Modern pollen-vegetation relationships in Ghana, tropical West Africa

Thesis submitted for the degree of Doctor of Philosophy

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Abstract

Understanding the pollen assemblages produced by modern tropical vegetation, and improving the taxonomic resolution of pollen identifications are vital in generating high quality interpretations of fossil pollen assemblages. This thesis explores the modern pollen-vegetation relationships of a forest-savannah transitional mosaic, a moist semi-deciduous forest and a wet evergreen rainforest in Ghana, using artificial pollen traps. It also tests the ability of Fourier Transform Infra-red Spectroscopy (FTIR) to identify Poaceae pollen to below family level. The work presented here will help to inform interpretations of the fossil pollen record of Lake Bosumtwi, Ghana, a million year old meteorite impact crater-lake. Pollen deposited in the lake has tracked vegetation change, including expansions and contractions of grass dominated landscapes evidenced by very high proportions of Poaceae pollen.

Characteristic taxa from the forest-savannah mosaic landscape were Poaceae (up to 61% of pollen sum) and Melastomataceae/Combretaceae (up to 73%). The moist semi-deciduous site was characterised by *Celtis* (up to 89%) and *Triplochiton* (up to 20%), and the wet evergreen rainforest was characterised by *Cynometra, Drypetes*, *Vitex* and *Homalium* (each around 10%). It was found that, using FTIR spectroscopy, it is possible to achieve an 80% classification success rate of pollen to sub-family level within the Poaceae. These results suggest that the threshold of 55% Poaceae pollen previously used to mark the transition between grass dominated and forested landscapes in the Lake Bosumtwi record may be too high, and should be closer to 40%. It is also concluded that the assemblages recovered from interglacial periods of the Lake Bosumtwi record may represent vegetation that was less similar to wet rainforest and closer to forest-savannah mosaic.

This thesis should inform future studies of fossil pollen assemblages recovered from West Africa, and further work on Lake Bosumtwi, to enable higher resolution interpretations of fossil pollen assemblages.

Acknowledgements

This thesis is dedicated to my Nan, whose wild and beautiful garden was where my love of plants first grew, and whose attention and curiosity towards the natural world inspired my own.

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Finally, thanks must go to my family for supporting me in my seemingly never ending pursuit of degrees. I promise that this really is the last one.

LES ÉTIQUETTES JAUNES

I picked up a leaf
today from the sidewalk.
This seems childish.

Leaf! you are so big!
How can you change your
color, then just fall!

As if there were no
such thing as integrity!

You are too relaxed
to answer me. I am too
frightened to insist.

Leaf! don't be neurotic
like the small chameleon.

Frank O'Hara, Meditations in an Emergency, 1957

Contents

List of Tables

List of Figures

Appendices (on memory stick supplied with thesis)

1   Chapter 1: Introduction

1.1   Overarching Rationale

Interpretations of fossil pollen assemblages are important because they contribute to our understanding of how vegetation responds to climate change over time (Maley & Brenac 1998). Anthropogenic emissions are currently causing widespread changes across the globe, with accompanying vegetation shifts (Niang et al. 2014). If past responses to climate change can be understood, this can be used to help inform predictions as to which areas may be most susceptible to changes in the coming decades and to parameterise models that are used in making these predictions (Jackson & Overpeck 2000; Goldewijk & Verburg 2013). It is particularly important to generate data and improve interpretations of fossil records from the tropics, which are important climatically and have been historically less well studied than temperate regions (Bush et al. 2011).

The relationships between modern vegetation types and the pollen which they produce are a crucial component of the evidence which informs interpretations of fossil pollen assemblages (Von Post 1916; Davis 1963; Fægri et al. 1989). A poor understanding of modern pollen-vegetation relationships may distort interpretations of the fossil record, due to factors such as which taxa over- and under- produce pollen relative to their occurrence (Molina et al. 1996; Molina et al. 1996; Bush & Rivera 2001), how distance from source affects the proportions of pollen taxa present in the record (Jacobson & Bradshaw 1981; Jackson & Lyford 1999), and whether indicator taxa can really be used as such (Bush 2002; Herzschuh & Birks 2010).

1.2   Overall aim

This thesis aims to generate the first modern pollen-vegetation study from Ghana and to investigate the possibility of improving the taxonomic resolution of Poaceae pollen. These areas of study are important as they will contribute to a better understanding of fossil pollen records, particularly that of Lake Bosumtwi, which is one of the longest terrestrial pollen records in Africa and is key to understanding vegetation change in the region over the past million years.

1.3   Background

The scientific approach of this thesis is based on forming a better understanding of modern pollen, both in terms of its chemistry and modern pollen assemblages captured from different vegetation types, in order to understand modern pollen-vegetation relationships. Here,

general introductions to the main issues addressed in this thesis; namely, pollen-vegetation relationships and pollen taxonomy, are presented. More specific background on forest-savannah transitions may be found in Sections 2.1-2.3, the identification of cryptic pollen taxa in Section 3.2, pollen deposition in Section 4.2, and the interpretation of fossil record in Section 5.2.

1.3.1   Pollen-vegetation relationships

The relationships between plants and the amount of their pollen that is captured and preserved in the fossil record are complex and depend upon a range of factors including pollen grain structure (Duffin & Bunting 2008; Borrell 2012) , the limits of pollen taxonomy (Bush 2002; Mander & Punyasena 2014), floral structure (Friedman & Harder 2004) , pollination syndrome (Bawa et al. 1985; Bush & Rivera 2001), pollen production (Theuerkauf et al. 2013), source area (Davis 2000), basin size (Jacobson & Bradshaw 1981; Prentice 1985), depositional environment (Wilmshurst & McGlone 2005), and taphonomy (Havinga 1967; Campbell 1999). It is therefore difficult for palaeoecologists, having recovered pollen from sediments, to accurately interpret how these assemblages represent their parent vegetation types (Goring et al. 2013).

The amount and nature of pollen produced by plants is dependent upon their pollination strategy, be this entomophilous (insect pollinated), zoophilous (animal pollinated) or anemophilous (wind-pollinated). In the tropics, less than 1% of tree taxa are anemophilous, with the majority being entomophilous or zoophilous (Bawa 1990). The amount of pollen produced by flowers varies dependent on their pollination syndrome, flower shape and size, anther size, whether they are monoecious, dioecious or hermaphrodite, and whether they are primarily out-crossing or selfing (Reddi & Reddi 1986; Bullock 1994; Molina et al. 1996). The majority of tropical trees are out-crossing, with high levels of self-incompatibility, factors which are linked to long-distance dispersal of pollen by pollinators such as bees being relatively common (Ward et al. 2005). Entomophilous trees are often under-represented in modern pollen rain studies due to their producing less pollen per flower, and that pollen potentially being adapted to adhere to insects, as opposed to being released into the air (Lézine & Edorh 1991; Linskens 1996; Bush & Rivera 1998).

Although it would theoretically be possible to predict the pollen assemblage produced by a particular vegetation type using the known characteristics of its constituent species, in reality, and particularly in the tropics, this is not yet possible. Factors including high diversity (Gentry 1992; Givnish 1999), a lack of vegetation surveys that capture all plants in an ecosystem, a lack of knowledge about the reproductive strategy and pollination ecology of the species in

question, and a paucity of pollen atlases and reference collections for tropical regions all confound efforts to predict pollen-vegetation relationships. It is, therefore, not yet possible to use these biological factors to accurately re-construct past vegetation based on fossil pollen assemblages. Modern pollen-vegetation studies can help to calibrate the fossil record and to identify potentially characteristic pollen assemblages.

Work has been carried out on a continent-wide scale (Vincens et al. 2000; Gajewski et al. 2002; Watrin et al. 2007) which has found that there are comparisons to be drawn between pollen, climate and vegetation in Africa. Gajewski et al. (2002) performed a meta-analysis of many different modern pollen studies, which demonstrated that pollen assemblages can predict climatic factors such as temperature and precipitation relatively well, even when different sampling media are used. Watrin et al. (2007) characterised a range of different taxa to assess their usefulness as indicators of climate and found that pollination syndrome and pollen taxonomy (how many botanical species a single pollen taxon represents) strongly affected the usefulness of taxa. Vincens et al. (2000) demonstrated that the transition between forest and savannah in Cameroon is recorded well by modern pollen samples (collected from soil), although when pollen assemblages are used to identify vegetation zones these vary from when botanical inventories are used, demonstrating some discrepancy between pollen and vegetation. Lézine & Edorh (1991) demonstrated that in Togo, although some ecosystems such as *Isoberlinia* woodland could be distinguished from their pollen spectra, others were swamped by non-arboreal taxa such as Poaceae, meaning that it was difficult to recover a regional signal from the soil samples. There is, clearly, not one simple to answer to the question of how pollen assemblages represent their parent vegetation types, regardless of sampling medium or geographical location.

1.3.2   Pollen taxonomy

Many pollen grains are not identifiable to below family or genus level based upon morphological observations made using a light microscope, which is the standard method of counting pollen (Fægri et al. 1989). A lack of taxonomic resolution in the fossil record can bring about problems when trying to reconstruct past vegetation types, because certain morphological pollen taxa may represent many different botanical taxa, which may occur in a range of different environmental conditions. One of the best illustrations of this problem is the Poaceae, or grass family, which has monoporate grains that are not easily identifiable beyond family level using light microscopy (Beug 2004).

The Poaceae is a diverse family, with members growing in a wide range of environments, from dry savannah to marshes and wetlands (Grass Phylogeny Working Group II 2012) . Poaceae

grains have often been interpreted as indicators of open and arid environments (van der Hammen & Absy 1994; Miller & Gosling 2014), although their presence in other, wetter environments has been acknowledged as an issue when interpreting fossil records (Meneses et al. 2015). The problem of how to interpret Poaceae pollen has been recognised by palaeoecologists working in the tropics (Bush 2002; Absy et al. 2014) and work-arounds have been proposed, such as removing wetland taxa (including Poaceae) from vegetation reconstructions (Crausbay & Hotchkiss 2012).

The walls of spores and pollen grains are composed of a highly decay-resistant biopolymer called sporopollenin, the chemical structure of which has been shown to remain stable over long time-periods and under extreme conditions (Ariizumi and Toriyama 2011; Fraser et al. 2012), which enables it to effectively protect the male gametes it transports. The chemical composition of sporopollenin is still not conclusively known, as it is a co-polymer composed of different chemical components, including aromatic and aliphatic compounds (Watson et al. 2007; Fraser et al. 2012). The mechanism by which it forms is also not fully understood, although developments have been made into understanding the genetics behind this (Ariizumi & Toriyama 2011).

There are various ways that cryptic pollen taxa can be identified, both within the Poaceae and other plant groups. These include:

- Detailed morphological observations, including measurements of annulus, whole grain size and surface patterning (Skvarla et al. 2003; Beug 2004; Joly et al. 2007; Schüler & Behling 2010). This technique is complicated by large over-laps in morphological characteristics between taxa, and is not practical when analysing hundreds of grains.
- Scanning Electron Microscopy (SEM) to observe differences in surface patterning between taxa (Andersen & Bertelsen 1972; Mander et al. 2013). This technique allows detailed observations of the surfaces of small numbers of grains to be observed, but requires specialised preparation and may not be suitable for analysing hundreds of grains.
- Transmission Electron Microscopy (TEM) to observe differences in ultrastructure of pollen walls between taxa (Peltre et al. 1987). TEM requires samples to be stained and sectioned before analysis, which is time consuming and not viable for large scale analyses.
- Confocal microscopy may be able to better resolve details of surface patterning in different taxa (Salih et al. 1997; Sivaguru et al. 2012). Although this technique is

promising, it requires considerable expertise and knowledge of detailed grass pollen morphology.

- Raman microspectroscopy, to use chemical differences between the composition of sporopollenin to identify taxa (Bağcıoğlu et al. 2015).

- Fourier Transform Infra-red Spectroscopy (FTIR) spectra of sporopollenin in pollen walls can be used to identify pollen grains to genus or species level (Pappas et al. 2003; Gottardini et al. 2007; Dell'Anna et al. 2009; Zimmermann 2010; Mularczyk-Oliwa et al. 2012; Parodi et al. 2013).

The majority of pollen counting work for palaeoecological reconstruction is undertaken using light microscopy, whilst counting hundreds of grains for each sample. Any technique that would be useful in distinguishing between taxa should, therefore, be able to be applied with minimum expense and preparation. It should also be feasible for use on single grains, not bulk samples and would also, ideally, be relatively fast.

FTIR meets these criteria as the requirements for sample preparation are minimal; pollen from standard palaeoecological pollen preparations can be used. Modern FTIR spectrometers can also be programmed to automatically scan slides, thereby freeing the user from having to individually locate and scan different pollen grains. Individual grains (down to around 10 µm in diameter) can be used to generate spectra. The other techniques outlined above often require extensive (and expensive) sample preparation or time intensive analysis.

1.4   Study Region

The understanding of tropical African ecosystems is of utmost importance because the continent is facing challenges related to population growth, disease and agriculture, all of which can be compounded by climate change (Niang et al. 2014). Palaeoecological studies have been carried out in tropical West Africa to investigate palaeo-precipitation, temperature, and accompanying vegetation type shifts (Lézine & Hooghiemstra 1990; Miller & Gosling 2014). Here, the main features of the study region are outlined, at regional, local, and plot level.

1.4.1   Tropical West Africa

For the purposes of this thesis, the term tropical West Africa is used to encompass the region of Africa stretching from Senegal to Togo, in which the Upper Guinean forests are located. The region supports almost 90 million people and has diverse politics, history and religion, but is

susceptible to the effects of anthropogenic climate change, in terms of crop production, water availability and the spread of disease (Githeko et al. 2000; Paeth et al. 2008). High rural populations of 50% or more in many tropical West African countries (World Bank) mean that the effects of climate change in the region are particularly profound. A better understanding of climate change and the types of challenges it will bring to the region would allow countries to anticipate, plan for and mitigate against these challenges and their impacts on livelihoods and quality of life.

1.4.1.1 Vegetation patterns
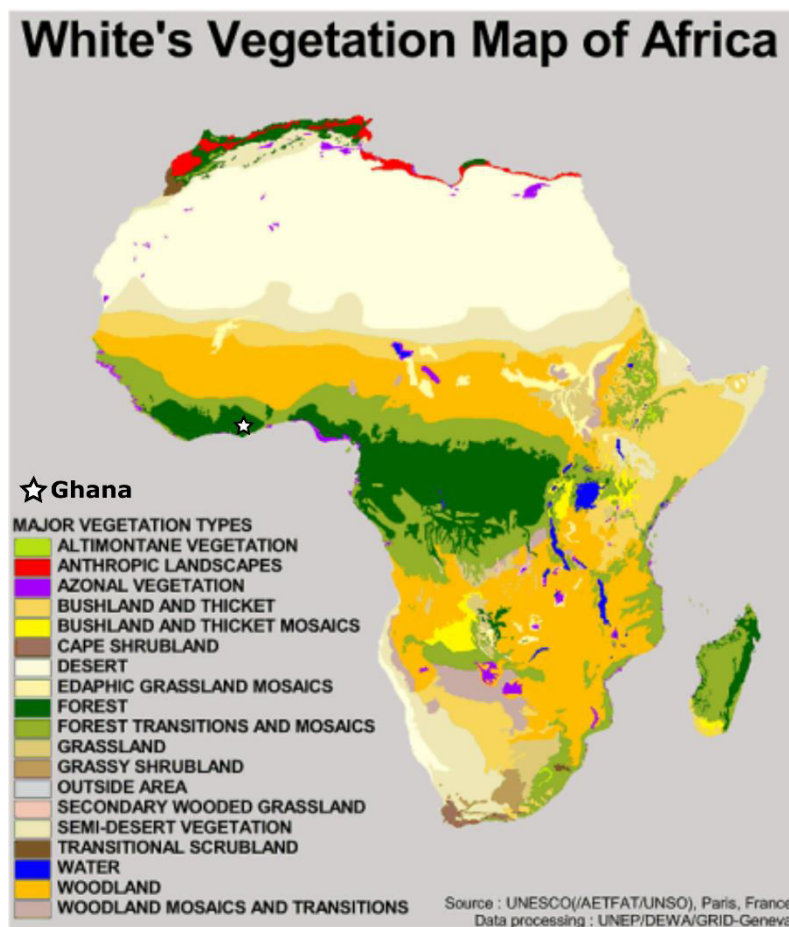
Figure 1.1: Simplified White's vegetation map of Africa.
Adapted from UNESCO, via UNEP (http://www.grid.unep.ch)

Tropical West Africa is an area of global importance for biodiversity, with 9,000 plant species of which 2,250 are endemic and many are threatened or rare (Myers et al. 2000; Poorter et al. 2004). The vegetation in tropical West Africa ranges from very wet evergreen rainforest nearer to the coast, to savannah and grassland ecosystems further north. The forests of tropical West Africa possess many endemic taxa; families such as the Acanthaceae, Anacardiaceae, Euphorbiaceae, Fabaceae, Rhizophoraceae, Rubiaceae, Sapindaceae and Zingiberaceae contain many of the endemics (Poorter et al. 2004).

The primary factor controlling vegetation cover in tropical West Africa is rainfall; there is a gradient of decreasing rainfall from South to North, which corresponds with the transition from forest to savannah and grasslands (Swaine 1992; Maharjan et al. 2011; Lehmann et al. 2011). In addition to rainfall, factors such as soil type, fire and herbivory are also important drivers of vegetation cover (Bongers et al. 2004; Azihou et al. 2013; Vaughn et al. 2015).

Soil type is an important factor in controlling vegetation cover due to the availability of nutrients and water. Soils of tropical West Africa are typically old, easily leached, acidic and have low cation availability (0-2 cmol/kg) (Jones et al. 2013). Nitrogen and phosphorus are key factors in determining the structure of forests; a positive association between above-ground biomass and clay rich soils, along with a negative correlation between C:N ratio and biomass have been observed (Lewis 2013). Nitrogen has been shown to be lower in savannah ecosystems, potentially acting as the limiting factor on biomass in those systems, whereas in forests, nitrogen content has been shown to be higher and phosphorus is potentially the limiting nutrient (Sugihara et al. 2014; Sugihara 2017). There is also evidence that, especially at the boundary between forest and savannah landscapes, when the two states exist on broadly similar soils, nitrogen and phosphorus soil content may be a result of, not a driver of vegetation patterns, which are then predominantly controlled by other factors such as fire (Gray & Bond 2015).

Fire is a particularly important factor in maintaining open, grass-dominated ecosystems (Stebbing 1937; Swaine et al. 1992). Fires, usually anthropogenic, may maintain savannah-like ecosystems in the presence of climatic conditions that would otherwise result in forest growth (rainfall of > 650 mm year^{-1}) (Maurin et al. 2014). The roles of grass and woody biomass in determining the intensity and effects of fires are intertwined; more grass biomass leads to higher intensity fires, which in turn suppress woody vegetation (Sankaran et al. 2005). Higher woody biomass results in less intense fires, as larger trees are less likely to succumb to burning, which therefore predominantly affects smaller trees and saplings (Higgins et al. 2000). The timing and frequency of fires are also important, with dry season fires affecting woody biomass more than wet season fires, and more frequent fires having a larger negative impact on woody vegetation than less frequent burns (Trapnell 1959; Smit et al. 2010).

Herbivory plays an important role in determining vegetation cover. Browsers (which eat leaves, green shoots and bark) and grazers (which eat ground level vegetation) affect the amount of biomass present in ecosystems as grass and woody cover, which can then affect the likelihood and intensity of fires (Van Langevelde et al. 2003). Herbivores fertilise soils, stimulating grass growth, which outcompetes sapling growth and can therefore supress woody

cover (van der Waal et al. 2011). Trampling and destruction of vegetation are also impacts of herbivores, particularly of larger animals such as elephants, which knock down larger trees and thus help to maintain the woody-grass balance in savannah ecosystems (McNaughton et al. 1988)

1.4.1.2   Climate

The climate of tropical West Africa is humid (between 70% in the dry season and 90% in the rainy season) and warm (average monthly temperature vary between 24-28°C) (Poorter et al. 2004). Weather is strongly influenced by the position of the sun throughout the year and its subsequent effect on rainfall and winds via the Inter-Tropical Convergence Zone (ITCZ), tropical rainbelt (located around 10° south of the ITCZ) and West African Monsoon (WAM). In the first half of the year, the ITCZ moves northwards, resulting in wet oceanic winds blowing from the Atlantic over the land, bringing heavy rainfall to the region (the WAM). In the latter half of the year, the ITCZ moves southwards, which may bring a second wet season to southern regions, but which is followed by dry, Harmattan winds blowing southwards from the Sahara. Sea Surface temperatures over the Atlantic, and pressure patterns arising from these drive the speeds and locations of the Tropical Easterly Jet and the Atlantic Westerly Jet, which have a large impact upon the amount of rainfall delivered to tropical West Africa (Nicholson 2009). The dynamics of this system are highly variable from year to year, and much of the region has experienced long periods of drought over the past 20 years (Cornforth 2012).

1.4.1.3   Past vegetation and climate change

Climate fluctuations in tropical West Africa have seen conditions in the region vary widely in terms of temperature, CO_2 levels, and precipitation over the Quaternary (past ca. 2.6 million years) (Lézine & Hooghiemstra 1990; Hooghiemstra et al. 2006; Shanahan et al. 2012). During glacial intervals, climate tended to be cooler and drier than in interglacials, and corresponding vegetation shifts occurred, with savannah regions expanding south during glacials, and rainforest ecosystems expanding north during interglacials (Dupont 2011; Miller & Gosling 2014; Miller et al. 2016). Precessional forcing, in particular, has been shown to influence the latitude of the ITCZ and the variability of monsoons, which control the timing and amount of precipitation delivered to the tropics (Clement et al. 2004; Braconnot et al. 2008; Gosling & Holden 2011). The interpretation of climate changes from pollen assemblages can be improved and independently verified by also considering other proxies of climatic change, such as nitrogen isotopes, carbon isotopes and palaeo lake levels (Brodie et al. 2011; Armitage et al. 2015).

1.4.1.4   The future of tropical West African vegetation

Projections of future climate change for tropical West Africa are not currently able to resolve whether precipitation will increase or decrease, but there are indications that dry season rain deficits will increase in frequency, and that extreme weather events will increase in frequency and severity (Roehrig et al. 2013; James et al. 2013; Niang et al. 2014). If precipitation decreases, this may reduce the extent of tropical West African rainforests, as they currently exist at the lower end of the range of precipitation levels at which rainforests are viable (Zelazowski et al. 2011). It is also possible, however, that increases in atmospheric CO_2 concentrations may bring about higher water use efficiency, although the details and magnitude of this effect are debated (Medeiros & Ward 2013; Smith et al. 2014; Shanahan et al. 2016; Becklin et al. 2017). Temperatures are also expected to rise 1-1.5°C by 2099, a factor that will put pressure on ecosystems and bring about vegetation change (Intergovernmental Panel on Climate Change 2014).

1.4.1.5   Anthropogenic influence

Anthropogenic influence on tropical West African vegetation has a long history, as humans have lived in the region in permanent settlements for at least 3,000 years and have existed in West Africa more widely for 20-30,000 years (Salas et al. 2002; Soares et al. 2012). Humans have affected landscapes primarily through burning, which could help to maintain savannahs and prevent tree-encroachment, and also through shifting cultivation, which is the practice of clearing a small patch of forested land, farming it for a few years and then moving on to a new area (Ickowitz 2006). Anthropogenic activity is likely to have interacted with climatic drivers of vegetation change in the past, and will continue to do so in the future, making the two difficult to examine separately (Herrmann et al. 2005; Hoscilo et al. 2015). This is particularly true of African forests, as they have been shown to be relatively more resilient to disturbance and climate fluctuations than their Neotropical and Asian counterparts. The resilience demonstrated by African rainforests is potentially due to the region experiencing larger climatic variability in the past, and due to the presence of humans in Africa for much longer than in other tropical forests (Fauset et al. 2012; Willis et al. 2013).

1.4.2   Ghana

Ghana is a West African country, whose capital is Accra and whose population was 28.3 million in 2016 (UN estimate). The climate of Ghana is tropical, but varies both longitudinally and latitudinally, with decreasing precipitation from both North to South and West to East (Figure 1.2), due to the effect of upwelling off its Atlantic coast interacting with air currents and the shape of the coastline (Poorter et al. 2004). The south of the country experiences two dry

seasons; one from December-March, and one in July and August, whereas more northern areas experience one wet season, from July – September, with the rest of the year being relatively dry (Nkrumah et al. 2014). Yearly rainfall varies from around 2,200 mm year^{-1} in the south-west of the country, to 1000 mm year^{-1} in the northern savannah regions, to 700 mm year^{-1} in the south-eastern coastal savannah (Figure 1.2) (Asante & Amuakwa-Mensah 2014). Sea surface temperatures in the Atlantic have a strong influence over the amount and timing of rainfall received by Ghana, especially in the south (Opoku-Ankomah & Cordery 1994). The average monthly temperature is never below 25 °C, with highs of 40 °C, and lows of 18 °C, with higher temperatures generally occurring in the north of the country (Asante & Amuakwa-Mensah 2014). The soils of Ghana are generally of poor quality and low organic content, and have suffered from over-farming and unsustainable farming practices (Bumb 2001).
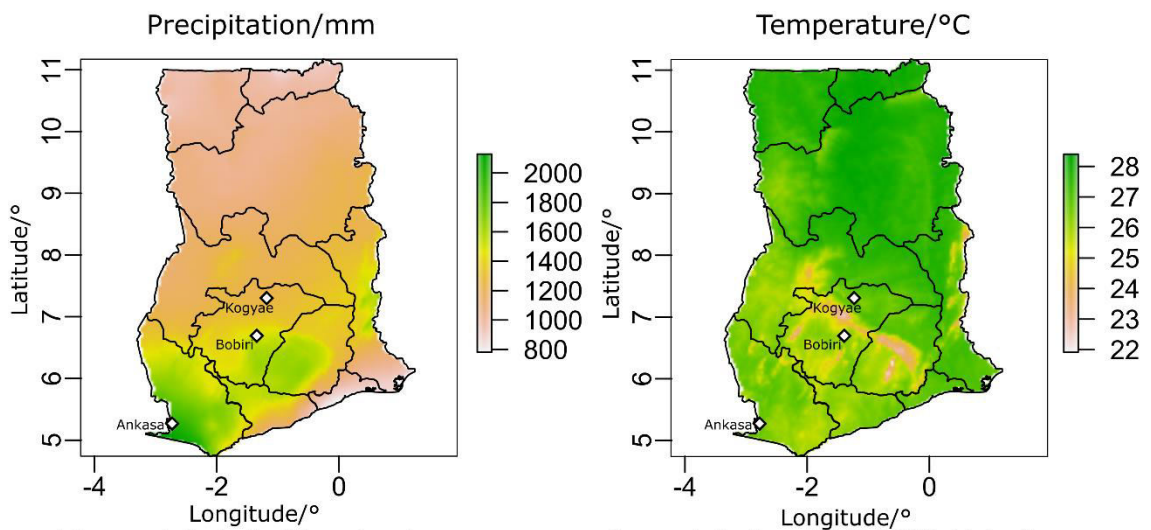
Figure 1.2: Left: Map showing average yearly precipitation 1970-2000 (data from WorldClim). Right: Map of Ghana showing average yearly temperature (data from WorldClim)

Ghana was chosen as the site for this study as it possesses of one of the few long term, terrestrial sediment records of the Quaternary in Africa (Lake Bosumtwi, see section 1.4.3), and yet no modern pollen-vegetation relationship studies have been published from the country. Modern pollen studies have been conducted in neighbouring Togo, Cote d'Ivoire and Burkina Faso (Lézine & Edorh 1991; Lézine et al. 2009), and so there is a clear gap in the literature for Ghana, which this thesis aims to address. Ghana also lies at an important geographic location climatically. It exists on the gradient between wet and dry conditions that characterises areas to the north and south of the tropical rainfall belt, and is therefore sensitive to changes in precipitation and extreme weather events brought about by climate change (Gonzalez et al. 2010). This sensitivity to change means it is important to understand the interaction between climate and vegetation at this geographical location.

