007	(0.0007)	0.002	(0.002)
Fresh shoots and leaf litter	0.001	(0.001)	0.0020	(0.0012)	0.0024	(0.0017)	0.005	(0.003)
0-6 cm soil	0.895	(0.105)	0.082	(0.013)	0.433	(0.074)	0.132	(0.008)
Forest Island								
Wood litter	0.000	(0.0000)	0.0003	(0.0000)	0.014	(0.010)	0.0004	(0.0000)
Leaf litter	0.005	(0.003)	0.0033	(0.0017)	0.012	(0.004)	0.0004	(0.0002)
0-6 cm soil	0.014	(0.005)	0.000	(0.000)	0.031	(0.004)	0.000	(0.000)
Savannah tree								
Wood litter	0.000	(0.000)	0.000	(0.000)	0.000	(0.000)	0.000	(0.000)
Fresh shoots and leaf litter	0.0002	(0.0002)	0.0010	(0.0005)	0.0008	(0.0004)	0.003	(0.001)
0-6 cm soil	0.331	(0.085)	0.090	(0.056)	0.224	(0.056)	0.049	(0.005)
Savannah swamp								
Wood litter	0.001	(0.0004)	0.0057	(0.0017)	0.003	(0.002)	0.013	(0.0071)
Fresh shoots and leaf litter	0.0003	(0.0002)	0.0019	(0.0013)	0.001	(0.000)	0.004	(0.003)
0-6 cm soil	0.046	(0.010)	0.008	(0.002)	0.118	(0.025)	0.019	(0.004)

Site	MC1		MC2		MC3		MC4	
Transition								
Wood litter	0.001	(0.0001)	0.0010	(0.0005)	0.001	(0.000)	0.006	(0.003)
Leaf litter	0.003	(0.002)	0.0021	(0.0002)	0.007	(0.001)	0.017	(0.002)
0-6 cm soil	0.224	(0.030)	0.086	(0.061)	0.252	(0.085)	0.040	(0.008)
Swamp forest								
Wood litter	0.0003	(0.0002)	0.00	(0.00)	0.0011	(0.0006)	0.006	(0.004)
Leaf litter	0.0003	(0.0001)	0.0007	(0.0004)	0.0012	(0.0005)	0.004	(0.002)
0-6 cm soil	0.009	(0.002)	0.064	(0.015)	0.156	(0.033)	0.020	(0.004)
Petroplinthite hill forest								
Wood litter	0.0003	(0.000)	0.0006	(0.0004)	0.0006	(0.0006)	0.006	(0.003)
Leaf litter	0.001	(0.001)	0.0008	(0.0006)	0.0030	(0.0028)	0.003	(0.003)
0-6 cm soil	0.177	(0.049)	0.003	(0.001)	0.106	(0.025)	0.011	(0.003)
Mixed tree spp. forest								
Wood litter	0.000	(0.000)	0.00	(0.00)	0.000	(0.000)	0.0007	(0.0006)
Leaf litter	0.0002	(0.0001)	0.0005	(0.0002)	0.0004	(0.0002)	0.002	(0.001)
0-6 cm soil	0.473	(0.067)	0.034	(0.008)	0.849	(0.081)	0.092	(0.010)