1.4.3   Lake Bosumtwi

The Bosumtwi crater is the largest known young (1.07 million years old) impact crater in the world, and the lake that has formed within it, Lake Bosumtwi (Figure 1.3), has a diameter of 8 km, a surface area of 52 km^2, and a maximum depth of 78 m (Koeberl et al. 2007). The sediments in the lake have accumulated from run off from the surrounding hills, and the lake is hydrologically isolated from the water table, with no regular inflow or outflow besides rainfall and evaporation (Shanahan et al. 2006).

Figure 1.3: Lake Bosumtwi, October 2014. Lake Bosumtwi is at 6°30'13 "N 1°24'39 "W

Lake Bosumtwi was cored initially in 1976, recovering a 27,500 year record which was at the time the longest Holocene terrestrial record in the region (Talbot et al. 1984). Another coring project was undertaken in 2004, which recovered the long cores that have given rise to extensive palaeoclimate and palaeovegetation reconstructions for the region (Shanahan et al. 2006; Shanahan et al. 2009; Shanahan et al. 2012; Miller & Gosling 2014; Shanahan et al. 2015; Miller et al. 2016). The sediment recovered from Lake Bosumtwi still represents one of the best terrestrial sediment records in Africa.

1.4.4   Forest plots
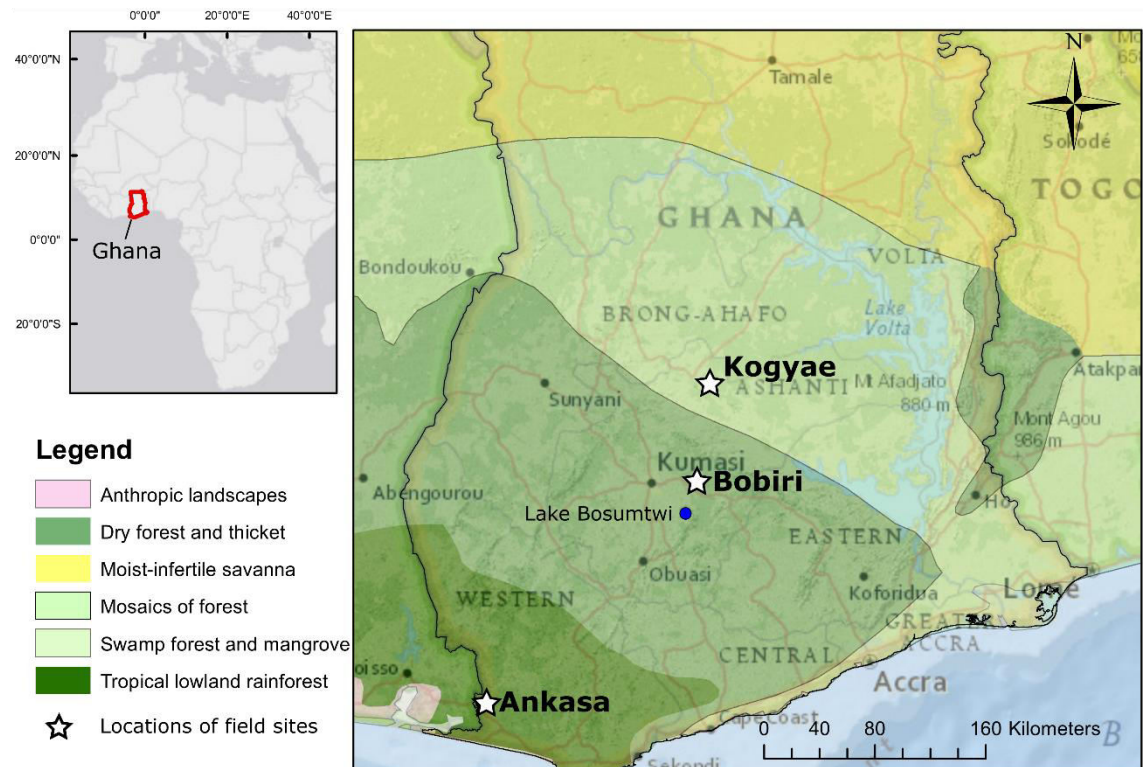
Figure 1.4: Map showing position of Ghana in Africa (top left) and vegetation types within Ghana (right). Vegetation typs of White (1983). White stars indicate locations of sites used in this study.

For detailed descriptions of the vegetation plots, photographs and most abundant taxa and diversity values, see 'Study Site description' sections in Chapters 2 (2.3.3.1-3 for Kogyae plots) and Chapter 4 (4.3.1 Ankasa and 4.3.2 Bobiri). Here, the plots are described with regards the broader ecological context that they represent.

 The characterisation of ecosystems and vegetation types in tropical West Africa is complex. White (1983) provided one of the most comprehensive summaries and vegetation maps (of the whole of Africa), whereas Hall and Swaine (Hall & Swaine 1981) provided more detail on the forest types of Ghana. Bongers et al. (2004) provided an overview of previous classifications used in tropical West Africa, and produced a synthesis which resolved issues of precipitation values overlapping between categories within previous classifications. Ankasa and Bobiri fit clearly into the 'wet evergreen rainforest' and 'moist semi-deciduous rainforest' categories, respectively, as outlined by Bongers et al (2004). The classification by Bongers et al. (2004), however, does not extend as far north as the Kogyae plots in this study. For the plots from Kogyae, therefore, the taxa were compared to White's 1983 classification to determine to which vegetation types the plots are best aligned.

Permanent vegetation study plots are established at all sites included in this study (Figure 1.4), as they are part of the Global Ecosystem Monitoring (GEM) project, which is run jointly by the University of Oxford and the Forestry Research Institute of Ghana (FORIG) (GEM website: gem.tropicalforests.ox.ac.uk/projects/african-forests-int). Vegetation data for the plots is available for download at forestplots.net (Lopez-Gonzalez et al. 2011). The plots are used to

Table 1.1: Information about vegetation plots.

	Ankasa	Bobiri	Kogyae Forest	Kogyae Transition	Kogyae Savannah
Location	South-West Ghana, Ankasa National Park	Central Ghana, near city of Kumasi, Bobiri Forest Reserve	North of Kumasi, on the Afram plains (a large expanse of arable land), Kogyae Strict Wildlife Reserve.	North of Kumasi, on the Afram plains (a large expanse of arable land), Kogyae Strict Wildlife Reserve.	North of Kumasi, on the Afram plains (a large expanse of arable land), Kogyae Strict Wildlife Reserve.
Rainfall	2000mm rainfall/year	1600 mm rainfall/year	1200-1300mm rainfall/year	1200-1300mm rainfall/year	1200-1300mm rainfall/year
GPS coordinates	5°16'06"N 2°41'38"W	6°42'15 "N 1°19'06 "W	7°15'41"N, 1°09'00"W,	7°18'07.7"N, 1°10'50"W	7°18'04.1"N, 1°09'53"W
Plot dimensions/m	100 x 100	100 x 100	100 x 100	100 x 100	100 x 100
Vegetation Type	Wet evergreen rainforest/Hygrophilous coastal evergreen Guineo-Congolian rainforest	Moist semi-deciduous forest/Drier peripheral semi-evergreen Guineo-Congolian rainforest	Drier peripheral semi-evergreen rainforest/Guineo-Congolian transition woodland	Guineo-Congolian Transition Woodland	Sudanian woodland/Guineo-Congolian secondary grassland and wooded grassland
Five most abundant tree taxa >10cm DBH	*Drypetes aylmeri* Hutch. & Dalziel (Putranjivaceae) *Cynometra ananta* Hutch. & Dalziel (Fabaceae) *Strephonema pseudocola* A.Chev. (Combretaceae) *Strombosia pustulata* Oliv. (Olacaceae) *Memecylon lateriflorum* (G. Don) Bremek. (Melastomataceae)	*Celtis mildbraedii* Engl. (Cannabaceae) *Funtumia elastica* (Preuss) Stapf (Apocynaceae) *Nesogordonia papaverifera* (A. Chev.) Capuron ex N. Hallé (Malvaceae), *Cola caricifolia* (G.Don) K.Schum. (Malvaceae), *Hymenostegia afzelii* (Oliv.) Harms (Fabaceae)	*Cola gigantea* A.Chev. (Malvaceae), *Dacryodes klaineana* (Pierre) H.J.Lam (Burseraceae), *Pouteria alnifolia* (Baker) Roberty (Sapotaceae), *Spathodea campanulata* P.Beauv. (Bignoniace*ae), Sterculia tragacantha* Lindl. (Malvaceae).	*Manilkara obovata* (Sabine & G.Don) J.H.Hemsl. (Sapotaceae), *Maranthes polyandra* (Benth.) Prance (Chrysobalanaceae), *Pterocarpus erinaceus* Poir. (Fabaceae), *Sterculia tragacantha* (Malvaceae). *Terminalia glaucescens* Planch. ex Benth. (Combretaceae).	*Anogeissus leiocarpa* (DC.) Guill. & Perr. (Combretaceae), *Bridelia ferruginea* Benth. (Phyllanthaceae), *Pterocarpus erinaceus* (Fabaceae), *Terminalia glaucescens* (Combretaceae), *Trichilia emetic* Vahl (Meliaceae),

monitor carbon flux, and as such are subject to yearly vegetation surveys, including measurements (Diameter at Breast Height (DBH), height and yearly growth) of all trees >10 cm DBH. Each site represents a different vegetation type, as outlined in Table 1.1, following those of White (1983) and Bongers (2004). Vegetation data have been provided by and are used with the permission of the University of Oxford and FORIG. The plots are monitored closely by FORIG staff throughout the year.

The plot locations were chosen to represent a range of ecosystems, from wet evergreen rainforest in the south to savannah in the north. On a smaller scale, the intra-plot differences between the three plots at Kogyae correspond to a forest-to- savannah transition zone (Sam Moore, pers. comm., but confirmed by analysis (see 1.4.4.3)).

1.4.4.1   Wet evergreen rainforest (Ankasa)

'Wet evergreen rainforest', as described and categorised by (Bongers et al. 2004) is forest that occurs between precipitation levels of 1400-2100 mm/year. In Ghana, this vegetation is present in the south-west of the country and is the most diverse vegetation type of the country (Hawthorne et al. 1998). The vegetation of the Ankasa reserve has been characterised

by Hawthorne, who identified seven distinct vegetation types within the reserve (Hawthorne et al. 1998). The vegetation of the plot used in this study most closely aligns to Hawthorne's (1998) 'VEG 1' type, which is present on well-drained hilltops and is characterised by high abundances of the tropical timber genera *Diospyros*, and *Cynometra*.

1.4.4.2   Moist semi-deciduous forest (Bobiri)

Moist semi-deciduous forest, as defined by Bongers et al. (2004) exists at precipitation levels of 1250-1750 mm/year. The Bobiri plot in this study is representative of this vegetation type, and although the plot is heavily dominated by the large tropical tree genus *Celtis* (Cannabaceae), it also contains other taxa, such as the buttressed, deciduous tree *Triplochiton scleroxylon* (Malvaceae) and the deciduous tree *Nesogordonia papaverifera* (Malvaceae) which are characteristic of this vegetation type.

1.4.4.3   Forest-Savannah Transitional Mosaic (Kogyae)

The plots at this site fall within the transitional mosaic and represent three different vegetation types within this mosaic; 'Forest', 'Transition' and 'Savannah'. Some woody taxa are unique to Forest and Savannah plots, whereas no taxa are unique to the Transition, which instead possesses taxa that are present in both Forest and Savannah plots (Figure 1.4). The distribution of taxa within the plots illustrates that they represent a gradient. This gradient is broadly equivalent to a shift from Drier peripheral semi-evergreen rainforest to Guineo-Congolian secondary grassland and wooded grassland (White et al. 1983 and Chapter 2). The plots can be considered together as representative of the band of transitional mosaic which is present in Ghana between the wetter forest of the south and the dry savannahs of the north (White et al. 1983), but can also, individually, represent points on the gradient of forest to savannah that are worth considering in their own rights, as is done in Chapter 2 of this thesis.
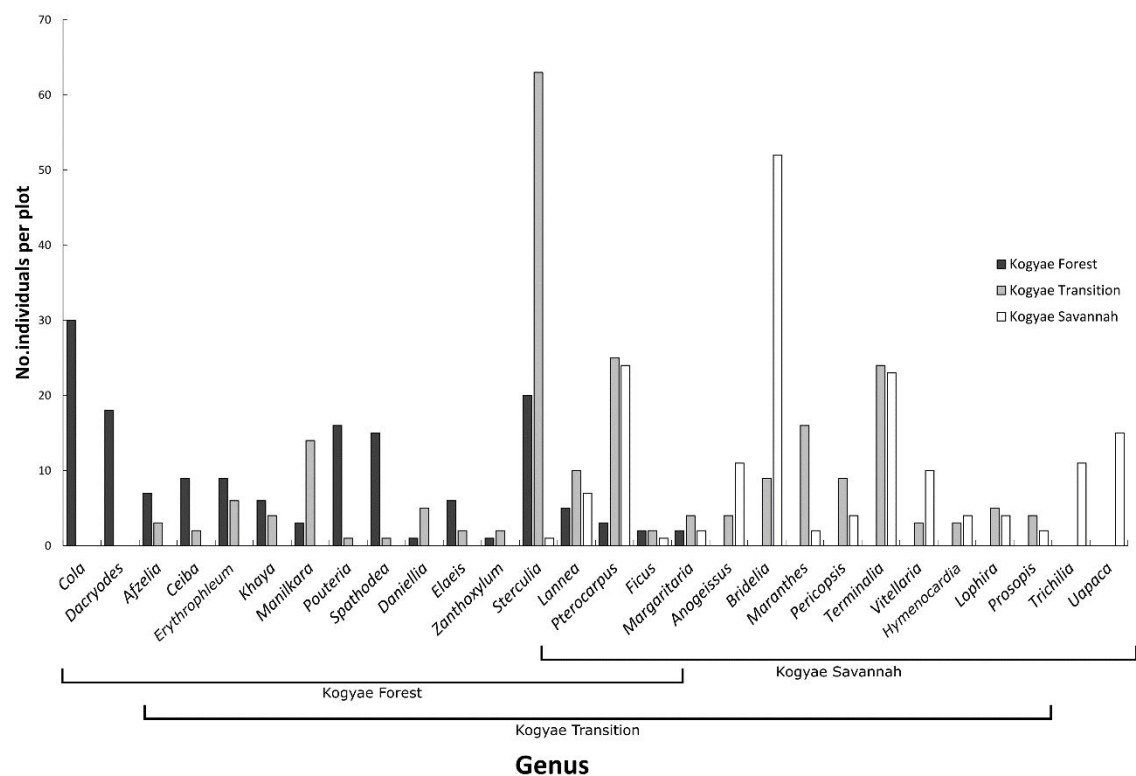
Figure 1.4: Bar plot of numbers of individuals of most common genera found in plots in Kogyae. Black bars indicate the abundance of the genus in forest plot, grey in transition and white in savannah. Each genus has three bars, illustrating its abundance in each plot.

1.4.5   Anthropogenic influence

Human influences on the vegetation of the plots are minimised by the fact that they are located in wildlife reserves where logging is strictly controlled. The Bobiri Wildlife Reserve was established in 1939, and plots there have not been logged for at least 50 years; the Ankasa National Park was established in 1976; and Kogyae Strict Nature Reserve was established as such in 1971 but had been a reserve since 1952. The impact of humans on African forests dates

back much further than the 20th Century, however, and it is therefore not realistic to declare any area of forest truly 'pristine' in Africa (Malhi et al. 2013)

1.5   Introduction to methods

Methods are detailed separately in each chapter. The methods presented here are intended to present more detail than is possible in a journal paper, or where clarification between chapters may be necessary.

1.5.1   Field methods

Artificial pollen traps, designed according to the protocol developed by Gosling (Gosling et al. 2003) were chosen for this study for the following reasons:

- Artificial traps enable a standardised amount of time to be sampled
- Artificial traps allow the surface area for collection of pollen to be standardised
- Artificial traps do not exhibit the same bias towards larger, saccate grains shown by moss pollsters (Pardoe et al. 2010)
- The environmental gradient over which sampling was needed encompassed a number of different soil types, which would have given rise to bias in preservation due to soil type (Elenga et al. 2000)
- The design of trap (see below) was able to withstand being deployed for a year at a time, and was unlikely to be interfered with by animals attempting to access water or glycerol, as can occur in some other artificial trap designs (Jantz et al. 2013)

Pollen traps were made in accordance with the technique of Gosling et al. (2003). They were composed of a plastic funnel (diameter 140mm), glass fibre filter paper affixed to the funnel using bathroom sealant, and cotton wool fibre filling the rest of the funnel, held in place with plastic netting secured around the funnel using plastic-coated wire (Jardine 2014). The traps were affixed to stakes hammered firmly into the ground or, in cases where that was not possible, to trees. All traps were positioned at approximately 50 cm above ground level to reduce the risk of inundation and to ensure a standard height of sampling across this study.

Traps were deployed at 10 m intervals along a straight line near to the middle of the plot; this was sometimes the 40 m line, but in Ankasa, the 60 m line, due to plot orientation. Five traps were chosen from each year for processing and counting, in order to give good spatial and statistical coverage (Gosling et al. 2005). Generally, every other trap within each plot for every year was processed and counted, although this was not always possible due to damage or loss

of traps. The resultant sampling resolution was consistent with or higher than other contemporary tropical pollen trapping studies (e.g. Gosling et al. 2009; Haselhorst et al. 2013) and enabled some redundancy in case of lost or damaged traps.

Traps were initially deployed to plots at Bobiri and Ankasa in 2011 and were collected and replaced at both sites in October of 2012, 2013 and 2014. Plots at Kogyae were set up in October 2013 and collected in 2014.

1.5.2   Pollen processing methods

Samples were processed following the method for cotton wool based traps from Gosling et al. (2003). Acetolysis, not washing, was used to extract pollen from cotton wool as this method was faster (acetolysis of 7-10 minutes) than performing multiple washes (five washes, with a centrifuge run of five minutes per wash), and also decreased the amount of other organic content in traps (fragments of leaves and soil). The samples were first split into cotton wool and filter paper elements, the cotton wool then dissolved using acetolysis, washed in sodium pyrophosphate to de-flocculate, then potassium Hydroxide, filtered at 180µm and washed the samples until supernatant was clear. The filter paper part of the trap was treated in the same way, but was first washed in 10% HCl, left in cold 60% HF overnight and then treated with hot 10% HCl. Some samples contained significant (wind-blown or termite-transported) siliceous material, and in these cases both the filter paper and the cotton wool were treated with 60% HF, separately. *Lycopodium* tablets were added as an exotic marker to enable the calculation of pollen concentrations (Stockmarr 1971); University of Lund batch number 124961, containing 12542 +/- 931 spores per tablet.

1.5.3   Pollen counting methods

Once processed, samples were mounted in glycerol and examined under x400 magnification using a Nikon Eclipse 50i light microscope. Pollen samples were counted using Keen's (2014) Model 1, which uses the richness and evenness of the first 100 grains of a sample to estimate a representative count size for the whole sample, and effectively resulting in less diverse samples returning higher count sizes.

1.5.4   Note on taxonomy

Pollen types were assigned codes, for example the 3rd grain (G3), photographed in Slide 28 (S28), Kogyae 2014 (K14), Transition vegetation plot (T), Trap 55 (T55) would be S28K14TT55G3. Types were numbered sequentially from 1 upwards, and Holotypes (sets of

images which acted as a standard for that pollen type) were designated. Holotypes were designated when the grain being photographed met the standards outlined below:

- images of only one grain

- includes both polar and equatorial views

- includes a scale bar

- grain not damaged

- images clear enough to see the diagnostic features of the grain such as pore shape and wall structure

When a new grain was found which did not meet the criteria to be designated as a holotype, it was photographed and given only a code, not a Type number, until a grain of the same morphotype was observed which could then be photographed as the holotype for that type. Pollen taxa were counted using their Type numbers, and taxonomic information was assigned to Types when this information became available.

1.5.4.1   *Fourier Transform Infra-red Spectroscopy (FTIR)*

FTIR was used to explore to feasibility of chemically differentiating between grass pollen taxa in this thesis for the following reasons;

- FTIR has been shown to successfully differentiate between pollen taxa at family and genus level (Zimmermann 2010; Mularczyk-Oliwa et al. 2012; Zimmermann & Kohler 2014)
- FTIR does not require large amounts of material; small groups of pollen grains, or even large single pollens (40 µm diameter and above) could be used to generate spectra. This is in contrast to the ~100 grains that would be needed to perform mass spectrometry, and means that the technique would be applicable to fossil samples.
- Preparing samples for FTIR is less time consuming (washing in acetone and allowing to air dry on slides) than for other techniques such as Scanning Electron Microscopy (SEM) or mass spectrometry, meaning that more samples could be analysed in the time available.
- FTIR analysis of pollen has been demonstrated to provide a proxy for UV radiation, and is therefore of interest to palaeoecologists regardless of taxonomic value of the technique, therefore using FTIR to generate taxonomic information would

create opportunities for studies to generate higher resolution climatic and vegetation records (Watson et al. 2007; Fraser 2008; Lomax et al. 2008; Fraser et al. 2011; Fraser, Lomax, et al. 2014; Jardine et al. 2016).

See Section 3.4 for a full overview of the methods used in FTIR analysis for this thesis.

1.5.4.2   Signal-to-noise ratio test

Before data collection on the FTIR spectrometer began, a signal-to-noise ratio test was carried out to optimise the number of scan per sample. 256 scans per sample was determined to be the optimal number of scans, which took approximately 9 minutes per run (4.5 minutes for background scans and 4.5 minutes for sample scans) and therefore represented a good balance between data quality and time taken for each analysis.

1.5.5   Statistical Methods

1.5.5.1   Pollen-vegetation statistics

Comparing the amount of pollen produced by an ecosystem to its vegetative abundance in the surrounding landscape is a controversial topic, and various ways have been suggested and used, including p/v (pollen percentage/ vegetation percentage of stems) and R-rel (percentage of pollen grains of a taxon/ the percentage basal area of that taxon in the vegetation) (Fægri et al. 1989; Bush & Rivera 2001; Gosling et al. 2009) .

Here, R-rel has been used, with variations depending on the chapter, to highlight different aspects and properties of the data. R-rel values are used and are defined as the percentage of the pollen taxon over the percentage of the taxon's basal area in the vegetation (Bush & Rivera 2001). This measure was used due to some taxa being present with many small, likely non-reproductive stems, which rendered p/v values (which use the percentage of the vegetation taxon's stems in the vegetation) less useful. Percentage values, not influx values were used to ensure data were comparable with other studies of modern pollen-vegetation relationships, and also due to influx values being quite variable in this study and possibly subject to some issues such as traps being shaded or covered by leaves for part of the deployment time.

In Chapter 2, R-rel$_{(av)}$ is used; that is, the average amount of pollen from the taxon in each trap sampled (so the total from the plot, divided by five, as five traps were counted from each plot at Kogyae). This is in order to capture a more even measure of the traps (i.e. a trap that has none of the pollen grain in question may moderate the effect of a trap that is 90% of that taxon).

In Chapter 4, R-rel$_{(ind)}$ is calculated for each trap, to illustrate the differences in the representation of certain taxa.

Finally, in Chapter 5, R-rel$_{(sum)}$ is used, as a bulk measure. This is because this chapter aims to take a broader view of the patterns observed, and this measure will be of interest to the broader palaeoecological community.

1.5.5.2   Ordinations

Ordinations were used to visualise the similarity of pollen assemblages to one another, and to highlight groupings in data that may not be readily apparent from a pollen diagram. Pollen diagrams are useful when comparing dominant taxa and broad scale differences between plots, whereas hulls can be overlain onto ordinations to highlight specific features such as year of sampling or physical position in plot (see Chapter 4). The influence of rare taxa, which are often excluded from pollen diagrams for practical reasons, can also be visualised more easily using ordinations.

Non-metric multidimensional scaling (NMDS) with Bray-Curtis dissimilarity, singletons removed, and Wisconsin double-standardisation was used to generate ordinations of pollen assemblages.

NMDS was used instead of principal component analysis (PCA) or detrended correspondence analysis (DCA) because the mean-variance distribution of the pollen assemblage data does not conform to a normal distribution, and NMDS (unlike PCA and DCA) is non-parametric and does not make assumptions about the underlying structure of the data, making it an appropriate metric in this instance (Oksanen et al. 2015). Bray-Curtis was chosen as the distance metric as it has been shown to be adept at detecting changes along gradients, and is a non-Euclidean distance metric. Singletons (taxa which were only present in one sample) were removed from the data set because they provided no useful information about the similarity of one sample to another. Wisconsin double standardisation, which standardises data sets first by column (sample) and then by row (taxon) helps to moderate the influence of a few dominant taxa, a common characteristic of pollen assemblages.

1.5.5.3   Diversity

Diversity metrics were calculated in order to compare pollen assemblages with one another, and with their parent vegetation types. Measures of diversity can provide a broader overview of the data, as pollen diagrams tend to reduce assemblages to only the most abundant data

and may not give a sense of structure (rare vs abundant taxa) that, using diversity and richness metrics, can be explored.

The Shannon Index, H', also known as the Shannon-Weaver index and the Shannon-Wiener index, has the equation $H' = -\sum P_i \ln(P_i)$, in which P_i is the proportion of individuals belonging to species i (Shannon 1948). The Shannon index is effectively the uncertainty that two species sampled from the population are the same (if only one species present, H'=0). This index was used to calculate diversity for both vegetation and pollen assemblages in this thesis, as it weights rare and abundant taxa more evenly than other metrics such as Simpson's or inverse Simpson's indices (Morris et al. 2014). The equal weighting of rare and abundant taxa is useful when analysing pollen diversity, as abundance does not necessarily indicate that the abundant taxon is important in the ecosystem.