Site	1,2,4-TMB		1,3,5-TMB	
Savannah grassland				
Wood litter	N/A		N/A	
Fresh shoots	0.016	(0.005)	0.024	(0.007)
0-6 cm soil	0.080	(0.018)	0.000	(0.000)
Savanah woodland				
Wood litter	0.169	(0.114)	0.147	(0.041)
Fresh shoots and leaf litter	0.038	(0.021)	0.361	(0.191)
0-6 cm soil	0.449	(0.050)	0.322	(0.036)
Forest Island				
Wood litter	0.033	(0.010)	0.023	(0.010)
Leaf litter	0.034	(0.010)	0.005	(0.001)
0-6 cm soil	0.030	(0.008)	0.004	(0.002)
Savannah tree				
Wood litter	0.008	(0.008)	0.021	(0.011)
Fresh shoots and leaf litter	0.000	(0.000)	0.179	(0.043)
0-6 cm soil	0.298	(0.038)	0.165	(0.018)
Savannah swamp				
Wood litter	0.040	(0.024)	0.000	(0.000)
Fresh shoots and leaf litter	0.012	(0.006)	0.023	(0.015)
0-6 cm soil	0.081	(0.007)	0.292	(0.076)
Transition				
Wood litter	0.062	(0.018)	0.015	(0.001)
Leaf litter	0.013	(0.005)	0.017	(0.004)
0-6 cm soil	0.058	(0.009)	0.030	(0.007)
Swamp forest				
Wood litter	0.054	(0.020)	0.000	(0.000)
Leaf litter	0.000	(0.000)	0.059	(0.010)
0-6 cm soil	0.083	(0.020)	0.026	(0.009)
Petroplinthite hill forest				
Wood litter	0.065	(0.027)	0.100	(0.006)
Leaf litter	0.000	(0.000)	0.401	(0.329)
0-6 cm soil	0.000	(0.000)	0.167	(0.066)
Mixed tree spp. forest				
Wood litter	0.035	(0.004)	0.043	(0.002)
Leaf litter	0.000	(0.000)	0.026	(0.008)
0-6 cm soil	0.000	(0.000)	0.075	(0.009)

Glossary

$[Ad/Al]_{G/S}$	$[Acid / Aldehyde]_{Guaiacyl / Syringl}$ ratio
1,2,3-TMB	1,2,3-trimethoxybenzene
1,2,4-TMB	1,2,4-trimethoxybenzene
1,3,5-TMB	1,3,5-trimethoxybenzene
A	A horizon: mineral horizon mixed with organic matter
Ah	As A horizon but with greater content of organic matter giving it a darker colour
Apx	As A horizon but with pisoplinthic material
ASE	Accelerated solvent extraction
a.s.l.	Above sea level
B	Mineral sub-horizon
Ba	As B horizon but with albic properties (a)
Bpx	As B horizon with pisoplinthic material (px)
Bv	As B horizon but with plinthic material (v)
Bvg	As B horizon but with plinthic material (v) and gleying (g)
C	Carbon
C (referring to lignin)	Cinnamyl
C/G	Cinnamyl / Guaiacyl ratio
CH_4	Methane
CO_2	Carbon dioxide
CsCl	Caesium chloride
CT	Condensed tannins
CTP1	Cellulose Thermochemolysis Product 1
CTP2	Cellulose Thermochemolysis Product 2

CTP4	Cellulose Thermochemolysis Product 4
CuO	Cupric oxide oxidation
CV	Coefficient of variance
D_b	Bulk density
DCM	Dichloromethane
DOM	Dissolved organic matter
Eag	Eluviated albic horizon (Ea) with evidence of gleying (g)
ENSO	El Nino Southern Oscillation
FAO	Food and Agriculture Organisation of the United Nations
FI	Forest island
G	Guaiacyl
G1	1,2-dimethoxybenzene
G14	*threo/ethryo* 1-(3,4-dimethoxyphenyl)-1,2,3-trimethoxypropane
G15	*threo/ethryo* 1-(3,4-dimethoxyphenyl)-1,2,3-trimethoxypropane
G18	*trans* 3-(3,4-dimethoxyphenyl)-3-propanoic acid methyl ester
G2	3,4-dimethoxytoluene
G3	3,4-dimethoxybenzeneethylene
G4	3,4-dimethoxybenzaldehyde
G6	3,4-dimethoxybenzoic acid methyl ester
G7	cis 1-(3,4-dimethoxyphenyl)-2-methoxyethylene
G8	*trans* 1-(3,4-dimethoxyphenyl)-2-methoxyethylene
GC	Gas chromatography
GHG	Greenhouse gases
GIS	Geographical information system
GLM	General linear model
GLSC	Guyana lands and surveys commission
GPS	Geographical positioning system

GtC	Gigatons of carbon
H	Hydrogen
HT	Hydrolysable tannins
IPSS	Intergovernmental Panel on Climate Change
IS	Internal standard
ISRIC	International Soil Reference and Information Centre
ITCZ	Inter-tropical convergence zone
IUSS	International Union of Soil Sceinces
KCL	Potassium chloirde
KI	Potassium iodide
KPF	Kokret palm forest
LCDS	Low carbon development strategy
LiCl	Lithium chloride
MC1	Methylated carbohydrate product 1 (3-deoxy-4,5,6-tri-*O*-methylgluconic acid, methyl ester)
MC2	Methylated carbohydrate product 2 (Tetra-*O*-methyl-3-deoxy-D-*arabino*-hexanoic acid, methyl ester)
MC3	Methylated carbohydrate product 3 (3-deoxy-4,5,6-tri-*O*-methylmannonic acid, methyl ester)
MC4	Methylated carbohydrate product 4 (Tetra-*O*-methyl-3-deoxy-D-*ribo*-hexanoic acid, methyl ester)
MeOH	Methanol
MF1	Mixed species forest 1
MF2	Mixed species forest 2
mg 100 mg OC	Milligrams per 100 milligrams of organic carbon

MRF	Mora forest
NSCD	National soil characterisation database
O_2	Oxygen
OC	Organic carbon
Oh	Horizon dominated by organic material, consisting of undecomposed or partially decomposed litter, (e.g. leaves, twigs, moss) which has accumulated on the surface
OM	Organic matter
P	P-hydroxyphenyl
P1	methoxybenzene
P18	*trans* 3-(-4-methoxyphenyl)-3 propenoic acid methyl ester
P3	4-methoxybenzeneethylene
P6	4-methoxybenzoic acid methyl ester
$PbCl_2$	Lead chloride
PCR	Polymer chain reaction
PF	Pisoplinthic forest
PgC	Petragram of carbon
PSG	Pisoplinthic savannah grassland
Py-GC/MS	Pyrolysis-gas chromatography/mass spectrometry
REDD+	Reducing Emissions from Deforestation and Forest Degradation
S	Syringyl
S/G	Syringyl / Guaiacyl ratio

S1	1,2,3-trimethoxybenzene
S14	*threo/ethryo* 1-(3,4,5-trimethoxyphenyl)-1,2,3-trimethoxybenzene
S15	*threo/ethryo* 1-(3,4,5-trimethoxyphenyl)-1,2,3-trimethoxybenzene
S4	3,4,5-trimethoxybenzaldehyde
S6	3,4,5-trimethoxybenoic acid methyl ester
S7	*cis* 1-(3,4,5-trimethoxyphenyl)-2-methoxyethylene
S8	*trans* 1-(3,4,5-trimethoxyphenyl)-2-methoxyethylene
SE	Standard error
SF1	Swamp forest 1
SF2	Swamp forest 2
SG1	Savannah grassland 1
SG2	Savannah grassland 2
SIC	Soil inorganic carbon
SOC	Soil organic carbon
SOM	Soil organic matter
SOTER-LAC	Soil and terrain project for Latin America and the Caribbean
SS	Savannah swamp
ST1	Savannah tree 1
ST2	Savannah tree 2
SW1	Savannah woodland 1
SW2	Savannah woodland 2
t C ha^{-1}	Tonnes of carbon per hectare
T1	Transition zone 1
T2	Transition zone 2
TBAH	Tetrabutyl ammonium hydroxide
TEAH	Tetraethyl ammonium hydroxide

THM	Thermally assisted hydrolysis and methylation
TIC	Total ion current
TMAH	Tetramethyl ammonium hydroxide
TOC	Total organic carbon
UNFCCC	United Nations framework for the convention on climate change
V	Vanillyl
V_s	Volume of soil
W_d	Weight of oven dry soil
WISE	World inventory of soil emission potential database
XRD	X-ray diffraction
θ	Volumetric water content
Λ	Lambda (sum of main lignin phenols)