The Shannon index was chosen over the Simpson's diversity indices, as Simpson's diversity index places more emphasis on abundant taxa, which, in terms of tropical pollen assemblages, may not be optimal. Hill numbers, which are the exponential of the Shannon index, are useful in comparing populations whose diversity may vary widely, as they represent the number of taxa that would result in the observed diversity, if all were present in equal proportions (Jost et al. 2010). This is done by converting the non-linear response of Shannon's index to species richness to a linear response, meaning that at high levels of species richness, Hill numbers do not level off as Shannon's indices do. These numbers are not presented in this thesis, however, as they tend to produce very low values when there is a dominant taxon and many rare taxa, as is the case in pollen assemblages, and therefore may not be the most appropriate measure to compare between pollen and vegetation diversity.

The diversity values calculated for the plots in this study and their true diversities differ, due to the bias introduced by the vegetation surveys, which measure trees of > 10 cm DBH. Although the taxa that contribute the majority of the biomass of the plot are captured (i.e. the largest trees), the surveys do not record plants of less than 10 cm DBH (see section 1.4.4) and therefore do not include the diversity of seedlings and saplings, epiphytes, herbaceous taxa and lianas of less than 10 cm DBH (lianas of more than 10 cm DBH were recorded). The diversity values calculated for the vegetation plots will therefore be lower than their 'true' diversity values, although the magnitude of this effect cannot be precisely quantified with the data available. See section 6.4.3 for possible solutions to this issue.

1.5.6   Notes on Terminology

Throughout this thesis, certain terms are used to describe plant reproductive techniques and pollination syndromes. These are defined as follows:

Monoecious: Species in which separate male and female flowers are borne on the same individual.

Dioecious: Species in which male and female flowers are borne on different individuals

Hermaphrodite: Species that possess flowers with both male and female parts.

Anemophilous: Taxa that are primarily wind pollinated

Ambiphilous: Taxa that may be both wind and animal (usually insect) pollinated

Zoophilous: Taxa that are primarily pollinated by animals other than insects

Entomophilous: Taxa that are primarily insect-pollinated

'X'-philous: Any other term denoting pollination ecology will refer to specific pollination strategies as sub-sets of zoophily or entomophily, such as chiropterophilous (bat pollination) or ornithophily (bird pollination)

1.6   Thesis aims

This thesis aims to characterise the modern pollen-vegetation relationships of a forest-savannah mosaic, moist semi-deciduous forest and wet evergreen rainforest in Ghana, tropical West Africa, and to investigate the use of Fourier-Transform Infra-red spectroscopy (FTIR) as a means of identifying grass pollen to below family level. The specific aims of this thesis are:

- To determine how tropical West African ecosystems, including a forest-savannah mosaic, moist semi-deciduous forest and wet evergreen rainforest are represented by their modern pollen rain, and specifically:
  - To investigate whether ecosystems within a forest-savannah mosaic landscape can be distinguished from one another using their modern pollen assemblages
  - To determine how modern pollen assemblages represent high-resolution vegetation patterns within closed-canopy moist semi-deciduous forest and wet evergreen rainforest
- To test whether FTIR spectroscopy can be used to improve the identification of Poaceae pollen.

- To consider how an improved understanding of modern pollen-vegetation relationships contributes to the interpretation of tropical West African fossil pollen record, particularly that of Lake Bosumtwi.

1.7   Structure of Thesis

This thesis is structured such that each data chapter (Chapters 2-5) stands alone as either a publication (Chapters 2 and 3) or is ready for submission to a journal (Chapters 4 and 5). The main aims of each chapter are outlined below.

Chapter 1: Introduction

Chapter 1 introduces the rationale, aims, background and methods used in this thesis.

Chapter 2: The modern pollen-vegetation relationships of a tropical forest-savannah mosaic landscape, Ghana, West Africa

Chapter 2 presents the pollen-vegetation relationships within a transitional mosaic of forest and savannah vegetation (Kogyae). This chapter aims to distinguish palynologically between the three plots (Savannah, Transition and Forest) and to identify the characteristic pollen taxa from each plot. The implications of this research for the fossil record are then considered and it is concluded that the threshold of Poaceae at which, in the fossil record of Lake Bosumtwi, has been taken to indicate a 'grassland' ecosystem, should be lowered from 55% to 40% or lower. A version of this chapter has been accepted for publication in *Palynology*.

Chapter 3: Chemotaxonomy as a tool for interpreting the cryptic diversity of Poaceae pollen

The high percentages (up to 50%) of Poaceae pollen observed in Kogyae, and in large sections of the fossil record of Lake Bosumtwi, highlight the need for a means of identifying Poaceae pollen to below family level. Chapter 3 presents the use of FTIR spectroscopy as a means of identifying Poaceae (grass) pollen grains. The identification of grass pollen is a challenge in palynology, as the taxonomic resolution of the fossil record suffers from the fact that most of the >11,000 species of Poaceae cannot be distinguished below family level using light microscopy. Modern Poaceae taxa, drawn from across the Poaceae phylogeny, were used to assess the feasibility of using FTIR spectroscopy to identify the pollen of this cryptic taxon to family level. This chapter was published in *Reviews in Palynology and Palaeobotany* in 2016 (Julier et al. 2016).

> Chapter 4: Does the pollen fall far from the tree? Spatial and temporal variation
>
> in pollen signals from wet evergreen rainforest and moist semi-deciduous forest
>
> in tropical West Africa (Ghana)

Following the investigation of the relatively dry (precipitation of 1000 mm year^{-1}) transitional mosaic landscape of Ghana in Chapter 2, Chapter 4 examines spatial and temporal differences in pollen deposition of wetter (precipitation of 1200- 2000 mm year^{-1}) tropical ecosystems. This chapter investigates, at plot-scale (1 ha), the pollen-vegetation relationships of two tropical forest vegetation plots (Bobiri and Ankasa) in high resolution, focussing on the most abundant taxa that occur in both pollen and vegetation, to improve our understanding of how vegetation is represented by its pollen rain in the tropics. High levels of variability, both spatially and temporally, were observed between how well the pollen assemblages reflected their parent vegetation types within both plots. The implications of this work for modern pollen trapping studies in the tropics are considered. This chapter will be submitted for publication in summer 2017.

> Chapter 5: Modern pollen rain across an ecological gradient from savannah to
>
> wet evergreen rainforest in tropical West Africa (Ghana)

Chapter 5 presents the pollen data from all five plots in this thesis, pulling out broad scale changes in pollen assemblages and considering how well the bulk pollen spectra recovered from these sites over the three years of sampling reflect their parent vegetation types. In this chapter, the implications of this study for interpretations of the fossil record of Lake Bosumtwi, Ghana, are considered. It is concluded that interglacial sections of the Lake Bosumtwi core may have seen vegetation around the lake more similar to forest-savannah mosaic, rather than the moist semi-deciduous forest or rainforest. This chapter will be submitted for publication in summer 2017.

> Chapter 6: Conclusions

Chapter 6 provides an overview of the main findings of this thesis and addresses the thesis aims as outlined in section 1.2. The limitations of this thesis and future research directions, taking into consideration the results of the thesis as a whole, are discussed.

2   Chapter 2: The modern pollen-vegetation relationships of a forest-savannah mosaic landscape, Ghana, tropical West Africa

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ACMJ collected and analysed data, generated figures, drafted and revised manuscript. PEJ, ALC, WTF, BHL and WDG commented on analyses and manuscript. PEJ, SAB, ADG, WTF, YM, SM and KOA contributed to data collection in field and commented on manuscript.

2.1   Abstract

Transitions between forest and savannah vegetation types in fossil pollen records are often poorly understood, due to over-production by taxa such as Poaceae and a lack of studies from these regions today. Pollen assemblages from within a forest-savannah transition zone in tropical West Africa are presented and compared, their characteristic taxa discussed, and their implications for the fossil record considered. Fifteen modern pollen traps were placed in the field for one year, to collect pollen rain from three vegetation plots within the forest-savannah transition zone in Ghana. High percentages of Poaceae and Melastomataceae/Combretaceae were recorded in all three plots. *Erythrophleum* characterised the Forest plot, *Manilkara obovata* the Transition plot, and *Terminalia* the Savannah plot, with *Pterocarpus* and *Uapaca* pollen also important in that plot. The results indicate that Poaceae pollen is not a good indicator of the Savannah plot when only percentage data are considered, and that influx data give a better representation of the forest-savannah gradient. Inconsistencies in percentage data were caused by the input of Melastomataceae/Combretaceae type pollen in some traps. It is concluded that when considering fossil records such as those from Lake Bosumtwi, Ghana, the threshold at which Poaceae pollen is indicative of Savannah-type vegetation may be lower than previously considered (55%), and potentially as low as 40%.

2.2   Introduction

The transition between closed canopy forest and open canopy savannah vegetation is one that can be observed at the present day along climate gradients, and in the fossil record during periods of climatic change (Mayle et al. 2007; Azihou et al. 2013; Miller & Gosling 2014; Miller et al. 2016). The transition between forest and savannah is, however, not always stable or clear cut (Cardoso et al. 2016), with large areas of land being classified as mosaics of forest and savannah (e.g. White et al. 1983; Torello-Raventos et al. 2013). Transitional ecosystems are today recognised as having conservation value in their own right, due to their ability to provide habitats for organisms from different ecosystems, and the potential for them to be especially sensitive to climate change (JNCC 2010; Ibie et al. 2016; Joyce et al. 2016). The identification of transitional ecosystems in the fossil record may be crucial in providing evidence to support efforts to conserve biodiversity, especially if the persistence of these transitional areas can be demonstrated over long time scales.

Projections of future environmental change suggest that modern forest-savannah boundaries will alter, and transitional zones shift (Niang et al. 2014), but little is known about what this change will look like in terms of vegetation composition (Cramer et al. 2001). Fossil pollen

records of forest to savannah transitions can serve as a guide to the likely future vegetation response; however, it has been difficult to observe forest to savannah transitions in the fossil pollen record because of a poor understanding of how they are represented. The relationship between taxa represented in the vegetation and in the pollen rain is known not to be directly proportional (Davis 1963). Anemophilous taxa, and zoophilous taxa with 'messy' pollination syndromes (open flowers with extruded anthers) are generally over-represented relative to their abundance in the vegetation, whereas those zoophilous taxa with more closed floral morphologies are more often under-represented or palynologically silent (present in vegetation but not pollen assemblages) (Bush & Rivera 2001).

To facilitate a better understanding of transitional periods in fossil pollen records, this study has explored modern pollen-vegetation relationships on a landscape scale (plots within 10 km of one another) within the forest-savannah transition zone in tropical West Africa. Data from pollen traps has been used to characterise the modern pollen rain produced by three vegetation types (forest, transition, and savannah) during one year. Modern pollen data were then compared with vegetation inventories, and an assessment was made as to whether it is possible to differentiate between forest, transition, and savannah vegetation on the basis of the pollen assemblage alone. The implications of this modern study for identification of past forest-savannah transitions in the fossil pollen record are then discussed.

2.2.1   Vegetation at the forest-savannah transition

The transition between forest and savannah ecosystems, on both spatial and temporal scales, is one that has been of interest to biogeographers, ecologists and palaeoecologists for decades (Beard 1953; Aubréville 1966; Ybert 1975). Although the botanical composition of savannah-type ecosystems on different continents has been shown to vary widely, it can, along with the height and canopy cover of savannah-transition ecosystems, be used in their classification (Torello-Raventos et al. 2013). The shift from forest to savannah is controlled on a macro-scale (100s of km) by climatic gradients, primarily rainfall (Swaine 1992; Lehmann et al. 2011), but on smaller scales other factors such as soil type and herbivory are also important. Where mean annual precipitation (MAP) is less than 650 mm, savannahs form because water availability limits tree growth and, consequently, forest cover (Sankaran et al. 2005). At precipitation levels of >650 mm MAP, savannahs can occur when other factors such as fire and herbivory inhibit the growth or enhance the mortality of trees (Swaine et al. 1992; Higgins et al. 2012; Dexter et al. 2015). Edaphic factors such as soil type and fertility may also influence local

vegetation dynamics, resulting in the formation of a mosaic of forest and savannah within regions that experience a more or less uniform set of climatic variables (Lehmann et al. 2011).

2.2.2   Pollen at the forest-savannah transition

On a large scale, pollen production by modern ecosystems in Africa has been found to differ between vegetation types (Gajewski et al. 2002; Lézine et al. 2009). For example, an extensive study of modern pollen-vegetation relationships in central Africa demonstrated that it is possible to differentiate broad vegetation types, such as tropical forest and tropical seasonal forest using the pollen signal, although difficulties were encountered in areas that were considered transitional (Lebamba et al. 2009). Within the forest-savannah transition zone of Cameroon, work has shown that it is possible to identify different vegetation types by their modern pollen output, although the characteristic pollen and vegetation taxa were different due to sampling bias towards larger woody plants in the botanical inventories, and pollen from entomophilous taxa being under-represented (Vincens et al. 2000). The forest-savannah transition has also been shown, in Côte d'Ivoire, to produce distinct pollen assemblages depending on the vegetation, with *Hewittia malabarica* L. (Suresh) indicating the forest-edge, and savannah being characterised by Poaceae pollen percentages of >40% (Ybert 1975). Studies of the Sudanian and Sahelian regions have shown that it is possible to differentiate between these regions by their pollen signals, and to also differentiate smaller scale vegetation types within them (e.g. *Isoberlinia* dry forest and dry or wet Combretaceae forest) (Lézine & Edorh 1991; El Ghazali & Moore 1998), although the arboreal pollen signal is often overwhelmed by very high (>90%) Poaceae abundances.

2.2.3   Forest-savannah transitions through time

Transitions between forest and savannah have been tracked through the last c. 1 Ma in tropical West Africa by 14 offshore pollen records, and several terrestrial records (Maley 1991; Elenga et al. 1994; Maley & Brenac 1998; Salzmann et al. 2002) of which only one record is older than 35 ka BP(Lake Bosumtwi, Ghana). The fossil pollen record obtained from Lake Bosumtwi is unique to tropical West Africa, and covers the last c. 500,000 years of the 1Ma sediment core that was recovered (Koeberl et al. 2007; Miller & Gosling 2014; Miller et al. 2016).Pollen from off-shore and terrestrial records identifies shifts in the vegetation correlating with climatic changes that broadly relate to orbital (glacial-interglacial) cycles during the Quaternary (Miller et al. 2016). The vegetation shifts observed are thought to be a consequence of the north- to south-wards movement of the tropical rain belt, and the intensity of the African Monsoon (Shanahan et al. 2015; Miller et al. 2016). The shifts between

wetter forest and drier savannah ecosystems are recorded in the pollen record of Lake Bosumtwi by fluxes in the Poaceae abundance, that varies from 0% to 90% of the terrestrial pollen sum; interpretations of the Lake Bosumtwi fossil pollen record placed the transition between forested and savannah vegetation types at 55% Poaceae (Miller et al. 2016).

2.3   Study region

2.3.1   Tropical West Africa

The vegetation of tropical West Africa has been classified into two main biomes; the 'Guineo-Congolian regional centre of endemism' and, further north, the 'Sudanian regional centre of endemism', that are distinct because of their endemic flora. The transition between the two is primarily driven by decreasing precipitation from south to north (White, 1983). The Guineo-Congolian region comprises areas of wet and semi-deciduous forest, transitional rainforests, secondary grassland and mosaics, while the Sudanian region contains drier woodland and more open savannah vegetation types (Table 2.1).

Table 2.1: Main components of the vegetation types of tropical West Africa as defined by White (1983). Taxa listed in this table are those which could feasibly be present in Ghana, i.e. not taxa specific to a certain soil type, elevation or geographic area outside of West Africa.

Biome	Vegetation Type	Characteristic tree taxa	Climatic information
Guineo-Congolian regional centre of endemism	Guineo-Congolian short forest and shrub forest	*Parinari excelsa* Is characteristic of this vegetation type. Other taxa include; *Albizia ferruginea, Alstonia boonei, Anthonotha obanensis, Clausena anisata, Diospyros monbuttensis, Hildegardia barteri, Harungana madagascariensis, Holarrhena floribunda, Newbouldia laevis*	Rainfall 1200-1600 mm per year.
Guineo-Congolian regional centre of endemism	Mixed moist semi-evergreen rainforest	*Canarium schweinfurthii, Entandrophragma angolense, E. candollei, E, cylindricum, E. utile, Guarea cedrata, G. thompsonii, Lophira alata, Lovoa trichilioides, Maranthes glabra, Nauclea diderrichii, Oxystigma oxyphyllum, Parkia bicolor, Pericopsis elata, Petersianthus macrocarpus, Piptadeniastrum africanum, Ricinodendron heudlottii, Scorodophloeus zenkeri, Sterculia Scorodophloeus zenkeri, Terminalia superba*	Rainfall 1600-2000 mm per year.

Guineo-Congolian regional centre of endemism	Drier peripheral semi-evergreen rainforest	*Afzelia africana, Aningera altissima, A.robusta, Aubrevillea kerstingii, Canarium schweinfurthii, Celtis mildbraedii, C.zenker, Chlorophora excelsa, Chrysophyllum perpulchrum, Cola gigantea, Hildegardia barteri, Holoptelea grandis, Khaya grandifoliola, Mansonia altissima, Morus mesozygia, Nesogordonia papverifera, Piptadeniastrum africanum, Ricinodendron heudlottii, Sterculia oblonga, S.rhinopetala, Terminalia superba, Triplochiton scleroxylon*	Rainfall 1200-1600 mm per year. Dry season usually fairly humid.
Guineo-Congolian regional centre of endemism	Secondary Guineo-Congolian rain forest	*Musanga cecropiodes, Pycnanthus angolensis, Harungana madagascariensis, Trema orientalis*	Rainfall 1600-2000 mm per year.
Guineo-Congolian regional centre of endemism	Old secondary forest	*Alstonia boonei, Antrocaryon micraster, Canarium schweinfurthii, Ceiba pentandra, Chlorophora excelsa, Discogylpremna caloneura, Funtumia africana, Holoptelea grandis, Khaya anthotheca, Morus mesozygia, Petnaclethra macrophyla, Petersianthus macrocapus, Pterygota macrocarpa, Pycnanthus angolensis, Ricinodendron heudlotii, Terminalia superba, Triplochiton scleroxylon, Xylopia aethiopica*	Rainfall 1600-2000 mm per year.
Guineo-Congolian regional centre of endemism	Guineo-Congolian transition woodland	*Afzelia africana, Antiaris toxicaria, Annona senegalensis, Anogeissus leiocarpus, Butryospermum paradoxum, Ceiba pentandra, Celtis brownii, Crossopteryx febrifuga, Daniellia oliveri, Diospyros mespiliformis, Maranthes polyandra, Parkia biglobosa, Piliostigma thonningii, Pseudocedrela kotschyi, Pterocarpus erinaceous*	Rainfall 1600-2000 mm per year.
Guineo-Congolian regional centre of endemism	Guineo-Congolian secondary grassland and wooded grassland	*Annona senegalensis, Afzelia africana, Borassus aethiopum, Bridelia ferruginea, Burkea afriana, Butryospermum paradoxum, Combretum collinum, Crossopteryx febrifuga, Cussonia arborea, Daniellia oliveri, Detarium senegalense, Dichrostachys cinera, Entada abyssinica, Gardenia ternifolia, Hymenocardia acida, Lophira lanceolata, Maranthes polyandra, Maytenus senegalensis, Nauclea latifolia, Parinari curatellifolia, Parkia biglobosa, Pericopsis laxiflora, Piliostigma thonningii, Pseudocedrela kotschyi, Psorospermum febrifugum, Pterocarpus erinaceus, Securidaca longepedunculata, Stereospermum kunthianum, Strychnos madagascariensis, S. spinosa, Syzygium guineense, Terminalia glaucescens, T. laxiflora, Uapaca togoensis, Vitex doniana, V. madiensis*	Rainfall 1600-2000 mm per year

Sudanian regional centre of endemism	Sudanian Woodland	*Acacia dudgeonii, A. gourmaensis, A. hockii, A. macrothyrsa, A. polyacantha subsp. campylacantha, A. sieberana, Afzelia africana, Amblygonocarpus andongensis, Annona senegalensis, Anogeissus leiocarpus, Antidesma venosum, Bombax costatum, Boswellia dalziellii, Bridelia ferruginea, Burkea africana, Butyrospermum paradoxum, Cassia sieberana, Combretum collinum, C. fragrans, C. glutinosum, C. molle, C. nigricans, Crossopteryx febrífuga, Cussonia arborea, Daniellia oliveri, Detarium microcarpum, Dichrostachys cinerea, Diospyros mespiliformis, Ekebergia capensis, Erythrophleum africanum, Faurea saligna, Ficus glumosa, Haematostaphis barteri, Hymenocardia acida, Khaya senegalensis, Isoberlinia doka, Lannea schimperi, Lophira lanceolata, Maprounea africana, Maranthes polyandra, Mitragyna inermis, Monotes kerstingii, Nauclea latifolia, Ochna afzelii, O. schweinfurthiana, Parinari curatellifolia, Parkia biglobosa, Pericopsis laxiflora, Piliostigma thonningii, Prosopis africana, Protea madiensis, Pseudocedrela kotschyi, Pterocarpus erinaceus, Steganotaenia araliacea, Sterculia setigera, Stereospermum kunthianum, Strychnos madagascariensis, Swartzia madagascariensis, Syzygium guineense subsp. guineense, Terminalia avicennioides, T. glaucescens, T. laxiflora, T. macroptera, Trichilia emetica, Uapaca togoensis, Vitex doniana, Xeroderris stuhlmannii, Ziziphus abyssinica, Z. mucronata*	Rainfall 500-1400 mm per year
Sudanian regional centre of endemism	Sudanian Transition Woodland	*Anogeissus leiocarpus, Antiaris toxicaria, Borassus aethiopum, Ceiba pentandra, Celtis integrifolia, Combretum molle, Diospyros mespiliformis, Khaya senegalensis, Kigelia africana, Parkia biglobosa, Piliostigma thonningii, Stereospermum kunthianum, Terminal laxiflora, Vitex doniana, Ziziphus mucronata*	Rainfall 500-1400 mm per year
Sudanian regional centre of endemism	Sudanian Grassland	*Balanites aegyptiaca, Combretum glutinosum, Diospyros mespiliformis, Gardenia ternifolia, Isoberlinia doka, Mitragyna inermis, Nauclea latifolia, Piliostigma reticulatum, Pseudocedrela kotschyi, Terminalia avicennioides, T. laxiflora, T. macroptera*	Rainfall around 1000 mm per year

At around 8°N there is a band of vegetation known as the 'Guineo-Congolian/Sudanian transition zone' (White 1983), that is between 100 and 500 km wide and runs approximately West-East across the West African countries, including Ghana (Gautier & Spichiger 2004). The transition zone comprises a patchwork of forest and savannah vegetation types and can be

considered as the boundary between the wet Guineo-Congolian forest types of the south and the drier Sudanian savannah regions of the north. It is within this transition zone that the Kogyae Strict Nature Reserve is situated, in which the plots for this study are located (Figure 2.1).

2.3.2   Kogyae Strict Nature Reserve

In 2012, three permanent vegetation study plots (KOG02 'Forest', KOG04 'Transition' and KOG05 'Savannah' see 3.3.1-3 for further details) were established in the Kogyae Strict Nature Reserve (Ghana) at the transition between the Guineo-Congolian and Sudanian biomes, to characterise the vegetation and monitor environmental changes at the ecotone (Figure 2.1). The Kogyae vegetation study plots were set up as part of the Global Ecosystem Monitoring (GEM) network (http://gem.tropicalforests.ox.ac.uk/) and are also part of the African Tropical Rainforest Observation Network (AfriTRON) (Lewis et al. 2013). Vegetation data from these plots are available at http://www.forestplots.net(Lopez-Gonzalez et al. 2011). The Kogyae Strict Nature Reserve contains a mosaic of ecosystems which differ in their floristic composition, tree cover, canopy height and density, and understorey vegetation. The Kogyae region typically receives around 1000 mm of rain/year, and experiences a dry season between October and April of each year (weather data from weather station at Kogyae Field Station). During the dry season the savannah vegetation in Kogyae usually burns, typically during February. The three vegetation study plots were established in three distinct vegetation types within Kogyae, but experience similar climatic conditions due to their close proximity; within 10 km of one another (Figure 2.1).

 The soils present in the Kogyae Strict Nature Reserve are shallow, prone to drying out and overlie horizontally bedded sandstones that are part of the Voltarian Group, however, depth and composition of soils differs between forested and savannah areas (Wildlife Department, Accra 1994). Animals present in the reserve that may exert grazing or browsing pressure include buffalo, baboons, antelopes, five species of monkey and domestic cattle (Danquah &

Owusu 2015).
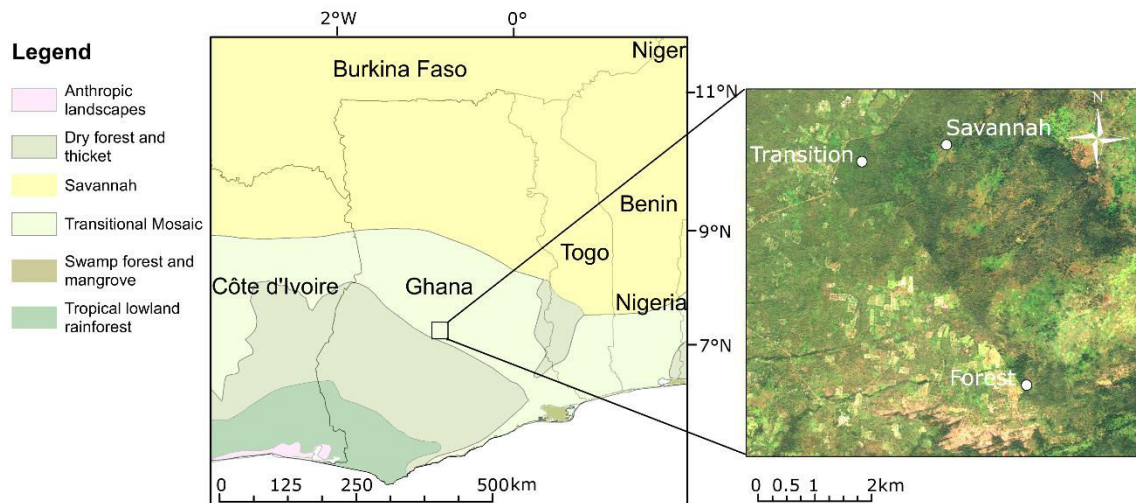
Figure 2.1: Location of vegetation plots relative to one another (right) and location of the field study site in Ghana as a whole, and relative to vegetation types (left).

2.3.2.1   Vegetation of study plots

The Forest (KOG02), Transition (KOG04), and Savannah (KOG05) vegetation study plots are each one hectare and were established following the standard GEM protocols (Marthews et al. 2014). As part of the GEM programme a vegetation inventory was generated for each plot, and a programme of yearly measurements of all trees >10 cm Diameter at Breast Height (DBH) was established measuring DBH, height, and yearly growth. Two genera occur only in the Forest plot (*Cola* and *Dacryoides*) and two genera occur only in the Savannah plot (*Trichilia* and *Uapaca*). The transition plot includes six genera which occur across all three plots (*Ficus*, *Anogeissus*, *Lannea*, *Margaritaria*, *Sterculia*, and *Pterocarpus*) and shares multiple genera with both the Forest and Savannah plots, but does not contain any unique genera (Figure 1.4).

Figure 2.2: Photographs of the vegetation in the three plots; a) Forest (KOG02) 7°15'41.9"N, 1°09'00.2"W, 197 m asl, b) Transition (KOG04) 7°18'07.7"N, 1°10'50.2"W and 190 m asl, and c) Savannah (KOG05) 7°18'04.1"N, 1°09'53.8"W and 186 m asl

2.3.2.1.1   Forest (KOG02)

The centre of the Forest plot is located at 7°15'41.9"N, 1°09'00.2"W, 197 m asl (Figure 2.1 and Figure 2.2). Thirty-seven species have been recorded in the Forest plot with DBH >10 cm, with

nine taxa occurring at >3% abundance (Table 2.2), the most abundant, by percentage of stems, being *Cola gigantea* A.Chev. (12.3%), *Sterculia tragacantha* Lindl. (10.7%) and *Dacryodes klaineana* (Pierre) H.J.Lam (9.6%). There are also understory monocotyledonous plant taxa, e.g. Zingiberaceae, Costaceae and Marantaceae, although the abundance of these taxa was not quantitatively assessed, as they are herbaceous and therefore were not included in the vegetation surveys. The taxa in the Forest plot fall largely into "Drier peripheral semi-evergreen rainforest" (seven taxa), or "Guineo-Congolian transition woodland" (six taxa), but with some "Sudanian transitional woodland" elements (five taxa), including *Ceiba pentandra* (see Table 2.1. and Figure 2.3). The average tree height is 20.8m. The diversity (Shannon index) of the Forest plot is 3.11.

Table 2.2: Species that occur at >3% in the Forest vegetation plot (KOG02), their reproductive strategy (Hermaphrodite, Monoecious or Dioecious), and their pollination syndrome (if known).

Family	Species	Stems (%)	Basal area (%)	Flower structure	Pollination syndrome
Malvaceae	*Cola gigantea* A. Chev	12.3	13.3	Monoecious	Entomophilous
Malvaceae	*Sterculia tragacantha* Lindl.	10.7	5.7	Hermaphrodite	Entomophilous
Burseraceae	*Dacryodes klaineana* (Pierre) H.J.Lam	9.6	13.7	Monoecious	Entomophilous
Sapotaceae	*Pouteria alnifolia* (Baker) Roberty	8.6	5.4	Hermaphrodite	Entomophilous
Bignoniaceae	*Spathodea campanulata* P.Beauv.	8.0	4.9	Hermaphrodite	Zoophilous
Malvaceae	*Ceiba pentandra* (L.) Gaertn.	4.8	5.4	Hermaphrodite	Zoophilous
Fabaceae	*Erythrophleum suaveolens* (Guill. & Perr.) Brenan	4.8	4.4	Hermaphrodite	Entomophilous
Fabaceae	*Afzelia africana* Pers.	3.7	5.8	Hermaphrodite	Entomophilous
Arecaceae	*Elaeis guineensis* Jacq.	3.2	3.0	Monoecious	Entomophilous

Figure 2.3: Bar chart showing the vegetation types as outlined by White 1983 to which the species in the three plots belong.

2.3.2.1.2   Transition (KOG04)

The centre of the Transition plot is located at 7°18'07.7"N, 1°10'50.2"W and 190 m asl. There are 34 species recorded at >10 cm DBH, with nine taxa occurring at >3% abundance (Table 2.3), the most abundant, by percentage of stems, of which are *Sterculia tragacantha* (26.9%), *Pterocarpus erinaceus* Poir (10.7%) and *Maranthes polyandra* (Benth.) Prance (6.8%). When compared to White (1983), the taxa in the plot are those characteristic of "Guineo-Congolian transition woodland" (12 taxa) and "Sudanian Woodland" (11 taxa), with some "Guineo-Congolian secondary grassland and wooded grassland" taxa (eight). The average tree height is 12.1 m. The diversity (Shannon index) of the Transition plot is 2.82.

Table 2.3: Species that occur at >3% in the Transition vegetation plot (KOG04), their reproductive strategy

(Hermaphrodite, Monoecious or Dioecious), and their pollination syndrome (if known).

Family	Species	Stems (%)	Basal area (%)	Flower structure	Pollination syndrome
Malvaceae	*Sterculia tragacantha* Lindl.	26.9	18.6	Hermaphrodite	Entomophilous
Fabaceae	*Pterocarpus erinaceus* Poir.	10.7	12.8	Hermaphrodite	Entomophilous
Chrysobalanaceae	*Maranthes polyandra* (Benth.) Prance	6.8	5.9	Hermaphrodite	Chiropterophilous
Combretaceae	*Terminalia glaucescens* Planch . ex Benth.	6.4	6.0	Hermaphrodite	Entomophilous
Sapotaceae	*Manilkara obovata* (Sabine & G.Don) J.H.Hemsl.	6.0	7.8	Hermaphrodite	Entomophilous
Phyllanthaceae	*Bridelia ferruginea* Benth.	3.8	2.3	Monoecious	Entomophilous
Fabaceae	*Pericopsis laxiflora* (Baker) Meeuwen	3.8	2.9	Hermaphrodite	Unknown
Combretaceae	*Terminalia avicennioides* Guill. & Perr.	3.8	3.7	Hermaphrodite	Entomophilous
Anacardiaceae	*Lannea velutina* A.Rich.	3.4	5.4	Monoecious	Entomophilous

2.3.2.1.3   Savannah (KOG05)

The Savannah plot is located 7°18'04.1"N, 1°09'53.8"W and 186 m asl. There are 26 recorded species at >10 cm DBH, of which eight occur at >3% (Table 2.4), the most abundant of which, by percentage of stems are *Bridelia ferruginea* Benth.(27.7%), *Pterocarpus erinaceus* (10.7%) and *Uapaca togoensis* Pax (8.1%). The vegetation of White (1983) represented by the highest number of species is "Sudanian woodland" (13 taxa), with "Guineo-Congolian secondary grassland and wooded grassland" as the second most represented biome (11 taxa). The understorey consists mainly of tall (>1 m in height) grasses, with other herbaceous taxa such as members of the Asteraceae. The average tree height is estimated to be between 10-12 m. The diversity (Shannon index) of the Savannah plot is 2.56.

Table 2.4: Species that occur at >3% in the Savannah vegetation plot (KOG05), their reproductive strategy (Hermaphrodite, Monoecious or Dioecious), and their pollination syndrome (if known).

Family	Species	Stems (%)	Basal area (%)	Flower structure	Pollination syndrome
Phyllanthaceae	*Bridelia ferruginea* Benth.	27.7	16.8	Monoecious	Entomophilous
Fabaceae	*Pterocarpus erinaceus* Poir.	10.7	16.7	Hermaphrodite	Entomophilous
Phyllanthaceae	*Uapaca togoensis* Pax	8.1	5.4	Dioecious	Unknown
Combretaceae	*Terminalia glaucescens* Planch. ex Benth.	6.4	11.1	Hermaphrodite	Entomophilous
Combretaceae	*Anogeissus leiocarpa* (DC.) Guill. & Perr.	6.0	10.3	Hermaphrodite	Unknown
Meliaceae	*Trichilia emetica* Vahl	6.0	3.1	Hermaphrodite	Entomophilous
Sapotaceae	*Vitellaria paradoxa* C.F.Gaertn.	5.4	7.9	Hermaphrodite	Entomophilous
Anacardiaceae	*Lannea velutina* A.Rich.	3.3	3.8	Monoecious	Entomophilous

2.4   Methods

2.4.1   Field Methods

Modern pollen rain was collected using pollen traps, composed of a plastic funnel (diameter 140 mm), glass fibre filter paper affixed to the funnel using bathroom sealant, and cotton wool fibre filling the rest of the funnel, held in place with plastic netting secured around the funnel using plastic-coated wire, following (Gosling et al. 2003).
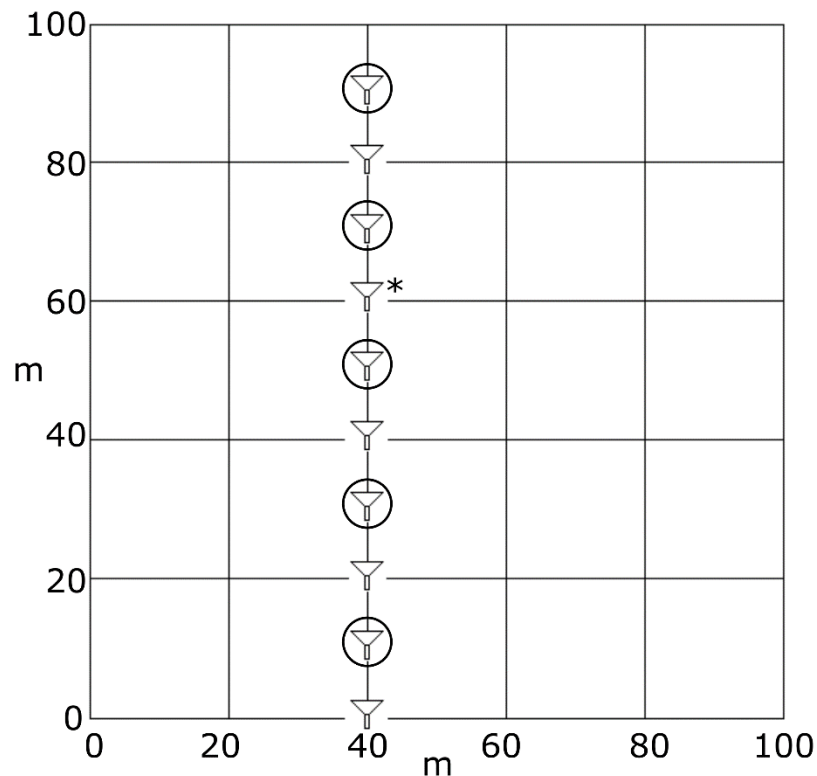
Figure 2.4: Diagram showing layout of pollen traps within vegetation survey plots. Circles indicate traps sampled (odd numbers are black circles e.g. Trap 51, Trap 53, Trap 55 in Savannah, Trap 61, 63 etc. in Transition and Trap 71, 73 etc. in Forest). The asterisk indicates Trap 76 in the Forest plot, which was sampled instead of Trap 75, due to poor condition of Trap 75.

A total of 30 traps, 10 per vegetation plot (1 ha; 100 m x 100 m) were deployed, along the 40 m line (i.e. a 100 m long transect across the plot, at the point 40 m along from the 0 m 0 m point of the plot) (Figure 2.4). All traps were positioned at approximately 50 cm above ground level to reduce risk of inundation and to ensure standard height of sampling across this study. The traps were affixed to stakes hammered firmly into the ground or, in cases where that is not possible, to trees. The distance from each trap to the closest large tree (>10 cm DBH) was recorded for all sites. Upon collection, traps were emptied of their contents, which was placed into sealed plastic bags and stored in a refrigerator.

The traps were in the field between October 2013 and October 2014, except for a short period during February during which all scientific equipment was removed from the plots to avoid destruction in the annual burn; equipment in the plots was removed and re-deployed by researchers from the Forestry Institute of Ghana (FORIG).

2.4.2   Laboratory Methods

Five traps per plot were processed and counted, to provide good statistical coverage of the plot whilst avoiding unnecessary effort (Gosling et al. 2005). Samples were processed following the method for cotton wool based traps from Gosling et al. (2003), in which cotton wool is removed by acetolysis and filter paper removed using hydrofluoric acid. Eight *Lycopodium* tablets per trap were added as an exotic marker to enable the calculation of pollen concentrations (Stockmarr 1971); University of Lund batch number 124961, containing 12542 +/- 931 spores per tablet. Samples were mounted in glycerol and counted at x400 magnification using a Nikon Eclipse 50i microscope. Pollen taxa were identified using atlases relating to tropical West Africa (van Campo 1974; Ybert 1979; Riollet & Bonnefille 1980; Gosling et al. 2013) and the African Pollen Database (Vincens et al. 2007).

2.4.3   Statistical Methods

Pollen counts were recorded digitally (Valencia 2014), and sample specific estimate count sizes required to capture the major features of pollen rain were generated (Keen et al. 2014), which returned count sizes that varied between 300-800 grains per sample. Data were visualised in plotting programme C2 (Juggins 2007). Pollen types were allocated numbers sequentially from 1 upwards during counting, and taxonomic information was later assigned to these types. Pollen type information can be found in Appendix IV and at (Julier & Gosling 2017).

The relationship between pollen and vegetation abundance was calculated by dividing average percentage abundance of pollen in each trap by percentage basal area of the corresponding taxon in the vegetation plot; the degree of under- or over-representation is then expressed as a R-rel$_{(av)}$ following Gosling (2009), i.e. values >1 indicate a taxon is over-represented in the pollen relative to the vegetation. Characteristic taxa were designated as taxa that occurred in at least four out of five samples from a plot, and at ≥3 % abundance in one sample or more. These conditions were chosen as they ensure that the taxa designated as characteristic are well-represented spatially in the plot (present in at least four of five traps) and represented by enough pollen grains (minimum of nine grains in smallest count of 300) to decrease the chance that they were anomalous or from far outside the bounds of the plots.

Non-Metric Multidimensional scaling (NMDS) using the Bray-Curtis distance metric was used to ordinate the data. Wisconsin double-standardisation, which standardises taxa counts to their taxon maxima and sample counts to their sample size, was used to minimise the effect of rare and very abundant taxa, and to reduce the effect of sample size. Singletons (taxa that only occur in one sample and as such provide no useful grouping information) were removed. NMDS is considered an appropriate ordination technique for count data as it is non-parametric (Oksanen et al. 2015) and has previously been used to analyse fossil and modern pollen assemblages (Jardine & Harrington 2008; Schüler et al. 2014). The Shannon index was used to calculate diversity, as this metric has been shown to give equal weight to rare and abundant taxa (Morris et al. 2014). All statistical analyses were conducted using R statistical software (R Core Team 2016) with R Studio (RStudio 2012) and with the package Vegan (Oksanen et al. 2015). Code for all analyses run in this section may be found in Appendix I.

2.5   Results

2.5.1   Modern pollen rain

Of the 30 traps deployed in Kogyae, 30 were recovered and every other trap (odd numbers) was processed and counted, to give five traps from each plot and an even coverage of the plot. Two taxa from Kogyae had mean abundances of > 10.0%: Poaceae (minimum 17.0%, maximum 60.9%, mean 28.8%) and Melastomataceae/Combretaceae (minimum 1.0%, maximum 72.7%, mean 18.8%), and four taxa were present in all traps: *Celtis*, *Alchornea*, Poaceae and Melastomataceae/Combretaceae (Figure 2.5). In total, 121 pollen taxa were defined in Kogyae, of which 37 were assigned botanical affinities.
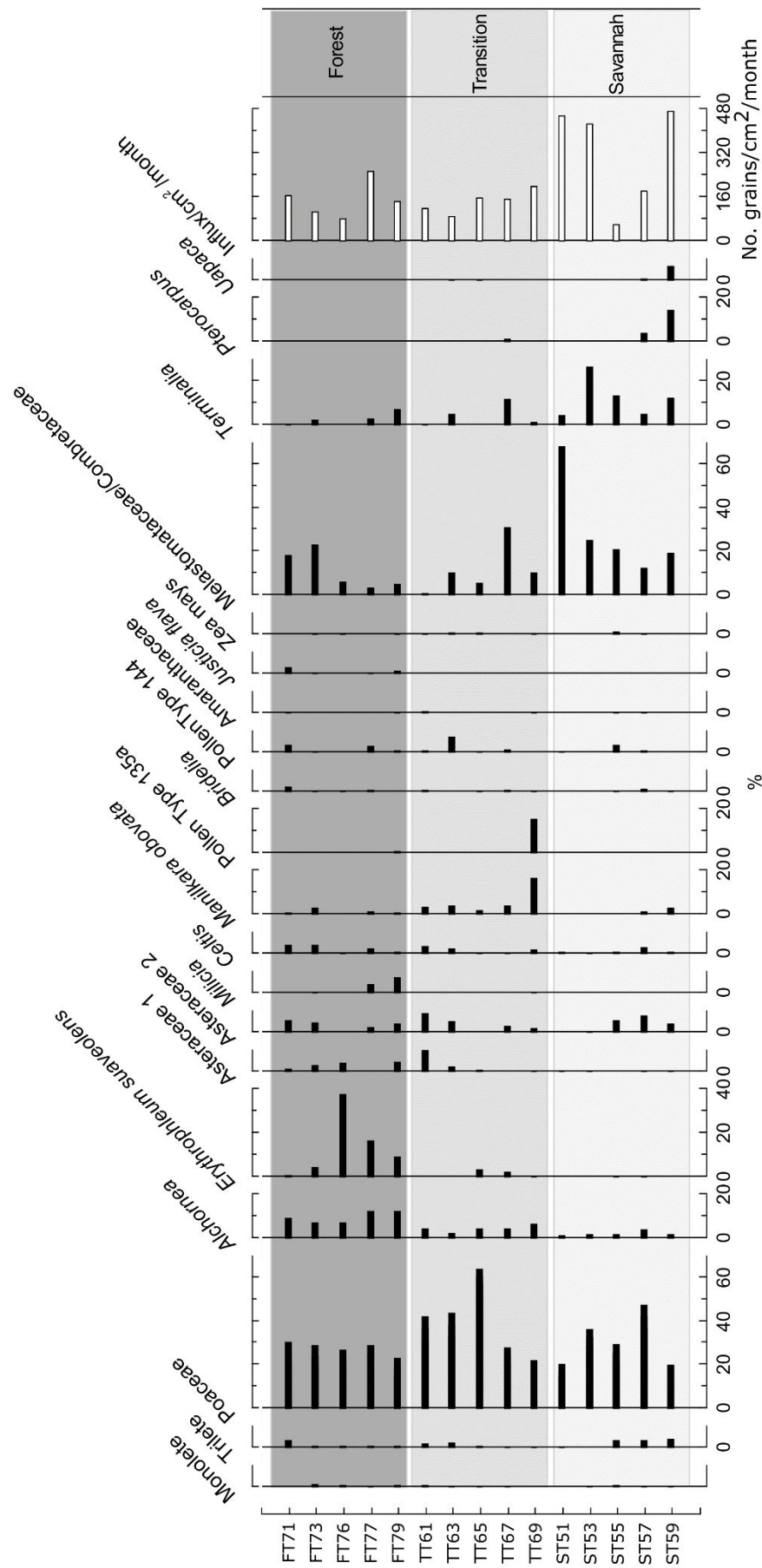
Figure 2.5: Pollen diagram showing pollen assemblages from each plot, along with total Influx values for each trap. Forest traps begin 'FT' Transition traps 'TT' and Savannah Traps 'ST'.

2.5.2   Forest

The Forest traps displayed broadly similar pollen assemblages, with Poaceae accounting for 19.0 – 25.0% of grains, and with a consistent presence of *Alchornea* (7.4-12.6%) and *Erythrophleum* (1.0-38.0%). Melastomataceae/Combretaceae was present in all traps, but varied in abundance from 3.6-23.0%. *Celtis*, although present in all of the traps, varied from 0.5-4.0% of the assemblage. Both Asteraceae types (Asteraceae 1 and Asteraceae 2) were present in four out of the five Forest traps, albeit in low abundances (1.8-5.8%). *Milicia* was present in three of the traps, from 0.3-7.0%. Nine taxa were identified in both the pollen and vegetation assemblages, these were *Afzelia africana* (R-rel$_{(av)}$ = 0.02)*, Bombax* (R-rel$_{(av)}$ = 0.02), *Celtis* (R-rel$_{(av)}$ = 1.44)*, Erythrophleum suaveolens,* (R-rel$_{(av)}$ = 3.64), *Ficus* (R-rel$_{(av)}$ = 0.24), *Lannea* (R-rel$_{(av)}$ = 0.05), *Manilkara obovata* (R-rel$_{(av)}$ = 0.61), Melastomataceae/Combretaceae (R-rel$_{(av)}$ = 6.37), and *Milicia* (R-rel$_{(av)}$ = 0.89) (Table 2.5, Figures 2.6 and 2.7). Within the Forest plot, 37 vegetation and 86 pollen taxa were identified, with nine taxa identified in both. The influx rates in this plot varied between 85 grains/cm^2/month and 258 grains/cm^2/month, with an average of 154 grains/cm^2/month and the diversity of the samples ranged from 2.0-2.9, with a mean of 2.5 (Figure 2.8).

Table 2.5: Taxa that occur in both pollen assemblages and vegetation survey for the Forest plot, their abundances in the data set and their R-rel$_{(av)}$ (Average pollen abundance in samples/% basal area of plot covered by vegetation taxon) values.

Taxon	No. stems > = 10 cm DBH	Stems in vegetation (%)	Basal area (% of plot area)	Average pollen abundance (%)	R-rel$_{(av)}$(Average pollen abundance/Basal area)
Afzelia africana	7	3.6	5.84	0.12	0.02
Bombax	4	2.0	2.56	0.05	0.02
Celtis	5	2.7	1.58	2.28	1.44
Erythrophleum suaveolens	9	4.8	4.42	16.10	3.64
Ficus	2	1.0	0.46	0.11	0.24
Lannea	5	2.7	2.19	0.10	0.05
Manilkara obovata	3	1.5	2.04	1.25	0.61
Melastomataceae /Combretaceae	2	1.1	1.67	10.64	6.37
Milica	2	1.0	2.62	2.33	0.89
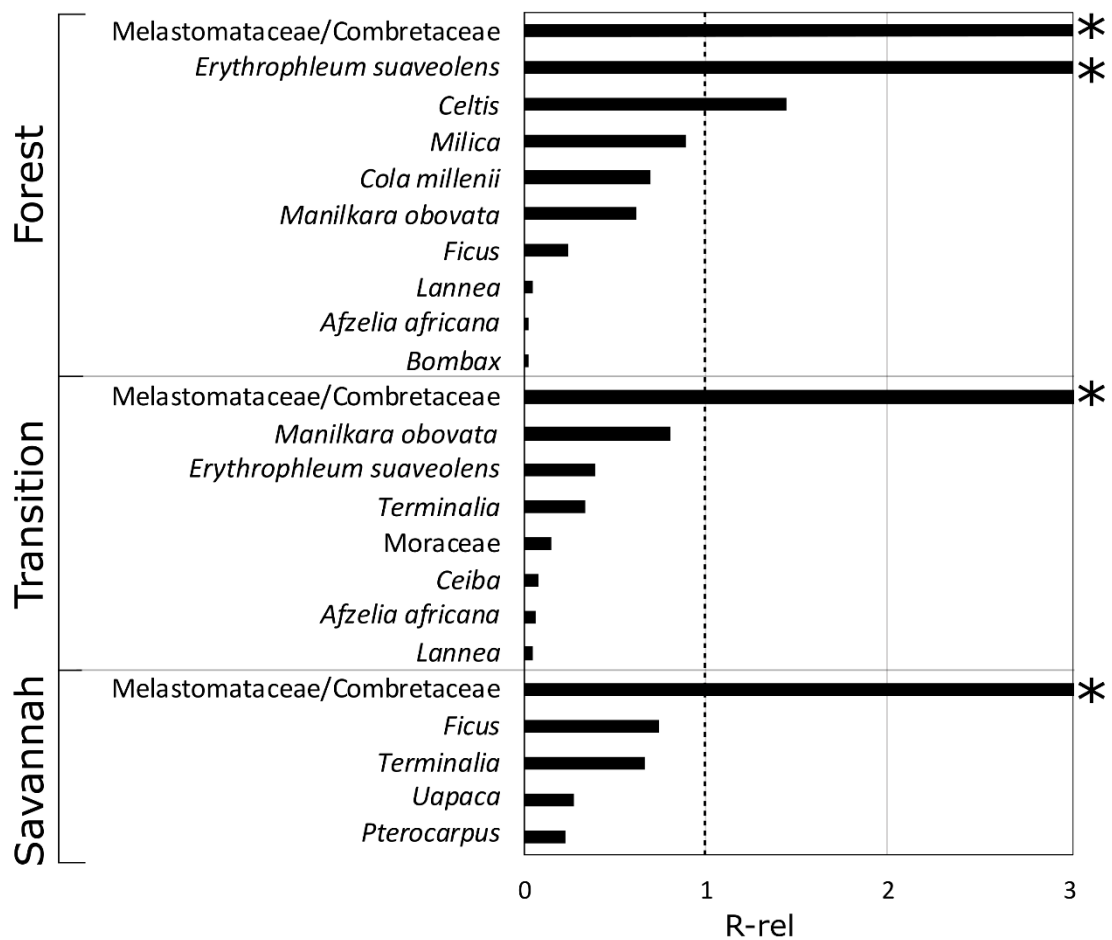
Figure 2.6: Bar chart showing R-rel values of taxa represented in both pollen and vegetation. The asterisks indicate that the R-rel values were too high to show on the figure; the Forest Melastomataceae/Combretaceae R-rel = 6.37 and *Erythrophleum suaveolens* = 3.64. The Transition Melastomataceae/Combretaceae R-rel = 5.18, and the Savannah Melastomataceae/ Combretaceae = 3.53

2.5.3   Transition

Poaceae pollen was present in all the Transition traps, accounting for at least 19.0% and at most 60.0% of the pollen sum. *Alchornea* was present in abundances of 2.0-7.0%. *Erythrophleum* was present in three traps in abundances of <5%. The Melastomataceae/Combretaceae signal ranged from 0.9% to 31.5% between the five traps. The two Asteraceae types accounted for up to 5.0% each of the pollen sum, but with variable percentage abundances (1.2-10.0%), and with neither being present in all five traps. *Manilkara obovata* accounted for between 2.3% and 4.5% of the pollen sum in four traps, but 16.9% in the fifth. 'Pollen type 135a' was only present in Trap 69, at 15.5%. Seven taxa that were present in both the vegetation and pollen assemblages were *Afzelia africana* (R-rel$_{(av)}$ = 0.06), *Ceiba* (R-rel$_{(av)}$ = 0.08), *Erythrophleum suaveolens* (R-rel$_{(av)}$ = 0.39), *Lannea* (R-rel$_{(av)}$ = 0.05), *Manilkara obovata* (R-rel$_{(av)}$ = 0.80), Melastomataceae/Combretaceae (R-rel$_{(av)}$ = 5.18), *Moraceae* (R-rel$_{(av)}$ = 0.15), and *Terminalia* (R-rel$_{(av)}$ = 0.34) (Table 2.6, Figures 2.6 and 2.7).

Within the Transition plot, 34 vegetation and 71 pollen taxa were identified, with eight taxa identified in both. Influx rates in this plot varied from 92-204 grains/cm^2/month, with an average of 146 grains/cm^2/month and diversity of the traps ranged from 1.8-2.5, with an

Table 2.6: Taxa that occur in both pollen assemblages and vegetation survey for the Transition plot, their abundances in the data set and their R-rel$_{(av)}$ (Average pollen abundance in samples/% basal area of plot covered by vegetation taxon) values.

Taxon	No. stems > = 10 cm DBH	Stems in vegetation (%)	Basal area (% of plot area)	Average pollen abundance (%)	R-rel$_{(av)}$ (Average pollen abundance/Basal area)
Afzelia africana	3	1.3	3.39	0.20	0.06
Ceiba	2	0.9	2.34	0.18	0.08
Erythrophleum suavolens	6	2.6	4.09	1.60	0.39
Lannea	10	4.3	5.81	0.28	0.05
Manilkara obovata	14	6.0	7.85	6.31	0.80
Melastomataceae /Combretaceae	4	1.7	2.25	11.65	5.18
Moraceae	2	0.9	0.53	0.08	0.15
Terminalia	24	10.2	9.70	3.28	0.34

average of 2.3 (Figure 2.8).

2.5.4 Savannah
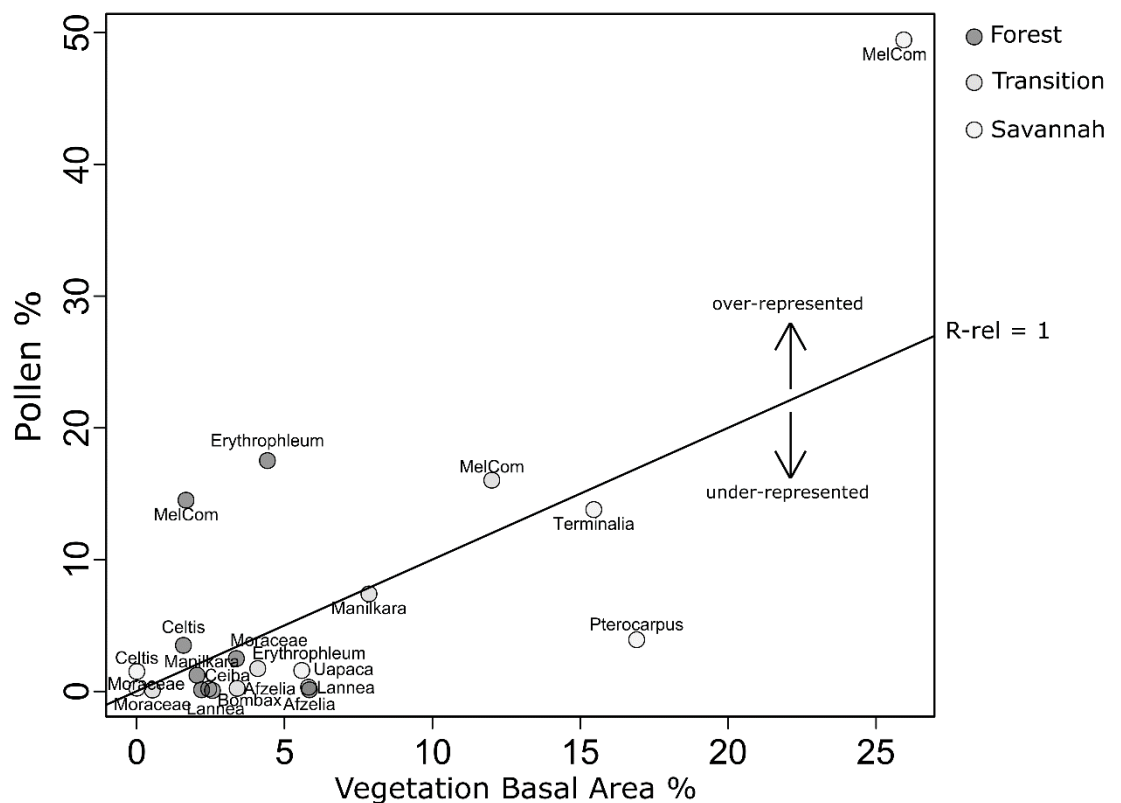
Figure 2.7: Scatter plot of Vegetation Basal Area % against Pollen Abundance %. Taxa names are as in manuscript, except Melastomataceae/Combretaceae, which is abbreviated to 'MelCom'. R-rel = 1 line is added to illustrate those taxa that are over-represented in the pollen compared to the vegetation (above the line) against those which are under-represented (below the line).

The traps exhibited largely similar pollen assemblages, with Poaceae and Melastomataceae/Combretaceae contributing more grains than any other taxa. Poaceae percentages were between 17.0-42.4%, and Melastomataceae/Combretaceae grains contribute 12.5-68.1%. *Alchornea* was present in all traps, but at <3.0% in four. Asteraceae 2 was present in three traps at percentages of >3.0%, but not in the other two. *Celtis* was present in four out of the five traps, but at abundances of <3.0%. *Pterocarpus* pollen was found at 4.3% and 14.9% in two traps. *Uapaca* was present in the same two traps (57 and 59) as *Pterocarpus*, at 0.9% and 6.5% respectively. *Terminalia* was present in all of the Savannah traps, from 4.6-27.1%. Five taxa were present in both the vegetation and pollen assemblages: *Ficus* (R-rel$_{(av)}$ = 0.74), Melastomataceae/Combretaceae (R-rel$_{(av)}$ = 3.53), *Pterocarpus* (R-rel$_{(av)}$ = 0.22), *Terminalia* (R-rel$_{(av)}$ = 0.66), and *Uapaca* (R-rel$_{(av)}$ = 0.27) (Table 2.7, Figure 2.6 and 2.7). Within the Savannah plot, 26 vegetation and 67 pollen taxa were identified, with five taxa identified in both. Influx rates for Savannah traps varied from 61-478 grains/cm^2/month (Trap 55 has an unusually low influx rate compared to the other traps) with an average of 322 grains/cm^2/month and the diversity of the traps ranged from 1.2-2.6, with an average of 2.1 (Figure 2.8).

Table 2.7: Taxa that occur in both pollen assemblages and vegetation survey for the Savannah plot, their abundances in the data set and their R-rel$_{(av)}$ (Average pollen abundance in samples/% basal area of plot covered by vegetation taxon) values.

Taxon	No. stems > = 10 cm DBH	Stems in vegetation (%)	Basal area (% of plot area)	Average pollen abundance (%)	R-rel$_{(av)}$ (Average pollen abundance/Basal area)
Ficus	1	0.5	0.23	0.17	0.74
Melastomataceae /Combretaceae	11	6.0	10.40	36.70	3.53
Pterocarpus	24	13.0	16.70	3.74	0.22
Terminalia	23	12.5	14.80	9.80	0.66
Uapaca	15	7.8	5.44	1.49	0.27
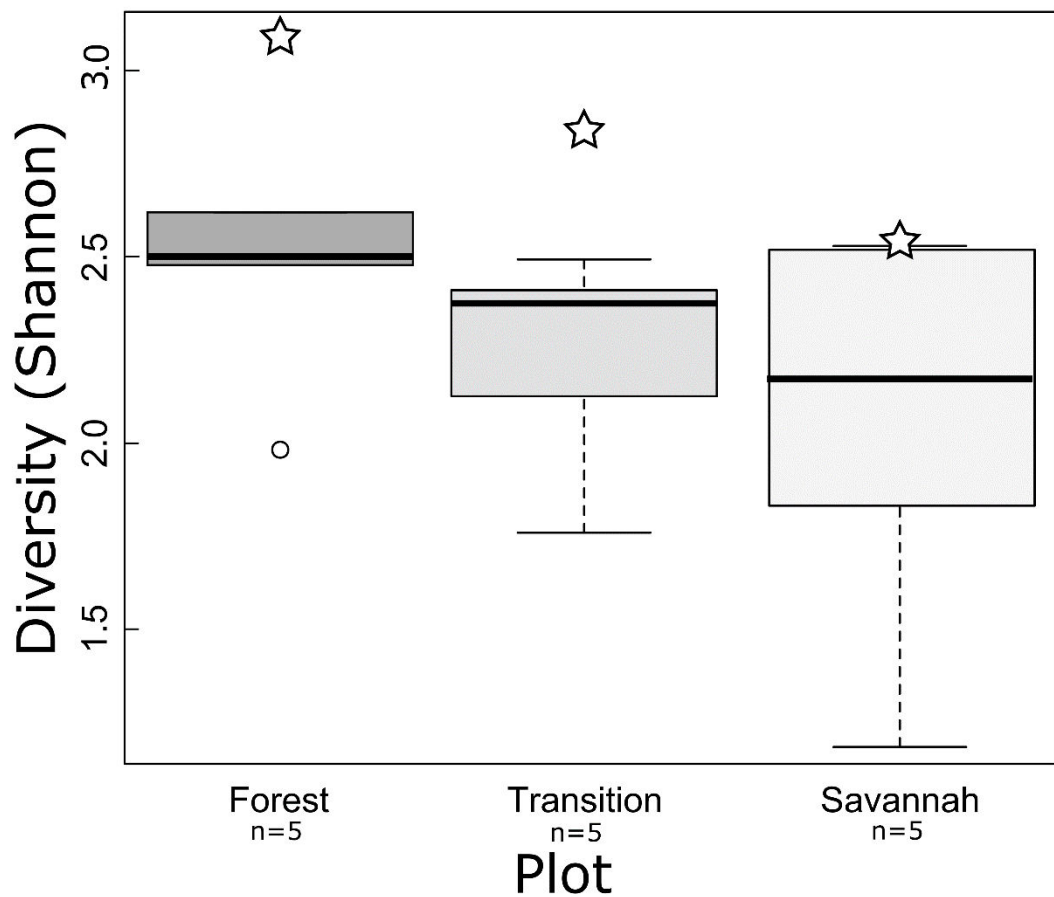
Figure 2.8: Diversity of the pollen assemblages (box and whisker plots) and the vegetation diversity (> 10 cm DBH) of the plots (star symbols). Boxes show 25th and 75th percentiles of data, bars near the middle of the boxes show the median value, and ends of whiskers show the extremes of the data, as long as those are not more than 1.5 inter-quartile ranges from the 25th and 75th percentiles. For 'Forest' the diversity data are not very variable, leading to its whiskers being short, and its outlying values not being joined by a whisker.

2.5.5   Multivariate analysis

The NMDS plot (Figure 2.9) shows the distribution of the samples (indicated by black, capital letter e.g. 'FT71' = Forest, Trap 71) in ordination space. The Forest and Savannah samples form two distinct groups in ordination space, separated on NMDS 2. The Transition samples cluster on NMDS 1, but are distributed widely over NMDS 2, and overlap with the ordination space occupied by Savannah samples. Abundant pollen taxa from the plots fall out with their parent vegetation types, such as *Erythrophleum suaveolens* with the Forest traps, *Manilkara obovata* with the Transition traps and *Uapaca* with the Savannah traps. Poaceae is positioned roughly centrally to the distribution of all of the samples.
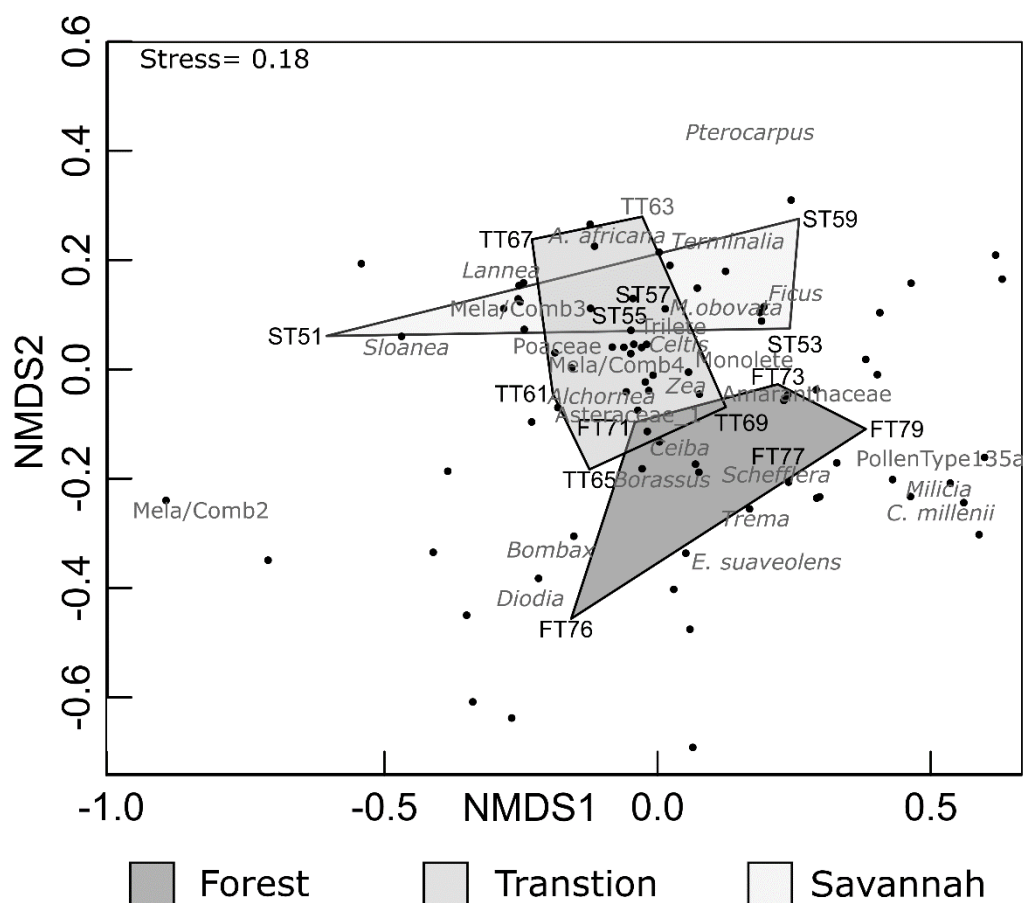
Figure 2.9: NMDS plot of pollen trap assemblages. Forest traps begin 'FT' Transition traps 'TT' and Savannah Traps 'ST'. Polygons encompass all traps of the each plot. Named taxa are included in grey. Un-identified pollen taxa were used in the analysis but are not shown on the figure.

2.6   Discussion

The data presented here suggest that it is possible to differentiate between vegetation types within a forest-savannah transition zone using their pollen assemblages. Here, characteristic pollen taxa from each plot are defined, along with more minor elements of the pollen rain

which do not meet the definition of 'characteristic' but still merit discussion based on their low abundance in a high number of traps, or high abundance in a small number of traps. Pollen-vegetation relationships are then discussed, the three plots are compared and the implications of these data for interpreting the fossil pollen record are considered, particularly with reference to the fossil pollen record of Lake Bosumtwi, Ghana (Miller & Gosling 2014; Miller et al. 2016).

2.6.1   Characterisation of plots by their pollen rain

2.6.1.1   Forest

The Forest traps are relatively homogenous in terms of pollen assemblage, influx values, and diversity (Figure 2.5 and Figure 2.7). The characteristic pollen taxa in the Forest plot were Poaceae, *Alchornea*, *Erythrophleum suaveolens* and Melastomataceae/Combretaceae (Figure 2.5). Of these characteristic taxa, *Erythrophleum suaveolens* (1.0-38.0% of the pollen sum) and Melastomataceae/Combretaceae (3.6-23.2% of the pollen sum) exhibited the most variation between traps. The other characteristic taxa had less variable signals, with Poaceae accounting for between 19.1-26.0% and *Alchornea* between 7.4-12.6% of the pollen sum.

Only one of the taxa that accounts for more than 3% of stems in the vegetation inventory was identified in the pollen signal as characteristic; *E. suaveolens*. *E.suaveolens* is widespread across tropical Africa (Hawthorne & Jongkind 2006), but is predominantly a tree of dry semi-deciduous Guineo-Congolian forest (Gorel et al. 2015), and as such provides a useful indicator of the Forest plot in this study. It is also strongly over-represented in this plot, with a R-rel$_{(av)}$ ratio of 3.64.

Melastomataceae/Combretaceae was a characteristic pollen taxon (up to 38.0% of the pollen sum). It did not, however, account for a large component of the vegetation inventory, comprising just 1.6% of the basal area of the plot. Consequently, the R-rel$_{(av)}$ value for this group was 6.37. This large R-rel$_{(av)}$ value could be due to the over-production of pollen by Combretaceae, in which andromonoecious inflorescences are known to occur (Watson & Dallwitz 1992). There are no woody Melastomataceae recorded in the Forest plot, but there may be understorey plants which contribute to the pollen signal. Any herbaceous Melastomataceae would likely, however, contribute only a small amount of pollen, as many Melastomataceae are buzz-pollinated, meaning that their pollen is not freely released into the air (Jones & Little 1983).

Together, the Asteraceae pollen types are characteristic of the Forest plot, although Asteraceae is absent from the vegetation survey. The absence of Asteraceae in the vegetation survey is likely due to it being represented by herbaceaous taxa which were not recorded in

the inventory, potentially including the invasive Asteraceae *Chromolaena odorata* (L.) King and Robinson which has been reported widely in Ghana and in the Kogyae Strict Nature Reserve (Castel 2012).

One of the characteristic pollen taxa of the Forest plot is *Alchornea*, which occurs in all traps at percentages of up to 12.6%. This taxon is not, however, present in the vegetation survey for the plot. This could be due to one of several factors: i) that *Alchornea* is genuinely not present in the vegetation plot, but is present in the surrounding vegetation; ii) that the pollen grain identified as *Alchornea* is a misidentification and originates from a different plant, or iii) that the *Alchornea* plants contributing to the signal possess stems that are too small to be included in the vegetation surveys, which only records plants of >10 cm DBH. It is likely that the latter is true of *Alchornea cordifolia* (Schumach. & Thonn.) Müll.Arg, which is a commonly recorded species in Guineo-Congolian Transition woodland and exhibits a scandent habit, making it likely that it could have been excluded from surveys and therefore account for the apparently orphan *Alchornea* pollen.

Taxa that occurred at >3% but in less than four traps included *Milicia* and *Celtis*. *Milicia* accounted for >3% of the pollen sum in two traps, although it was present in three, and *Celtis* only occurred at >3% in two traps, although was present in four. The low abundances of *Milicia* and *Celtis* mirror their abundance in the vegetation, which is surprising, as both the Moraceae (the family to which *Milicia* belongs) and *Celtis* have previously been found to be over-represented in pollen rain (Gosling et al. 2005; Bush et al. 2011). Possible reasons for their relatively low representation compared to similar studies could relate to over-production by Melastomataceae/Combretaceae and Poaceae swamping the traps. It is also possible that due to only one year of data being available, the climatic conditions may not have favoured flowering that year, or a biological factor such as a fungal infection or pest could have prevented the *Celtis* individuals from producing as much pollen as usual.

There were 27 vegetation taxa whose pollen was not identified in the pollen assemblages, including all but two of the most abundant species recorded in the vegetation surveys (*Erythrophleum suaveolens* and *Afzelia africana*). This abundance of palynologically silent taxa could be due to their entomophilous pollination syndromes (Table 2.1), climatic conditions not favouring flowering in the year 2013-2014, or the individuals of those taxa being located in the plot in such a way as to make pollen deposition unlikely. Although the main over-producing taxa such as Poaceae and Melastomataceae/Combretaceae may have 'messy' or anemophilous pollination syndromes, there does not seem to be a clear link between taxa that are entomophilous or zoophilous and absent from the pollen assemblages in this system,

despite this having been shown to be the case in other studies of modern pollen rain (Bush & Rivera 2001; Gosling et al. 2005).

2.6.1.2   Transition

Characteristic taxa for the Transition plot were Poaceae, *Alchornea*, Melastomataceae/Combretaceae and *Manilkara obovata*. The percentage abundances of Poaceae (19.1-61.1%), Melastomataceae/Combretaceae (1.0-31.5%) and *M. obovata* (2.3-16.9%) vary considerably between traps. *Alchornea* (2.7-6.8%) does not exhibit such variability.

Melastomatacee/Combretaceae were over-represented (R-rel$_{(av)}$ = 5.18), likely due to over-production by Combretaceae. All other taxa identified in both pollen and vegetation in this plot, besides Poaceae, were under-represented. *M. obovata* exhibits the closest to 1:1 ratio in this plot, of 0.8 (Figure 2.6). Although *M. obovata* is primarily a forest species (White et al. 1983), it is characteristic of this particular plot's pollen assemblage. It has entomophilous, hermaphroditic flowers whose anthers protrude from the perianth, potentially allowing pollen to be released more freely than from a closed flower and resulting in a higher R-rel value of this taxon than might be expected for an entomophilous taxon.

Four taxa are present at percentages of >10% in only one trap. These are Asteraceae types 1 and 2, Type 135a and *Terminalia*. *Terminalia* accounts for 10.2% of the stems in this plot, but does not contribute a characteristic level of pollen to the assemblages, instead being under-represented (R-rel$_{(av)}$=0.34).

The Moraceae in the Transition plot were under-represented (R-rel$_{(av)}$ = 0.15), as were *Ceiba* (R-rel$_{(av)}$ = 0.08), *Afzelia africana* (R-rel$_{(av)}$ = 0.06) and *Lannea* (R-rel$_{(av)}$ = 0.05). Of these, the Moraceae, *Ceiba*, and *A. africana* were all only represented by a small number of individuals (two, two and three individuals respectively), meaning that pollen production may have been genuinely low for these taxa. *Lannea* is represented by 10 individuals, however, and has the lowest R-rel ratio (0.05), indicating that it is most under-represented. These taxa are all monoecious or hermaphroditic, and insect pollinated, leading to no clear explanation of why they might be under-represented to different extents.

Alchornea and *Celtis* were present in the pollen (2.5-6.7% and 0.7-3.8% of pollen sum respectively) but not in the vegetation. As *Celtis* pollen was present in all three plots, but only in one of the vegetation surveys (Forest), this could be the result of transport of pollen from outside the plot.

2.6.1.3   Savannah

The characteristic pollen taxa of the Savannah plot were Poaceae (14.1- 42.4%),

Melastomataceae/Combretaceae (12.5-68.1%), and *Terminalia* (4.6- 27.1%) and *Alchornea* (1.8

– 4.4%). *Celtis* (0.6 – 2.9%). was present in at least four out of five of the traps, but did not

account for more than 3% of the pollen sum in any trap. Taxa that accounted for >3% in at

least one trap but were not present in at least four included *Pterocarpus* (0.0- 14.9%) and

Uapaca (0.0- 6.6%)

The pollen assemblage in the Savannah plot exhibited an over-representation of

Melastomataceae/Combretaceae (R-rel$_{(av)}$ = 3.53). Under-represented taxa were *Pterocarpus*

(R-rel$_{(av)}$ = 0.22)*, Terminalia* (R-rel$_{(av)}$ = 0.66) and *Uapaca* (R-rel$_{(av)}$ = 0.27). *Pterocarpus*, although

not a characteristic pollen type of the Savannah pollen assemblage, is a genus of Sudanian

Woodland (Novinyo et al. 2014). *Uapaca* is also a genus of wooded savannah ecosystems, but

is dioecious, and the genders of the trees in the Savannah plot are unknown, meaning that this

could be a reason for its under-representation despite its abundance in the vegetation.

One trap (Trap 55) was found to have a low pollen accumulation rate (62 grains/cm^2/month) in

comparison to the other Savannah plot traps, which exhibited concentration of between 187

and 475 grains/cm^2/month. Despite its low pollen accumulation rate, Trap 55 did not contain

an anomalous pollen assemblage, with similar percentages of abundant taxa to the other

traps, and a high diversity. It is possible that it was covered by a leaf or other debris for part of

the year, leading to less pollen accumulating in this trap than the other traps. We decided,

however, to include Trap 55 in the analysis despite its anomalously low pollen accumulation

rate, as we consider that it represents the pollen assemblage in a similar way to its sister traps.

The Savannah plot contains low levels of *Alchornea* (1.7- 4.4%), despite none being present in

the vegetation survey of the site. *Celtis* was present in the pollen assemblages at low

abundance (present in one trap at 2.9%) but was not recorded in the vegetation, therefore

possibly representing transport from outside the plot.
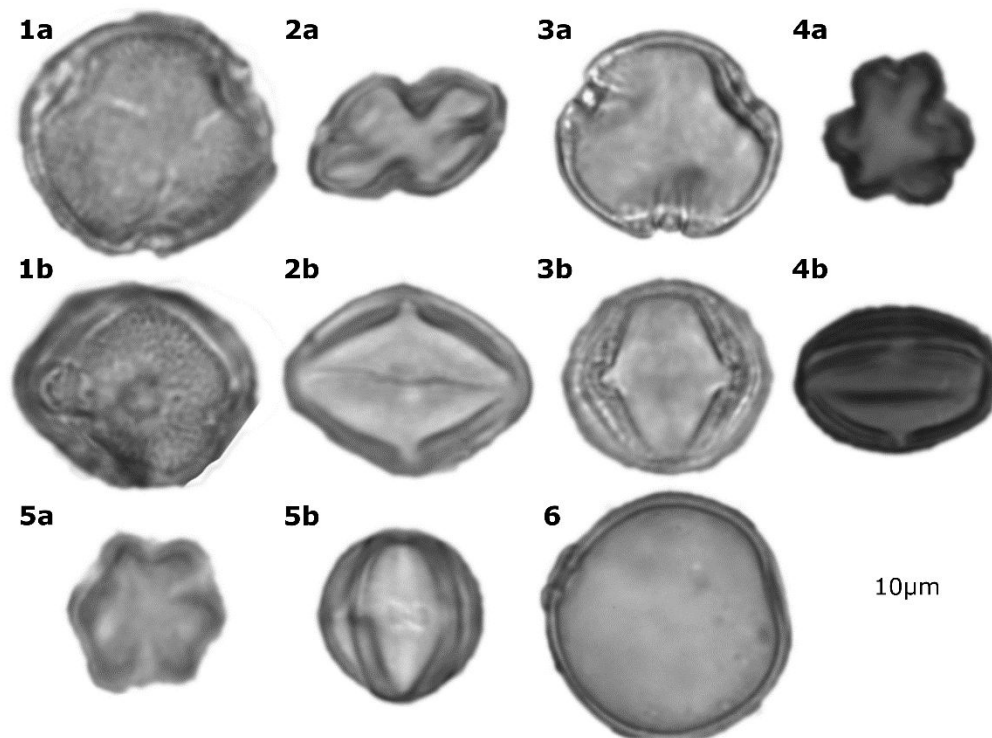
Figure 2.10: Plate showing characteristic taxa from all plots. 1 = *Erythrophleum suaveolens*, 2 = *Manilkara obovata*, 3 = *Alchornea*, 4 = Melastomataceae/Combretaceae 5 = *Terminalia* sp 6 = Poaceae sp. 'a' and 'b' denote polar and equatorial views of the same grain.

2.6.2   Differentiation of Plots

Despite their close geographical proximity within the forest-savannah mosaic, the plots give rise to different palynological assemblages, in both the relative abundances of common taxa and the presence or absence of rarer taxa. The pollen assemblages differ in abundance of dominant, over-producing taxa, such as Poaceae and Melastomataceae/Combretaceae, but there are also differences in the less abundant components of the assemblages such as *Erythrophleum suaveolens* in the Forest plot, *Manilkara obovata* in the Transition, and *Pterocarpus* in the Savannah plot. The differences in pollen assemblage composition reflect the unique vegetation assemblage in each plot. The R-rel values for taxa are often not consistent across plots; although Melastomataceae/Combretaceae is consistently over-represented in the pollen sum, the degree of over-representation varies widely (Figure 2.6 and Figure 2.7).

Erythrophleum suaveolens, whilst over-represented in the Forest plot (R-rel$_{(av)}$ = 3.64), is under-represented in the Transition plot (R-rel$_{(av)}$ = 0.39). As there are relatively few individuals in each plot (nine in the Forest and six in the Transition), this discrepancy in representation may be due to factors such as tree location in the plot, wind direction, tree height or openness. Although there are fewer individuals of *E. suaveolens* in the Transition plot, they account for a similar total percentage of the basal area as in the Forest plot, indicating that the Transition

plot individuals may be larger; a factor which does not appear to have influenced their representation in the pollen rain.

Terminalia is under-represented in both the Transition and Savannah pollen assemblages, but has a lower R-rel value in the Transition plot (R-rel$_{(av)}$ = 0.34) than in the Savannah plot (R-rel$_{(av)}$ = 0.66). This is despite it being more vegetatively abundant in the Transition plot than it is in the Savannah plot (24 stems in Transition, 23 in Savannah). *Terminalia* does account for a greater percentage of total stems and has a larger basal area percentage coverage in the Savannah plot than in the Transition, however, suggesting that *Terminalia* trees in the Savannah plot may be larger than those of the Transition.

Alchornea, is statistically 'characteristic' of all plots in this study, as it is present in the majority of traps and occurs above 3% in at least one trap in each plot. *Alchornea* is, however, a wind-pollinated taxon that is widely distributed across tropical Africa (Watrin et al. 2007). Anemophily, and its prevalence in all plots and means it is not a useful taxon when distinguishing between vegetation types within a forest-savannah transitional mosaic.

Poaceae, one of the most abundant taxa in all three plots, does not display an increase in percentage abundance from Forest to Savannah (Figure 2.5). Its influx values, however, increase along the gradient with the Forest having an average of 36 grains/cm^2/month, the Transition traps 51 grains/cm^2/month and the Savannah traps 79 grains/cm^2/month. Melastomataceae/Combretaceae pollen shows an increase in percentage abundance along the Forest-Transition-Savannah gradient, but also displays very high influx values in the Savannah plot (an average of 54 grains/cm^2/month) compared to the other two plots (an average of 8 grains/cm^2/month in the Forest plot, and 9 grains/cm^2/month in the Transition). This very high Melastomataceae/Combretaceae influx effectively masks the Poaceae signal in the Savannah plot and accounts for its similarity to the Transition and Forest plots in the percentage data.The absence of a clear differentiating signal from Poaceae percentage abundance in this study is illustrated both by its lack of clear increase from forest to savannah (Figure 2.5), and by its position in the ordination roughly centrally to all three plots (Figure 2.8). The absence of variation in the Poaceae signal differs from the findings of Vincens (Vincens et al. 2000) and Ybert (Ybert 1975), who both observed a strong increase in the percentage abundance of Poaceae pollen along the forest-savannah gradient in Cameroon and Côte d'Ivoire respectively. The discrepancy between this study and previous studies could be due to the swamping of pollen counts in previous studies by arboreal taxa such as *Celtis*, and a lack of Melastomataceae/Combretaceae type pollen. The studies by Vincens (2000) and Ybert (1975) encompass wider vegetational gradients than the plots used in this study, with samples

encompassing swamp wet forest types. Vincens (2000) used soil samples, meaning that their results may be representative of a longer time-period than ours. The clear increase in the absolute abundance of Poaceae pollen from Forest to Savannah observed in this study may, however, indicate that concentration data might be able to provide a more accurate representation of grass-dominated pollen assemblages than percentage data alone.

There are 67 pollen taxa in this dataset whose abundance is low enough (<3% in any trap) that it would not be informative or practical to include them in a pollen diagram, but whose presence may nevertheless contribute to the separation of the plots palynologically. The differences between the pollen assemblages of the plots are highlighted by the fact that the Forest and Savannah samples form two clearly separate groups in ordination space, with Transition traps overlapping with the Savannah (Figure 2.8). There were, however, a variety of pollen taxa which contributed to the spread of the data but which are not recorded in the vegetation plots including *Borassus*, *Schefflera*, *Sloanea*, *Diodia*, *Trema*, *Nesogordonia,* and *Pycnanthus*, as well as multiple distinctive pollen morphotypes. These taxa may represent extra-plot pollen, or may have been present in the plot, but not recorded due to being too small (< 10 cm DBH).

2.6.3   Pollen and vegetation diversity

In comparison to the diversity of the vegetation plots, the pollen assemblage diversity indices were lower: Forest pollen 2.7 against vegetation 3.1, Transition pollen 2.4 against vegetation 2.8, and Savannah pollen 2.2 against vegetation 2.6. The diversity data show a trend of increasing pollen diversity with increasing vegetation diversity (Figure 2.7), with the Forest traps showing less variation than the Transition traps, which in turn showed less than the Savannah traps. The large variability in the diversity of the Savannah plot pollen traps could have been due to a more open canopy, allowing for more extra-plot components to feature in the pollen rain. Statistical tests of this relationship were not attempted due to the small number of vegetation diversity measures (three) available. The positive relationship between vegetation and pollen diversity has been demonstrated in modern pollen traps (Jantz et al. 2014), and in lake sediments (dependent on the situation of the lake in question) (Felde et al. 2016), although there are difficulties involved with using these data to interpret fossil pollen assemblages due to features of depositional environments that may affect the relationship between pollen and parent vegetation in the fossil record (Odgaard 1999).

2.6.4   Implications for the Lake Bosumtwi record

In Lake Bosumtwi, shifts from forested to grassland ecosystems have been inferred from high levels (>55% abundance) of Poaceae in fossil samples (Miller & Gosling 2014; Miller et al. 2016). If this criterion were applied to the data presented here, just one of the traps would be considered grassland (Transition trap 65).

The palynological over-production of Poaceae likely accounts for the very high percentages of pollen observed during 'savannah' periods of the Bosumtwi record, as opposed to these representing an ecosystem that is more grass dominated and open than the Savannah plot of this study. If these periods were representative of a more grass dominated landscape, it might be expected that Sahelian elements would be observed within these, which is not the case (Miller & Gosling 2014). Determining which grass taxa contribute to the signal of sections of the Bosumtwi record that are very high in Poaceae may help to improve the interpretation of the parent vegetation; although Poaceae pollen is very difficult to identify to below family level using light microscopy, techniques are now being developed which may allow this analysis to be undertaken (Mander et al. 2013; Julier et al. 2016).

Even with the strong bias towards anemophilous taxa displayed in the fossil record, the forest stages identified in the Lake Bosumtwi record have higher Moraceae abundances than the traps in this study, indicating that the inference of some form of wooded environment is well-justified. However, in the forest zones of the Lake Bosumtwi record, taxa used to indicate a moist, broadleaf forest vegetation type include *Uapaca*, *Alchornea* and *Celtis*. *Uapaca*, in the samples analysed here, is only present in the Savannah plot (although it is not a characteristic taxon of this plot). *Alchornea* and *Celtis* are present in the pollen rain of the majority of trap samples from all three plots, at similar abundances as those observed in the Bosumtwi record. Melastomataceae/Combretaceae, along with Poaceae percentages of up to 40%, are used to indicate a moist, rainforest environment in the Bosumtwi record, but here are indicative of the Savannah plot. It is possible, therefore, that the ecosystems characterised in the Bosumtwi record as 'moist broadleaf forest' may represent drier vegetation types (the inferred rainfall of the Bosumtwi forest zones were 1000-3300 mm/year, whereas the rainfall at Kogyae, and within the transitional zone in general is 900-1200 mm/year) (Miller & Gosling 2014).The forest zone may include the transitional mosaic explored here, an observation that could have implications in the reconstruction of past climate change.

Data obtained from an artificial pollen trap (radius 70 mm) and a lake such as Lake Bosumtwi (radius 4 km) will vary considerably, with the former likely capturing a very local signal (within tens of metres of the trap), but the latter a heavily regional signal (likely 90% or

more of pollen regional) (Jacobson & Bradshaw 1981; Sugita 1994). The individual source areas for the traps in this study are likely to have varied, depending on the structure of the vegetation immediately surrounding them. Traps are, therefore, able to be considered as capturing a 'snapshot' of the pollen produced by a specific vegetation type, as opposed to the regional, time-averaged signal captured by large lakes (Kidwell & Flessa 1995). The spatial and temporal differences in the pollen signal obtained from traps and lakes should be taken into consideration when interpreting fossil records.

The observation that the Poaceae percentages in this study differ from those of the fossil record is not, alone, an indication that the interpretation of the fossil record needs to be re-examined. Poaceae is wind dispersed, highly productive, and therefore more likely to appear in high percentages in the sediment record as opposed to traps under the canopy, in relation to other, entomophilous or zoophilous taxa. When treated alone, Poaceae is an unreliable indictor taxon (Bush 2002). It is, therefore, sensible to use other taxa along with Poaceae to distinguish between vegetation types; the taxa outlined here may help to provide better constraints on the interpretation of transitional zones within pollen records.

2.7   Conclusions

This work has demonstrated that by using modern pollen traps deployed within vegetation plots, differences can be identified between the pollen assemblages produced by three vegetation types within a forest-savannah transitional mosaic landscape. Although there is not a straight forward relationship between pollen and vegetation assemblages, certain taxa can be used to indicate the different vegetation types, such as *Erythrophleum suaveolens* for the Forest, *Manilkara obovata* for the Transition, and *Pterocarpus* and *Uapaca* for the Savannah vegetation type. These taxa, and many other rarer taxa, contribute to the plots producing pollen assemblages that can be separated using multivariate methods, and to the biodiversity of the pollen assemblages reflecting that of the vegetation. The plots also differ in their percentage and influx values of Poaceae, with the influx of Poaceae better reflecting the gradient of forest to savannah than percentage data, a finding that has implications for the interpretation of the fossil record. Based on the insights gained from the modern pollen data, a re-examination of the fossil pollen record from Lake Bosumtwi may benefit from a lower threshold of Poaceae pollen to categorise an assemblage as being from a savannah or transitional ecosystem. Depending on other taxa present, this threshold could be as low as 40%. Future high resolution sampling of the Lake Bosumtwi record, from periods of transition between forest and savannah may benefit from the results outlined in this study, thereby improving knowledge of how vegetation responds to climate fluctuations in the past and

helping to shape predictions of how ecosystems might respond to climate change in the future.

3   Chapter 3: Chemotaxonomy as a tool for interpreting the cryptic diversity of Poaceae pollen

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3.1   Abstract

The uniform morphology of different species of Poaceae (grass) pollen means that identification to below family level using light microscopy is extremely challenging. Poor taxonomic resolution reduces recoverable information from the grass pollen record, for example, species diversity and environmental preferences cannot be extracted. Recent research suggests Fourier Transform Infra-red Spectroscopy (FTIR) can be used to identify pollen grains based on their chemical composition. Here, we present a study of 12 species from 8 subfamilies of Poaceae, selected from across the phylogeny but from a relatively constrained geographical area (tropical West Africa) to assess the feasibility of using this chemical method for identification within the Poaceae family. We assess several spectral processing methods and use K-nearest neighbour (k-nn) analyses, with a leave-one-out cross-validation, to generate identification success rates at different taxonomic levels. We demonstrate we can identify grass pollen grains to subfamily level with an 80% success rate. Our success in identifying Poaceae to subfamily level using FTIR provides an opportunity to generate high taxonomic resolution datasets in research areas such as palaeoecology, forensics, and melissopalynology quickly and at a relatively low cost.

3.2   Introduction

The correct identification of pollen grains is an important factor in any research area that uses pollen assemblages to make inferences about vegetation. These research areas can be as diverse as palaeoecology (Germeraad et al. 1968; Mander & Punyasena 2014), forensics (Horrocks et al. 1998; Mildenhall et al. 2006) and melissopalynology (Herrero et al. 2002; Martin 2005), as they all share a reliance upon the taxonomic resolution of pollen identification to maximise the accuracy and usefulness of their data. Looking further back into geological time, palynological research has played a fundamental role in understanding plant origination and radiation (e.g. the origin and radiation of vascular plants (Rubinstein et al. 2010) and the radiation of the angiosperms (Lupia et al. 1999) and shaped our understanding of how the terrestrial biosphere responded to mass extinction events (Tschudy et al. 1984; Looy et al. 2001). This highly diverse group of studies all share a reliance upon the taxonomic resolution of pollen identification to maximise the accuracy and usefulness of their data. The utility of pollen and spores as an archive becomes reduced, however, when taxonomic resolution leads to a loss of information (Bush 2002).

The Poaceae (grass) family exemplifies this problem, as it comprises 11,554 currently accepted species in 759 genera (The Plant List 2013), which exist across a wide climatic gradient, from Antarctica to tropical lowland rainforest. Yet pollen grains from this family are almost indistinguishable below family level using light microscopy, therefore they are generally not classified below 'Poaceae' by the majority of palynologists (Fægri et al. 1989; Holst et al. 2007; Strömberg 2011). Consequently Poaceae pollen are essentially a rich yet currently underdeveloped archive ripe for palynological research.

Extensive research over the last four decades has used a variety of tools to determine if the identification of Poaceae pollen to below family level is possible. This analysis has been on individual grains using: (i) surface pattern analysis of images of pollen grains obtained through scanning electron microscopy (SEM) (Andersen & Bertelsen 1972; Mander et al. 2013; Waikhom et al. 2014), (ii) detailed morphometric analysis considering whole grain and pore morphology (Joly et al. 2007; Schüler & Behling 2010), and (iii) confocal microscopy of pollen exines (Salih et al. 1997). A success rate in identifying Poaceae pollen to species level of 85.8% has been achieved through SEM (Mander et al. 2013), and this technique has even allowed differentiation of cultivars (Datta & Chaturvedi 2004). These methods, although successful, are time consuming and require considerable sample preparation, laboratory work, and expertise. Therefore, from a practical perspective, the application of these techniques to palaeobotanical questions has not yet occurred.

Fourier Transform Infra-Red spectroscopy (FTIR) has recently been used to differentiate pollen taxonomically, demonstrating it is possible to distinguish between plant orders, and in some cases to species level (Pappas et al. 2003; Dell'Anna et al. 2009; Zimmermann 2010; Zimmermann 2016). FTIR analysis has also been successfully used in characterising pollen surface compounds (Pummer et al. 2013). FTIR analysis generates absorbance spectra, with bands relating to chemical bonds within specific functional groups. The size, shape and position of these bands provides information about the type of bonds present and their chemical environments, which, in the case of biopolymers such as sporopollenin, can be very complex (de Leeuw et al. 2006; Watson et al. 2007; Fraser et al. 2011; Watson et al. 2012; Fraser, Watson, et al. 2014). Interpretation of FTIR spectra relies upon knowledge of the type of bonds likely to be present in a substance, and how they might vary. In this study, we treat spectra statistically and use classification algorithms to identify pollen, thus removing the need for in-depth biogeochemical analysis.

Spectra produced by FTIR analysis are affected by a number of operational factors, such as intensity of beam, thickness of sample and thickness of slide (if using a microscope enabled FTIR). Spectra may be noisy if the sample to be scanned (and therefore aperture size) is small, or the material is of poor quality, for instance if pollen grains are degraded. Degradation of the samples used in this study is not expected to be significant, although may be present, as some chemical changes have been observed over short time (hours-days) periods (Zimmermann et al. 2015). Changes in spectra driven by degradation can, however, be accounted for by using statistical processing techniques prior to analysis (Zimmermann & Kohler 2013). For example, use of algorithms such as Savitsky-Golay smoothing can alleviate noisiness, but potentially remove useful information such as subtleties in shape of bands from spectra if their parameters are not calibrated properly, whereas generating first and second derivatives of spectra may result in degradation of the signal-to-noise ratio (Brown et al. 2000; Zimmermann & Kohler 2013). The chemical structure of sporopollenin, is known to be very stable over geological time (Fraser et al. 2012) and resistant to diagenetic alteration (Watson et al., 2007; Fraser et al., 2014a), meaning that the interpretation of the fossil record may benefit from the application of this technique.

Here we show that analyses of FTIR spectra from a selection of Poaceae taxa can be used to successfully identify pollen grains. Using a simple nearest neighbour classification algorithm our results have very similar levels of success when compared to much more expensive and labour intensive methods currently deployed, such as SEM (Mander et al. 2013). Therefore, FTIR based analyses raise the possibility of a further exploration of the grass pollen record.

3.3   Methods

3.3.1   Sample collection and preparation

A total of twelve grass taxa were analysed from eight subfamilies (Table 3.1) across the grass phylogeny, as outlined in the latest publication by 'The Grass Phylogeny Working Group' (Grass Phylogeny Working Group II 2012). The sampling strategy employed ensured a wide phylogenetic spread whilst also enabling analysis of lower-order identification by sub-sampling some subfamilies, such as the Ehrhartoideae. Poaceae pollen was obtained from herbarium specimens at the Royal Botanic Gardens, Kew, London, UK by dissecting out stamen from individual florets. Where possible, two or more specimens for each species were sampled, and specimens from Ghana or neighbouring tropical West African nations were preferentially sampled, to complement current palaeoecological (fossil pollen) investigations at Lake Bosumtwi, Ghana (Miller & Gosling 2014), and to reduce large-scale environmental variability as much as possible.

Table 3.1:Subfamily and species of grass sampled for pollen FTIR.

Tribe	Species
Bambusoideae	*Bambusa vulgaris* Schrad.
Pharoideae	*Leptaspis zeylanica* Nees ex Steud.
Puelioideae	*Puelia olyriformis* (Franch.) Clayton
Ehrhartoideae	*Oryza sativa* L.
Ehrhartoideae	*Oryza longistaminata* A.Chev. & Roehr.
Ehrhartoideae	*Leersia drepanothrix* Stapf.
Arundinoideae	*Phragmites karka* (Retz.) Trin. Ex Steud
Chloridoideae	*Ctenium elegans* Kunth
Chloridoideae	*Enteropogon macrostachys* (A.Rich.) Munro ex Benth.
Panicoideae	*Pennisetum pedicellatum* Trin.
Panicoideae	*Cenchrus setiger* Vahl
Pooideae	*Triticum aestivum* L.

3.3.2   Chemical analysis

The pollen was washed in acetone and allowed to air-dry on zinc-selenide slides. Groups of two or more pollen grains clustered together were examined using a continuum IR-enabled microscope with a 15× reflechromat objective lens and nitrogen-cooled MCT-A detector in transmission mode. The microscope was linked to a Thermo Nicolet Nexus (Thermo Fisher Scientific, Waltham, MA, USA) FTIR bench unit at The Open University. Spectra were averaged over 256 scans per sample, and background scans were taken before each sample to alleviate any atmospheric contributions. Visual inspection of spectra and atmospheric suppression correction was conducted using OMNIC software (Thermo Fisher Scientific, Waltham, MA, USA).

3.3.3   Data processing and analysis
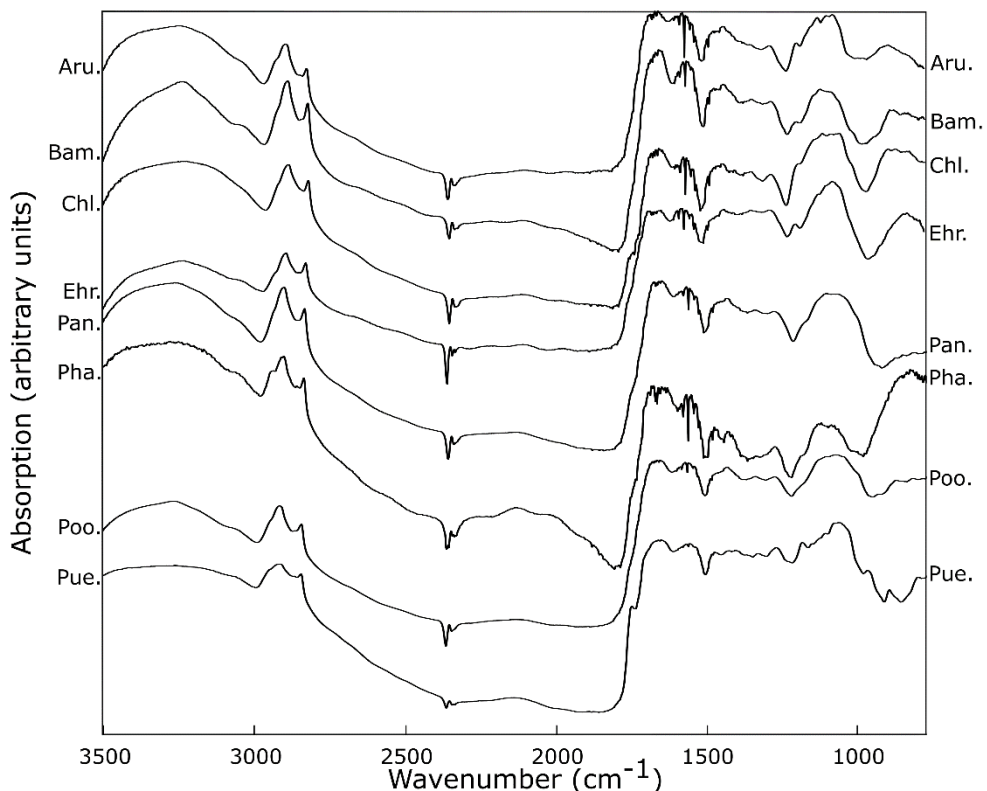
Figure 3.1: Average standardised spectra of one representative individual from each subfamily. Aru.= Arundinoideae, Bam.= Bambusoideae, Chl.= Chloridoideae, Ehr.= Ehrhartoideae, Pan.= Panicoideae, Pha. = Pharoideae, Poo. = Pooideae, Pue.= Puelioideae.

Average spectra were calculated from multiple replicates for every sample (Figure 3.1). These average spectra were inspected visually, and comparisons of selected absorbance bands were compiled (after Steemans (2010)) to determine potential structural drivers of the statistical patterns observed (Table 3.2). The absorbance bands chosen were based on those used by

other researchers investigating sporopollenin composition. Bands that do not vary between taxa are omitted from visual inspection; for instance, the broad OH band at 3300 cm^{-1} is omitted, as it is present in all taxa in the same form and thus provides no visually quantifiable classification information. The bands included in the visual inspection and references to papers which have used them in investigations of sporopollenin are as follows: C=C band at 3070 cm^{-1}, (Fraser 2008); vasCH$_2$ and vsCH$_2$ at 2925 cm^{-1} and 2850 cm^{-1} respectively, and vC=O at 1710 cm^{-1}, (Watson et al. 2007; Fraser et al. 2012); vasCH$_3$ at 2960 cm^{-1}, (Steemans et al. 2010); vsCH$_3$ at 2890 cm^{-1}, (Fraser 2008); C=C non-conjugated at 1660 cm^{-1}, (Steemans et al. 2010; Fraser, Watson, et al. 2014; Zimmermann & Kohler 2014), OH at 1630 cm^{-1} (Fraser 2008); C=C (aromatic ring stretch) at 1500 cm^{-1}, (Watson et al. 2007; Lomax et al. 2008; Fraser, Watson, et al. 2014); CH$_n$ (asymmetric bending) at 1460 cm^{-1} and CH$_3$ (symmetric bending) at 1375 cm^{-1}, (Fraser et al. 2012); C=C or CH$_n$ at 720 cm^{-1}, (Fraser et al. 2012; Zimmermann & Kohler 2014).

All average spectra were z-score standardised (i.e. standardised to zero mean and unit variance) by finding their mean amplitude, subtracting the mean from the actual values, and dividing by the standard deviation. When no other treatments were applied, these z-score standardised spectra are referred to as 'Unprocessed Spectra' (see Fig. 2 for information on processing). These standardised spectra were not subject to variations in signal amplitude due to variable sample thickness (Duarte et al. 2004; Jardine et al. 2015). Standardisation of spectra (and all other statistical manipulations) were performed in R v. 3.1.2, using R Studio (RStudio 2012) (see Appendix II for full details of code used).

Further processing of raw data was conducted to investigate various extraneous factors that may have impacted upon the analyses. Such processing involved one or more of the following: (i) truncation, (ii) baseline correction, and/or (iii) atmospheric suppression of the region from 1800-2700 cm^{-1} (Figure 3.2) to remove the effects of CO_2 and scattering from that region ('Truncated Spectra'). Baseline correction using the R package baseline (Liland & Mevik 2015) was conducted because some unprocessed spectra exhibited climbing baselines on visual inspection ('Baseline Corrected Spectra'). Atmospheric suppression using the OMNIC atmospheric suppression algorithm and z-score standardisation was performed to assess the impact of water vapour across the spectra ('Atmospheric Suppressed Spectra'). Collectively, this approach allowed us to compensate for possible noise and/or atmospheric effects and to directly compare against the 'unprocessed spectra'. Figure 3.2 shows a visual summary of the stages performed during spectral processing.
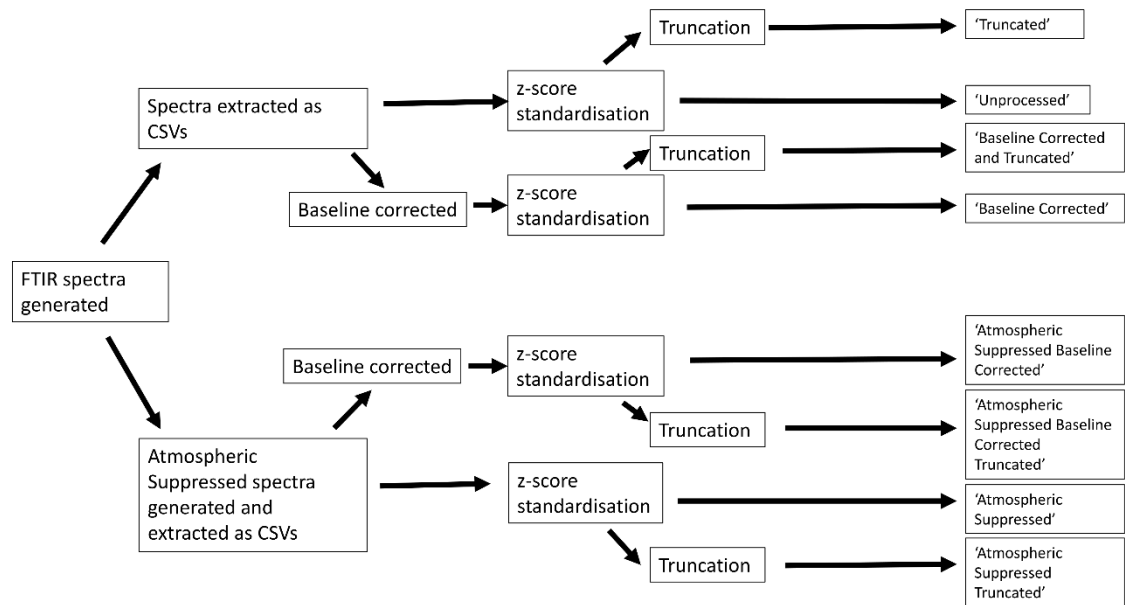
Figure 3.2: Flowchart showing the processing steps taken to produce the different pre-processed spectra upon which we based further analyses. Results for all of these treatments may be found in Appendix II.

The final stage of processing was the application of 'spectral pre-processing' *sensu* (Zimmermann & Kohler 2013) to both the raw spectra and all of the modified spectra. This final stage of the processing involved generating first and second derivatives of spectra, and applying Savitsky-Golay spectral smoothing (Zimmermann & Kohler 2013). The Savitsky-Golay smoothing technique applies an algorithm which approximates the spectrum using polynomial least-square fitting to a moving window. Both polynomial order, and window size used affect the resultant spectrum. As different window sizes may be optimal for different regions of the spectrum, and this study aimed to provide a simple tool for pollen identification and thus used the whole spectrum, a window size of 11 was chosen; this falls within the range of optimal values defined by Zimmermann (Zimmermann & Kohler 2013). The R package Prospectr was used to perform Savitsky-Golay smoothing and derivation of spectra (Stevens & Ramirez-Lopez 2013). Principal Component Analysis (PCA) was used to visualise all resultant data from the processing steps detailed above.

Using the R-package, Class (Venables & Ripley 2002), k-nearest neighbour (k-nn) analyses were performed, using leave-one-out cross-validation to generate identification success rates. This analysis uses a Euclidean distance matrix to classify individual spectra based upon their nearest neighbours in the matrix, and then removing one data point (a single spectrum) before re-introducing it for classification. The number of nearest neighbours is instrumental in classification success, as increasing k expands the analysis to include the next closest points, with classification decided by majority vote (the sample is classified based on the most common taxon among its nearest neighbours). So for k=1, only the closest point is included in

the analysis, whereas in k=5, the 5 nearest neighbour points are included, with the classification being decided by majority rule, or the most common taxon among those points. To test whether or not the success of the classification algorithm was due to sample size, we used a null model which assigns names to points randomly within the Euclidian distance matrix generated from the original data, and then applies the classification test to the new matrix, repeating the process 999 times. This process allows the distribution of random successful classifications to be compared to the actual classification success.

3.4   Results

FTIR spectra of pollen from different grass subfamilies display broadly similar characteristics (Figure 3.1). Nevertheless, there are small variations in the spectra between subfamilies and lower orders of classification (Table 3.2) (de Leeuw et al. 2006; Watson et al. 2007). Some absorbance bands are present in, and are similar between, all taxa analysed, such as the C=C stretching band of C=C-H group at 3070 cm^{-1}, the $vasCH_2$ and $vsCH_2$ bands at 2925 cm^{-1} and 2850 cm^{-1} respectively, and the C=C aromatic ring stretch at around 1500 cm^{-1} (Table 3.2). Other bands are variable both between and within subfamilies, such as the $vasCH_3$ and $vsCH_3$ bands at 2960 cm^{-1} and 2890 cm^{-1}, respectively (Table 3.2). Absorbance bands that vary between and within subfamilies include the non-conjugated C=C band at 1600 cm^{-1}, the asymmetric bending CH_n at 1460 cm^{-1}, the symmetric bending CH_3 band at 1375 cm^{-1} and the cis-substituted C=C/CH_n rocking band at 720 cm^{-1}. Some absorbance bands exhibit more within-subfamily variation (* in Table 3.2), such as the band at 1630 cm^{-1}, whereas others display less within-subfamily variation, but differ in presence and shape between subfamilies, as exemplified by the band at 720 cm^{-1}. Codes used to characterise bonds in Table 3.2 follow Steemans et al. 2010.

Table 3.2: Peak properties; 'sh' = shoulder, '+' = weak absorbance band, '++' = strong absorbance band, '+++' = very strong, * = absorbance band differs within subfamily, '-' = absence of absorbance band. '~' before Wavenumber = the position of a band may vary.

Group	Wavenumber/ cm^{-1}	Arundinoideae	Bambusoideae	Chloridoideae	Ehrhartoideae	Pharoideae	Pooideae	Puelidoideae
C=C	3070	sh	sh	-	sh	sh	sh	sh
$_{vas}CH_3$	2960	sh	sh	*	sh	+	-	*
$_{vas}CH_2$	2925	++	++	++	++	++	++	+++
$_{vs}CH_3$	2890	*	*	*	sh	sh	-	*
$_{vs}CH_2$	2850	++	+	+	+	+	+	+
$_vC=O$	~1710	sh	sh	sh	sh	sh	-	sh
C=C (non-conjugated)	1660	+	*	*	*	+	+	+
OH	1630	*	*	*	*	+	sh	+
C=C (aromatic ring stretch)	~1500	sh	sh	sh	sh	sh	sh	sh
$CH_{2/3}$	1460	*	+	sh	*	+	sh	*
CH_3 (symmetric)	1375	sh	*	+	*	sh	sh	*
C=C (cis-substituted) or CH_n (rocking)	720	*	*	+	+	-	+	-

67
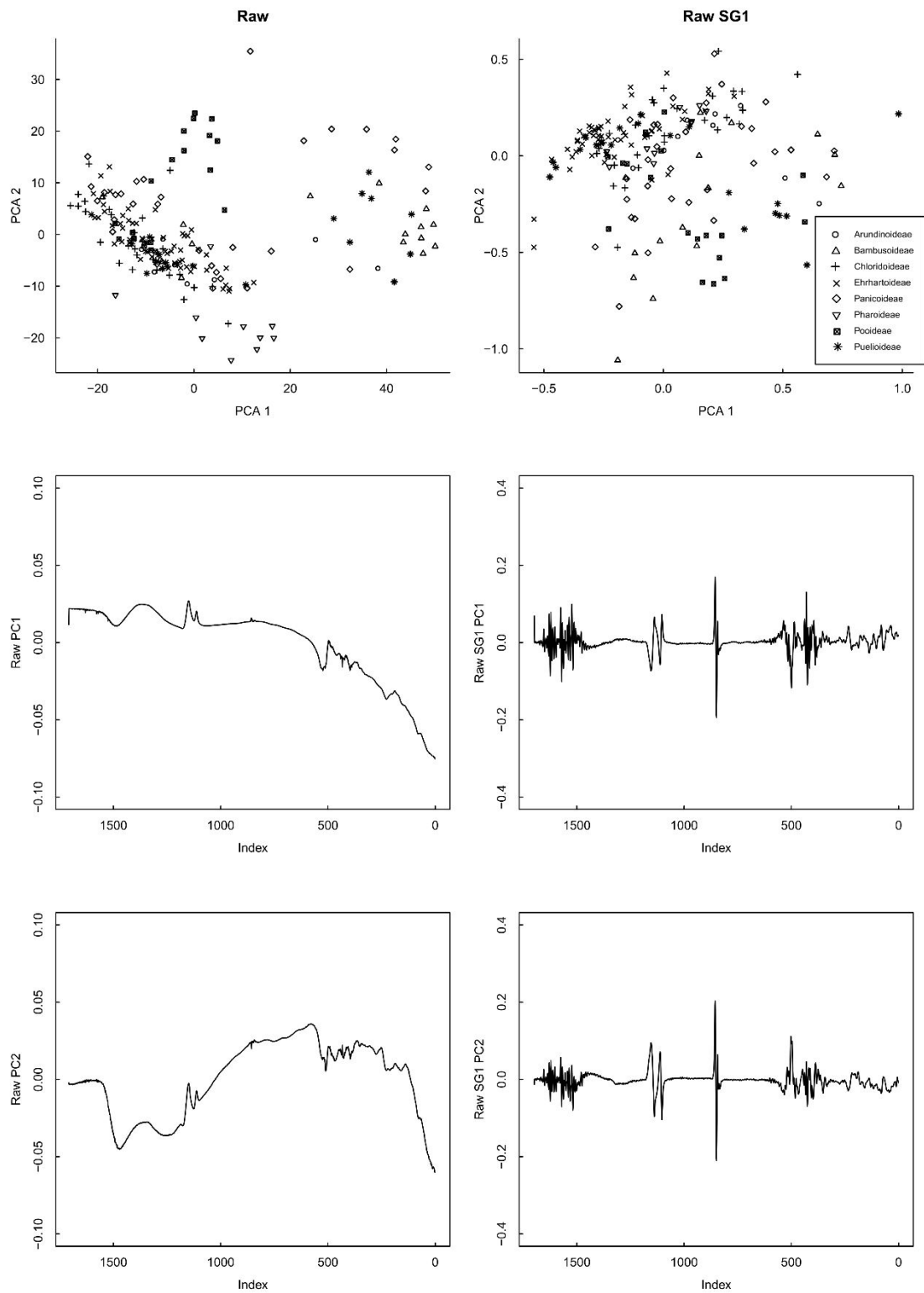
Figure 3.3: PCA plots of Raw and Raw SG1 (Savitsky-Golay smoothed, first derivative) spectra and, below them, their loadings plots, illustrating areas of the spectrum which contribute most strongly to the signal. X axis does not represent wavenumber directly.

PCA of the pre-processed spectra (Figure 3.3) shows that groupings are present but complex. Most subfamilies appear broadly grouped together but with significant spread over one or both PCA1 or PCA2. The Ehrhartoideae, for instance, are distributed along PCA1, but more closely grouped along PCA2. The Bambusoideae, however, are less clearly grouped, with a wide spread across both axes. No subfamily is clearly separate from the others, with all

exhibiting some degree of overlap with other subfamilies. Neither are there clear phylogenetic groupings, as subfamilies do not cluster according to degree of relatedness (Grass Phylogeny Working Group II 2012). Loadings plots of the PCA show significant contributions from CO_2 (at around 900 on the x-axis). This contribution is not as prevalent in treated spectra (see Appendix II for details).

The combined treatment of first derivative with Savitsky-Golay smoothing returns the most accurate classification, with successful classifications at subfamily level reaching 80%. Other treatments, including no pre-processing, first and second derivatives, and second derivative with Savistsky-Golay smoothing did not result in as high a proportion of successful classifications (Figure 3.4) at any taxonomic level. The maximum success achieved by the algorithm to genus level was 77%, to species level 77%, and individual level 69%. A random permutation test confirmed that the chances of achieving the successful classification proportions observed if no pattern was present were 0.01%.

+ Savitsky-Golay smoothing   ■ No treatment

⊠ Savitsky Golay + first derivative   ● First derivative

✳ Savitsky-Golay + second derivative ▲ Second derivative
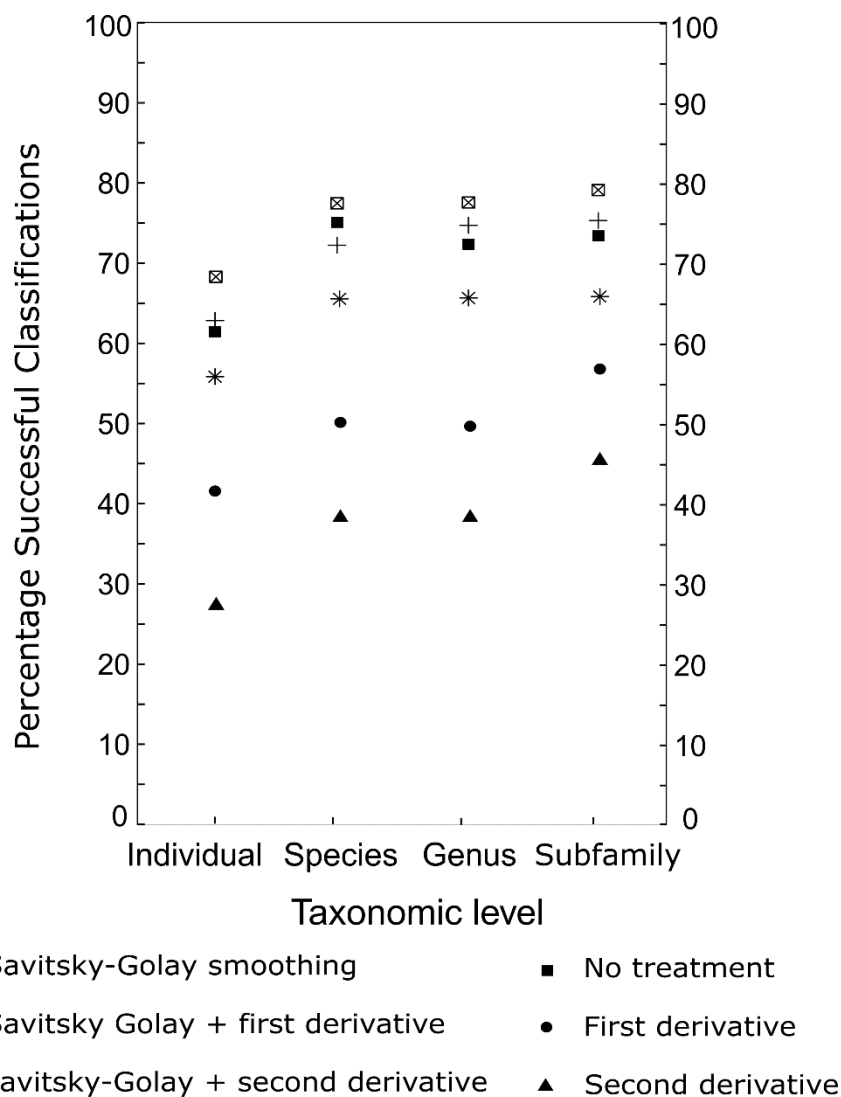
Figure 3.4: Plot showing maximum classification success of processing techniques for spectra at a range of taxonomic levels, with values representing most successful number of nearest neighbours for each pre-processing treatment and level. For full k-nn results see Appendix II.

The success rates at sub-family level for the different treatments outlined in Figure 3.2 are presented in Table 3.3. This shows that unprocessed and truncated spectra have the highest success rates, at 80% and 82% respectively, while all Atmospheric Suppressed Spectra show lower identification success rates. Sub-family level results have been presented here for the sake of brevity and comparison, but for full results, including k values, see Appendix II.

3.5   Discussion

From visual (Figure 3.1) and PCA (Figure 3.3) analyses of the FTIR spectra it was possible to discern subtle differences between subfamilies via visual analysis of absorbance band strength and nature although, as is demonstrated by Table 3.2, chemical differences between taxa are complex and subtle. The exact composition of sporopollenin remains enigmatic, due to its inert nature and resistance to decay or chemical degradation, even over very long periods of

geological time (Fraser et al. 2012). Sporopollenin is, however, known to comprise aromatic components such as *para*-coumaric acid and ferulic acid, linked by ester linkages and aliphatic components (Boom 2004; de Leeuw et al. 2006; Watson et al. 2012; Fraser, Watson, et al. 2014). Based upon Table 3.2, and a knowledge of the broad structure of sporopollenin, it is likely that chemical variation between taxa arises from variation in aliphatic chain length (shown by the variation in shape and presence of CH_n bands in Table 3.2) and degree of saturation (shown by variation in C=C bands). Further chemical analyses via gas chromatography/mass spectrometry (GC/MS) should be able to confirm these tentative conclusions, but as we are primarily concerned with the development of an identification tool, not the chemical structure of sporopollenin, this analysis is not pertinent here.

Some scattering artefacts and noise are evident in the spectra, likely the result of either: (i) the absence of physical atmospheric suppression during sample analysis, and/or (ii) for 8% of samples, sufficiently large groups of pollen grains were not present on the slide, so that some single-grain measurements had to be taken (see Appendix II for details of numbers of grains scanned for each sample). We consider, however, that these artefacts and noise are random in their nature and would affect all samples equally. A full analysis of the impact of pollen grain number of identification success rate can be found in Appendix II.

The first and second derivatives, Savitsky-Golay smoothing and combinations thereof, comprise some of the most commonly used processing techniques used in analysis of FTIR generated spectra (Zimmermann & Kohler 2013). Using first and second derivatives without Savistky-Golay smoothing on unprocessed spectra shows a decrease in classification success across all taxonomic levels compared to raw spectra, from a raw data success rate at species level of 74% to 49% success for first derivative spectra and 37% for second derivative data (see Figure 3.3). This is likely due to the exaggeration of the contribution of noise to the analysis of un-smoothed data, which is eliminated by the point-averaging smoothing effect of the Savitsky-Golay smoothing algorithm (Zimmermann & Kohler 2013).

Truncated Spectra show the highest success rate, at 82%, with Unprocessed Spectra at 80% and Baseline Corrected Spectra at 81% (Table 3.3). Spectra which were subjected to atmospheric suppression showed large reductions in identification success rates (Table 3.3). As the Atmospheric Suppression was performed using an algorithm provided by the OMNIC software, we consider that it is therefore likely the suppression algorithm may remove useful information from the grass pollen spectra. This is because FTIR, and the associated OMNIC software are most commonly used to analyse pure chemical samples, whereas the identification of grass pollen relies on subtle chemical variations in sporopollenin. Further, we

believe the OMNIC atmospheric suppression may remove useful information from spectra because Truncated Spectra return higher identification success rates, whilst having wavenumbers removed which do not contain useful information, only CO_2 contributions and scattering effects (Mohlenhoff et al. 2005).

Table 3.3: Maximum success rate at sub-family level for different treatments, and their corresponding pre-processing treatment.

Treatment	Maximum Identification Success (% at Sub-Family level)	Pre-processing
Truncated	82	Savitsky Golay
Unprocessed	80	Savitsky Golay
Baseline Corrected Truncated	81	Savitsky Golay
Baseline Corrected	79	Savitsky Golay
Atmospheric Suppressed	61	None
Atmospheric Suppressed	60	Savitsky Golay
Atmospheric Suppressed	46	Savitsky Golay
Atmospheric Suppressed	64	None

Pollen grains used in this study were washed in acetone, which would have removed surface chemicals and traces of insecticide or other contaminants. It is likely, however, that internal material such as intine and possibly cytoplasm remained present in the grains. This means that the signal observed from grass pollen is not that of 'pure' sporopollenin, but, rather, represents the pollen grain as a whole entity (Blokker et al. 2006). For the purposes of modern pollen identification, this does not pose an immediate issue, as it is likely that cytoplasmic and pollen-wall associated compounds contribute to the taxonomic signal detected in this study, and others which have focussed on other taxonomic groups such as the Pinales (Zimmermann 2010). For fossil pollen samples, or pollen that has been acetolysised, however, some of this information may be lost, either due to chemical alteration during processing (Jardine et al. 2015), or lack of non-sporopollenin pollen components in preserved grains. In these cases, it may be beneficial to use FTIR analysis in conjunction with other, structural methods of distinguishing between similar taxa, such as those described by Sivaguru and Mander (Sivaguru et al. 2012; Mander et al. 2013).

Environmental factors could affect success of classification, as it has been demonstrated that factors such as UVB irradiance (Lomax et al. 2008; Fraser, Lomax, et al. 2014), year-on-year environmental factors (Zimmermann & Kohler 2014) and heat stress (Lahlali et al. 2014; Jiang et al. 2015) have an impact upon the chemical composition of pollen grains and spores. Our samples were also taken from a relatively narrow geographical and temporal range (tropical West Africa, and within the 20th Century), compared to the time ranges and geographical ranges dealt with in the fossil record. Therefore, we suggest that the effects of any UVB fluxes are likely to be minimal in this sample set, as UVB changes occur over longer time scales and wider geographical areas (Magri 2011). The fact that classification success improves from individual to species level, where individuals from the same species have been collected in different areas and different years (Appendix II) demonstrates a clear taxonomic signal. The absence of a significant improvement between species and subfamily level suggests that although this is a successful classification technique, it is not a phylogenetic one. Although the number of samples varies between taxa, the randomisation algorithm demonstrates that successful classification is not due purely to chance.

These results show that differentiation between Poaceae taxa below family level is possible using the relatively fast and inexpensive method of FTIR microspectroscopy. At subfamily level, it is possible to achieve an 80% classification success rate, and at species level, a 77% classification success rate. The ability to identify grass pollen to subfamily level, or below, allows for a more detailed interrogation of grass pollen record. One specific benefit of increased taxonomic resolution could be the recovery of a more complete ecological picture from the fossil pollen record and the determination of previously hidden plant-climate relationships. For instance, the identification of pollen from the Puelioideae subfamily would indicate a forest-origin for the grass pollen observed, whereas Ehrartoideae might suggest a more open habitat (Grass Phylogeny Working Group II 2012).

3.6   Conclusions

We have shown that FTIR analysis followed by spectral and statistical processing has the potential to significantly improve pollen identification within the Poaceae. Our data demonstrate that it is possible to achieve an 80% successful classification rate to subfamily level for Poaceae pollen, which, when applied, will allow new insights into taxonomic resolution in fossil pollen records. The rapidity and relative low costs of FTIR analyses make this a potentially very useful method for subfamily identification of Poaceae pollen.

4   Chapter 4: Does the pollen fall far from the tree? Spatial and temporal variation in pollen signals from wet evergreen rainforest and moist semi-deciduous forest in tropical West Africa (Ghana)

Note on format: This chapter has been structured for submission to a journal specialising in vegetation science, with a predominantly ecological audience in mind. It therefore presents data in a highly detailed manner, with information on individual taxa that may be of interest to ecologists or vegetation scientists.

4.1   Abstract

How pollen moves within and between ecosystems can affect factors such as genetic structure of populations, how resilient they are to environmental change, and the amount and nature of pollen deposited in the sedimentary record. Artificial pollen trap data from traps in two 100 m by 100 m vegetation plots, one in a wet evergreen forest, and one in a moist semi-deciduous forest in tropical West Africa (Ghana), with 10 traps collected annually in 2012, 2013 and 2014, were used to examine spatial and temporal variation in the pollen rain of the most abundant taxa present in both pollen and vegetation assemblages. Samples from the wet evergreen plot exhibit high variability spatially, with the most abundant pollen types changing between samples, and many pollen taxa being over-represented relative to their vegetative abundance in some traps whilst entirely absent from others. The most abundant plant taxa of the wet evergreen plot (*Drypetes* and *Cynometra*) do, however, constitute major components of the pollen rain. There is less variation between samples from the moist semi-deciduous plot on a spatial scale, as this plot is heavily dominated by *Celtis*, which typically comprises >70% of the pollen assemblages. *Triplochiton scleroxylon* exhibits a strong temporal signal in the moist semi-deciduous plot, being most abundant in the relatively dry year, 2013. It is concluded that pollen rain in these tropical ecosystems is highly heterogeneous, and suggest that pollen assemblages obtained by trapping are susceptible to small-scale variations in forest structure. A high spatial resolution (five or more samples per 100 m by 100 m plot) when sampling pollen from modern tropical forest ecosystems is recommended in order to obtain a representative pollen assemblage.

4.2   Introduction

Pollen production and dispersal are crucial to the maintenance of plant populations, their genetic diversity, and ultimately, their ability to adapt and evolve to changing conditions (Ellstrand & Elam 1993). Pollen dispersal in the tropics has, until recently, been largely under-

studied compared to temperate regions (Giesecke et al. 2010), although molecular work has been carried out which allows parentage of individuals to be inferred, thereby inferring how far their parental pollen travelled (Dick et al. 2003; Gonzales et al. 2006). How pollen moves within and between ecosystems is of particular interest to palaeoecologists, who use pollen from lakes, swamps, or oceanic sedimentary records to re-construct past vegetation and therefore need to understand biases that could be affecting these records (Overpeck et al. 1985; Fægri et al. 1989).

In order to interpret fossil pollen records, it is necessary to understand which taxa produce an excess of pollen relative to their abundance in the vegetation, which under-produce, and how far pollen travels. Ideally, to obtain analogues of ancient depositional environments, lake, swamp or oceanic surface samples would be taken from a range of modern environment, along with robust vegetation surveys of the surrounding vegetation, to infer how these different environments might be represented by their pollen in the fossil record (Hicks & Hyvärinen 1999; Birks et al. 2016).

 Although there are some studies that use the surface sediments in modern lakes as a sampling medium (Correa-Metrio et al. 2011; Matthias et al. 2015a), it is often not possible to locate suitable depositional environments in each vegetation type, so other means of collecting pollen, such as soil samples, moss polsters or artificial pollen traps are necessary. These sampling techniques are all subject to bias; soil samples may affect the preservation of pollen grains, favouring those with thicker exines (Wilmshurst & McGlone 2005; Jantz et al. 2013), moss polsters preferentially capture and retain larger, bisaccate grains (Pardoe et al. 2010; Lisitsyna et al. 2012), and both are subject to uncertainty regarding the amount of time pollen is sampled for (Lisitsyna & Hicks 2014). Artificial pollen traps alleviate the issue of time because they can be deployed for set periods, but are not free of bias, favouring under-storey pollen taxa and under-representing rarer tree pollen (Jantz et al. 2013). Pollen traps are not perfectly analogous to lake sediments, but can provide useful data on the relative over or under-production of taxa in ecosystems, and have been shown to reflect vegetation changes across ecological gradients relatively well in comparison to other methods of pollen rain collection (Jantz et al. 2013).

The movement and dispersal of pollen has been studied extensively using molecular markers to identify offspring of individuals (Ellstrand 1992; Austerlitz et al. 2004; Smouse & Sork 2004; Ward et al. 2005; Gonzales et al. 2006; Dick et al. 2007) In the tropics, high levels of out-crossing and pollen dispersal over long distances (up to tens of kilometres) are observed despite the majority of tree taxa being entomophilous and therefore presumed to have

relatively limited pollen dispersal capabilities (Ward et al. 2005). These studies measure successful pollination events and subsequent population structure, but do not show how the bulk of pollen produced by plants moves within an ecosystem, whether driven by air currents, rain, or gravity (DiLeo et al. 2014). The pollen distribution curve (the amount of pollen deposited against distance from source) has been shown to be strongly leptokurtic, or negative-exponential, meaning that a large proportion of all pollen produced is deposited very close to the plant (Miller 2016). Modern pollen studies in the tropics have largely corroborated this idea, observing high levels of local (within 10s of metres of samples) pollen in traps (Bush & Rivera 1998; Gosling et al. 2005).

Here, modern pollen assemblages are presented that were collected in artificial pollen traps deployed at two sites; one wet evergreen rainforest (Ankasa) and one moist semi-deciduous forest (Bobiri). The following aims are addressed:

- Characterise assemblages from each ecosystem in terms of their most abundant and consistently occurring pollen taxa
- To explore drivers of variation in representation in each of the most abundant taxa.
- To consider the implications of this work for models of pollen distribution and pollen trapping studies.

4.3   Site descriptions

The two sites, Ankasa and Bobiri, in this study are located in tropical West Africa, within the Guineo-Congolian centre of endemism (White et al. 1983; Gautier & Spichiger 2004) (see Chapter 1). Guineo-Congolian forest accounts for much of the forest cover across tropical West Africa, from Senegal to Togo, and comprises many endemic species and multiple different vegetation types (Bongers et al. 2004). The Guineo-Congolian region is characterised by very high rainfall (up to 4 m per year) with the amount of rainfall being a determining factor in the vegetation type. The tropical rain belt is an important driver of rainfall in the region, and it is estimated that changes to this, or to the intensity of the African Monsoon, have caused vegetation change in the past (Shanahan et al. 2015; Miller 2016), and will continue to do so in future climate scenarios (Intergovernmental Panel on Climate Change 2014).

The weather in Ghana over the duration of this study was not exceptional, although in 2013, there was a dry rainy season (OCHA 2013). Rainfall estimates were available for both plots (see Section 1.4.2) from local weather stations, but temperature was not.
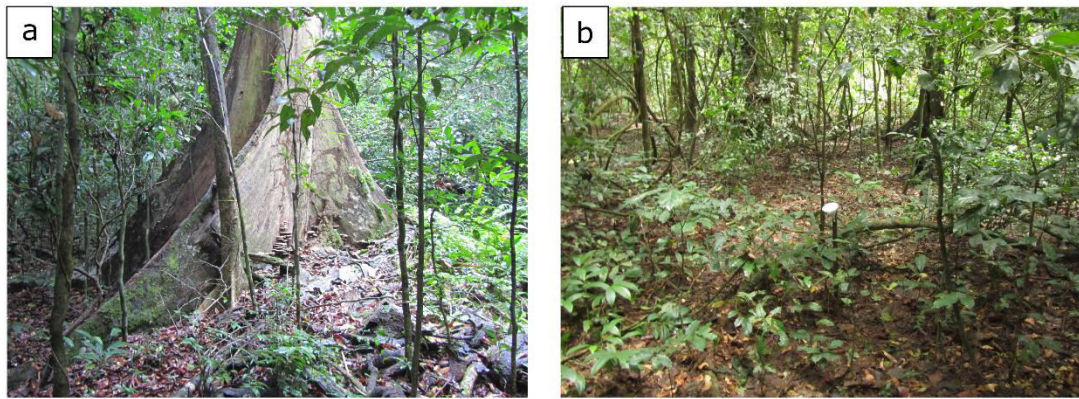
Figure 4.1: Photographs of Ankasa (a) and Bobiri (b) taken within plots in October 2014. The large buttressed tree in (a) is that referred to in the text (Section 4.6.3.1).

4.3.1   Ankasa (ANK02)

The first site, Ankasa is located in the Ankasa Conservation Area in South-West Ghana, and the plot is located at 5°16'06"N, 2°41'38"W (See Figure 1.4 and Figure 4.1a). The plot was established in 2011 and a vegetation survey of all trees >10 cm Diameter at Breast Height (DBH) was conducted. The vegetation type of the Ankasa plot is wet evergreen rainforest, and is the most biodiverse area of Ghana, with multiple species of very high conservation priority (Hawthorne et al. 1998). The most abundant taxa in this plot, by percentage of stems, are *Drypetes aylmeri* Hutch. & Dalziel (8.9%), and *Cynometra ananta* Hutch. & Dalziel (7.1%) (see Table 4.1). Herbaceous plants were not surveyed, but include taxa such as *Psychotria*, and members of the Zingiberaceae, Orchidaceae and Commelinaceae, along with various fern species (Hawthorne et al. 1998). The diversity of the surveyed vegetation in the Ankasa plot is 4.0 (Shannon Index) and there were 449 individual trees recorded (Figure 4.2a), of 100 different taxa.

The heights of the trees (>10 cm DBH) in the Ankasa plot range from 4.1 m to 41.6 m, with an average of 18.9 m. The soil is a Forest Oxysol, with a pH of 3.5 – 4, which is prone to leaching and very infertile (Wildlife Division Forestry Commission 2000). Ankasa experiences some of the highest rainfall in Ghana; in 2011-2012, the total rainfall this plot experienced was 1902 mm, in 2012- 2013 was 1788 mm, and in 2013-2014 was 2089 mm.

Table 4.1: Most abundant vegetation taxa Ankasa >10 cm DBH. (p) indicates presumed, based upon floral structure.

Family	Species	Stems (%)	Basal area (%)	Flower structure	Pollination syndrome
Putranjivaceae	*Drypetes aylmeri* Hutch. & Dalziel	8.9	9.2	Dioecious	Entomophilous
Fabaceae	*Cynometra ananta* Hutch. & Dalziel	7.1	8.1	Hermaphrodite	Chiropterophilous
Combretaceae	*Strephonema pseudocola* A.Chev.	5.6	5.8	Hermaphrodite	Zoophilous
Olacaceae	*Strombosia pustulata* Oliv.	4.9	4.5	Hermaphrodite	Entomophilous (p)
Melastomataceae	*Memecylon lateriflorum* (G. Don) Bremek.	3.3	3	Hermaphrodite	Entomphilous
Rhizophoraceae	*Cassipourea hiotou* Aubrév. & Pellegr.	3.1	3.8	Hermaphrodite	Entomophilous
Ebenaceae	*Diospyros sanza-minika* A. Chev.	2.9	2.7	Monoecious	Entomophilous
Clusiaceae	*Pentadesma butyracea* Sabine	2.9	2.3	Hermaphrodite	Zoophilous
Malvaceae	*Heritiera utilis* (Sprague) Sprague	2.7	3.1	Monoecious	Entomophilous (p)
Dichapetalaceae	*Tapura ivorensis* Breteler	2.2	2.5	Hermaphrodite	Entomophilous

4.3.2   Bobiri (Bobiri Strict Nature Reserve; BOB01)

Bobiri is located at 6°42'15 "N 1°19'06 "W, in the Bobiri Forest Reserve, (Figure 1.4 and Figure 4.1b). The plot was last logged over 50 years ago, and is designated as a 'Strict Nature Reserve' in which no logging was allowed. This forest plot was established in 2011 and a vegetation survey of all trees >10 cm DBH was conducted. The vegetation type of this plot is moist semi-deciduous rainforest (Hall & Swaine 1981), and fits into the category of 'Drier peripheral semi-evergreen Guineo-Congolian rain forest' (White et al. 1983). The most abundant taxa in this plot, by percentage of stems, are *Celtis mildbraedii* Engl. (14.7%) and *Funtumia elastica* (Preuss) Stapf (5.0%) (Table 4.2). Herbaceous taxa were not surveyed, although may comprise families such as the Marantaceae and Verbenaceae (White et al. 1983). The diversity of the

Bobiri plot (Shannon index) is 3.8, and there were 483 individual trees recorded (Figure 4.2b), of 87 different taxa.

The height of trees (>10 cm DBH) in Bobiri are around 37 m, with some emergents of up to 60 m (Hall & Swaine 1981). The soil is a Forest Ochrosol, which is red, well drained, and is relatively high in organic content near the surface but with leaching further down in the profile. The average rainfall per year at this site over the years sampled was 1219 mm. Rainfall was measured at the Forestry Research Institute of Ghana (FORIG), which is around 30 km away from the site, and there were some missing measurements, meaning that accurate yearly averages were not available.

Table 4.2: Most abundant vegetation taxa >10 cm DBH Bobiri (p) indicates presumed, based on floral structure.

Family	Species	Stems (%)	Basal area (%)	Flower structure	Pollination syndrome
Cannabaceae	*Celtis mildbraedii* Engl.	14.7	17.3	Monoecious	Anemophilous
Apocynaceae	*Funtumia elastica* (Preuss) Stapf	5.0	3.4	Hermaphrodite	Entomophilous (p)
Malvaceae	*Nesogordonia papaverifera* (A. Chev.) Capuron	4.8	6.5	Hermaphrodite	Entomophilous (p)
Malvaceae	*Cola caricifolia* (G.Don) K.Schum.	4.1	3.6	Monoecious	Entomophilous (p)
Fabaceae	*Hymenostegia afzelii* (Oliv.) Harms	4.1	3.6	Hermaphrodite	Zoophilous
Simaroubaceae	*Hannoa klaineana* Pierre & Engl.	3.9	5.2	Hermaphrodite	Entomophilous (p)
Fabaceae	*Albizia zygia* (DC.) J.F.Macbr.	3.1	2.5	Hermaphrodite	Entomophilous
Fabaceae	*Baphia nitida* Lodd.	2.7	1.6	Hermaphrodite	Entomophilous
Malvaceae	*Cola gigantea* A.Chev.	2.7	3.1	Hermaphrodite	Entomophilous
Olacaceae	*Strombosia glaucescens* Engl.	2.7	1.9	Hermaphrodite	Entomophilous (p)

4.4   Methods

4.4.1   Field methods

Ten pollen traps were deployed in 100 x 100 m vegetation plots in both the Ankasa Nature
Reserve and the Bobiri Strict Nature Reserve in October of 2011, 2012 and 2013, and were
collected yearly. Sample dates are referred to by their year of collection e.g. '2012' is
equivalent to October 2011- October 2012. The traps were placed at 10 m intervals along the
60 m Y line in Ankasa, and the 40m X line at Bobiri (see Figure 4.2). Pollen traps were made
according to Gosling et al. (2003), comprising of a plastic funnel of diameter 140 mm, with
filter paper sealed at the bottom to prevent pollen from washing out, and filled with cotton
wool to capture pollen rain. The traps were covered with plastic mesh, and attached to stakes
at around 50 cm above ground level, to minimise the likelihood of inundation.
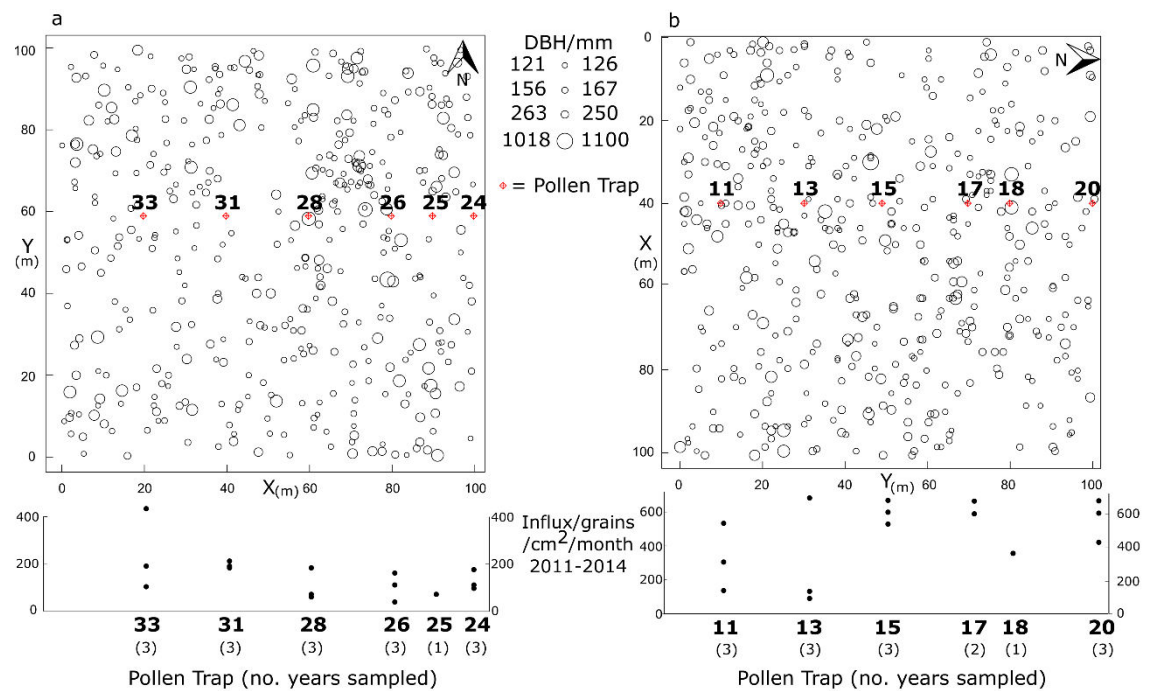
Figure 4.2: Maps of all trees in plot (100m x 100m), scaled by the square root/10 of their
DBH. a) is Ankasa, b) is Bobiri. Red symbols indicate trap positions and bold numbers
trap numbers. Pollen influx is shown below for all samples from each trap. Bobiri plot is
rotated by 90° in this plot, in order to display influx values below map.

4.4.2   Laboratory methods

Traps for processing were selected to give a relatively even spatial coverage of the plot across
years, with at least five traps per year being processed and counted. It was not always possible
to use the same traps each year due to issues such as termites colonising traps and poor
preservation of trap contents. Traps were processed following the method of Gosling (2003),
with acetolysis being used to remove cotton wool and hydrofluoric acid to remove filter paper.

Samples were washed with potassium hydroxide and sodium pyrophosphate, sieved at 180 μm and mounted in glycerol. Lycopodium tablets (batch number 124961, containing 12542 +/- 931 spores per tablet) were added to enable calculations of pollen concentration (Stockmarr 1971). Samples were counted at x400 magnification using a Nikon Eclipse 50i microscope. Pollen counts were recorded digitally (Valencia 2014) and were counted to a statistically representative number (Keen et al. 2014). Pollen taxa were identified using literature on tropical West Africa (van Campo 1974; Ybert 1979; Riollet & Bonnefille 1980; Gosling et al. 2013), the African Pollen Database (Vincens et al. 2007) and the reference collection at the University of Amsterdam, Netherlands.

4.4.3   Statistical methods

Pollen assemblages were visualised in C2 (Juggins 2007). Statistical analyses were carried out in R statistical software (R Core Team 2016) with Rstudio version 1.0.136 (RStudio 2012). Trees in plots were mapped using code from Finley and Banerjee (2010). The package Vegan (Oksanen et al. 2015) was used to carry out Permutational Analysis of Variance (PERMANOVA) tests to test for differences between groups of samples when grouped by Year and Trap, using the function 'adonis' with 999 permutations and Bray-Curtis dissimilarity matrix (Anderson 2001).Non-Metric Multidimensional Scaling (NMDS) analysis was also undertaken in Vegan, excluding singletons (taxa which occur in only one sample) and using double Wisconsin standardisation (which corrects for sample size and effects of very rare or abundant taxa by diving each taxon by its column maximum, and then by its row total) with dimensions = 3 (Bray & Curtis 1957). Results were plotted in two-dimensions, as this captured the dominant patterns observed in three dimensions but in a simpler graphical presentation. Pollen to vegetation ratios, or R-rel$_{(ind)}$ values, were calculated by using the percentage of pollen for one sample and dividing it by the basal area of the plot occupied by its parent taxon in the vegetation (Davis 1963). 'Sample' is used to mean the pollen from one trap in one year, whereas 'Trap' refers to multiple samples from the same position within the plot over three years, unless stated otherwise.

4.5   Results

Overviews of the pollen assemblages recovered from Ankasa and Bobiri over the three years of sampling (Figures 4.4 and 4.5) are presented, and figures of R-rel$_{(ind)}$ for selected taxa from each plot (Figures 4.7-49). A plate of pollen taxa identified can be seen in Figure 4.9. These figures show the most abundant taxa that have been identified in both the pollen and the vegetation, and how their R-rel$_{(ind)}$ values vary over space and time. Two pollen taxa that are

often of interest to palaeoecologists, Melastomataceae/Combretaceae and Moraceae, are presented from both plots, as although they are not among the most abundant taxa in Ankasa, the differences in their representation between wet evergreen rainforest and moist semi-deciduous forest is note-worthy.
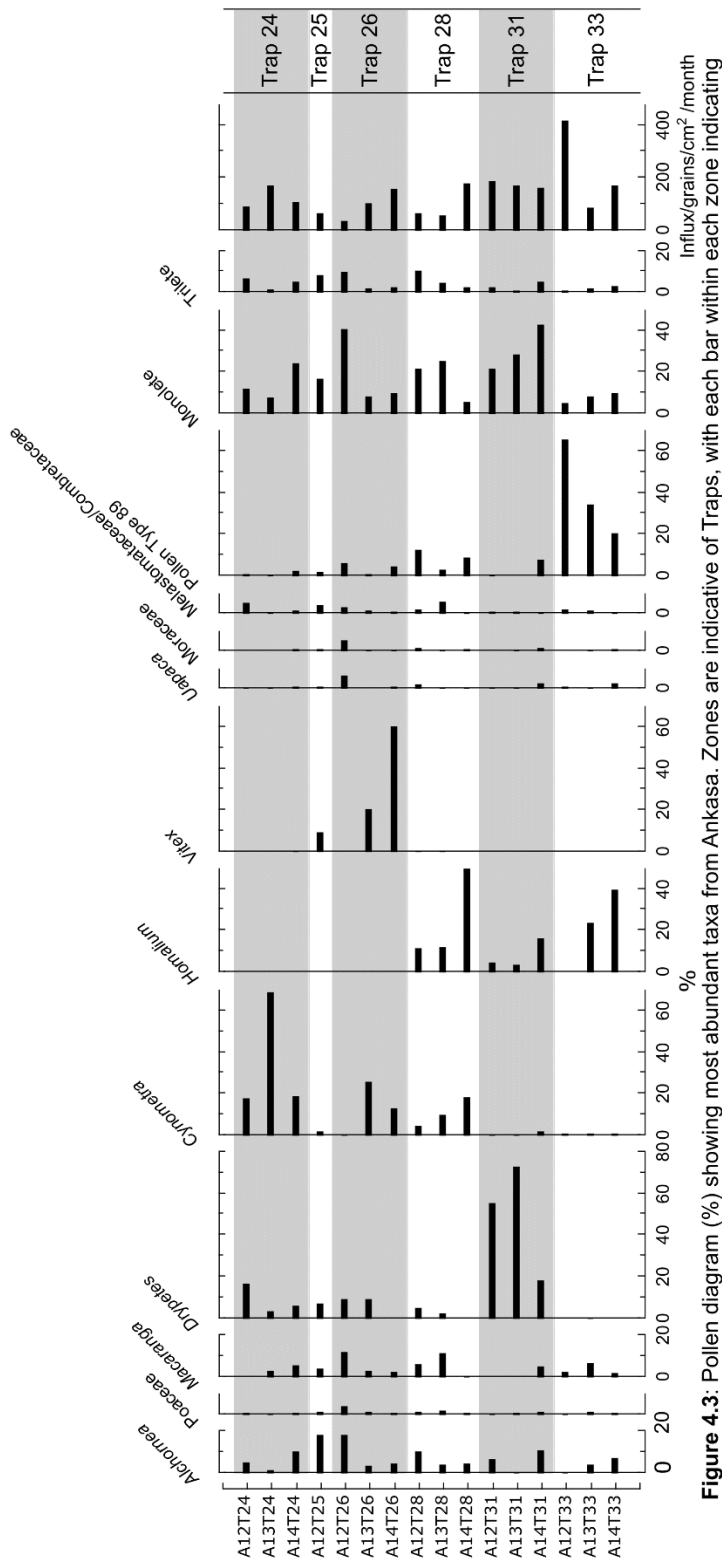
Figure 4.3: Pollen diagram (%) showing most abundant taxa from Ankasa. Zones are indicative of Traps, with each bar within each zone indicating one year of sampling. 'A12T24= Ankasa 2012, Trap 24'.
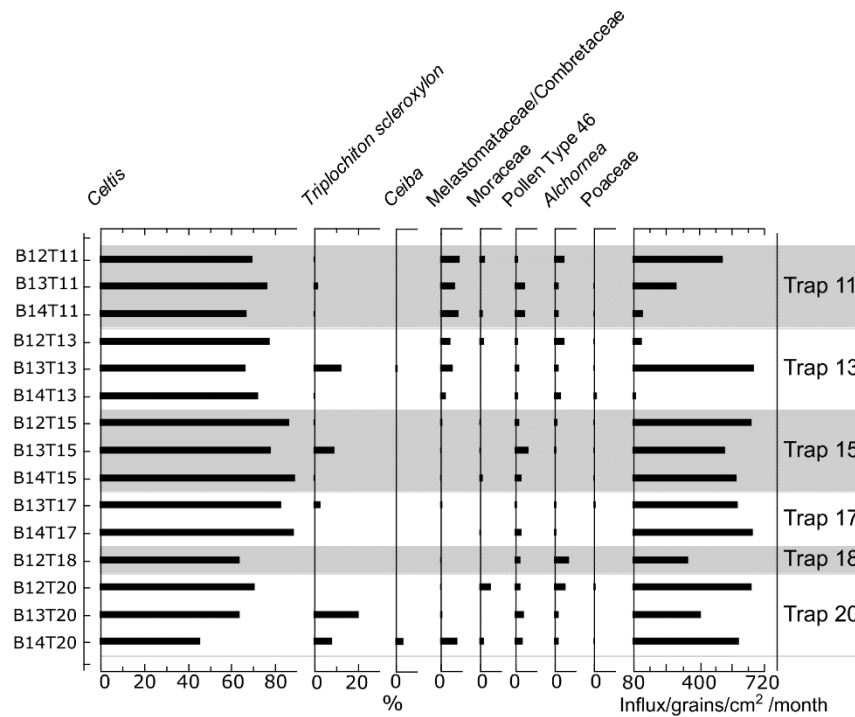
Figure 4.4: Pollen diagram (%) from Bobiri, showing most abundant taxa. Zones are indicative of Traps, with each bar within each zone indicating one year of sampling. 'B12T11= Bobiri 2012, Trap 11'.

4.5.1 Ankasa

Pollen assemblages from the Ankasa samples were variable, both between years and traps (Figure 4.5). Taxa which occurred in all samples over all three years are *Cynometra* (0.3 – 68.8%), *Alchornea* (0.7 – 18.7%), Type 12 (0.3 – 6.6%), and Poaceae (0.3 – 4.2%). Monolete and Trilete spores are also present in all samples (but with percentages calculated outside of the pollen sum), with abundances ranging from 5.2 – 43.0% and 1.0 – 10.7% of the pollen sum respectively (Figure 4.3). The pollen influx varied from 36 – 422 grains/cm^2/month, with an average of 141 grains/cm^2/month. A total of 144 pollen taxa were recorded, of which 49 were assigned botanical affinities. Count totals for samples from Ankasa ranged from 183 to 743 grains, with an overall count of 5670 grains.
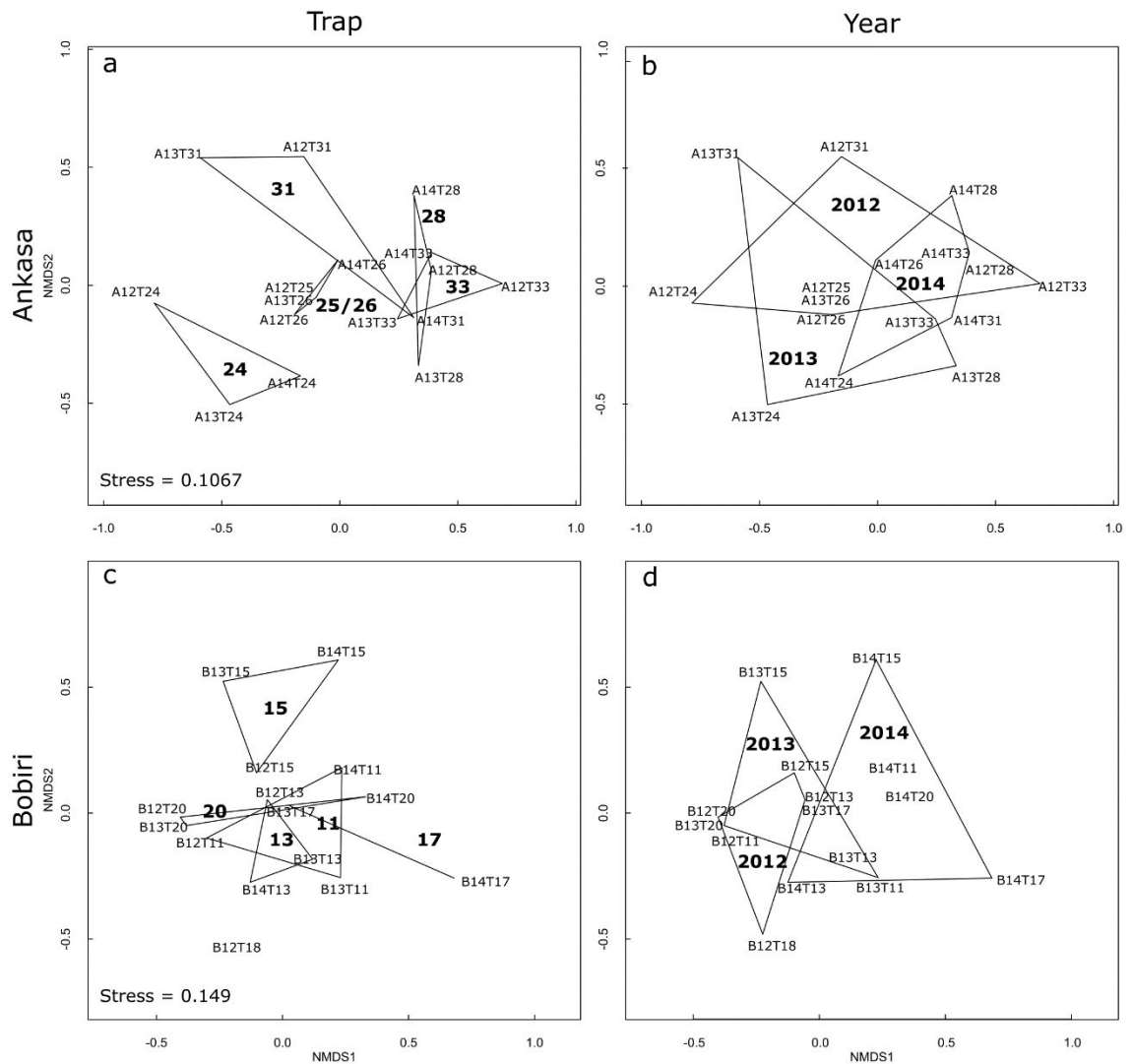
Figure 4.5: NMDS plots showing Ankasa (a and b) and Bobiri (c and d), with hulls overlain by Trap (a and c) and by Year (b and d). Bold numbers refer to Traps, with non-bold labels referring to year and trap i.e. A12T24= Ankasa 2012, Trap 24.

4.5.1.1   Major Taxa

The five most abundant pollen taxa which were also recorded in the vegetation of the plot, ranked by their average percentage abundance per trap, were *Drypetes* (13.1%)*, Cynometra* (11.7%)*, Homalium* (10.1%), *Vitex* (5.7%) and *Uapaca* (1.5%)*.* Melastomataceae/Combretaceae (2.1%) and Moraceae (0.9%) are also presented here, to enable comparison with Bobiri; Figure 4.6 and 4.7 illustrate the R-rel$_{(ind)}$ values reported below.
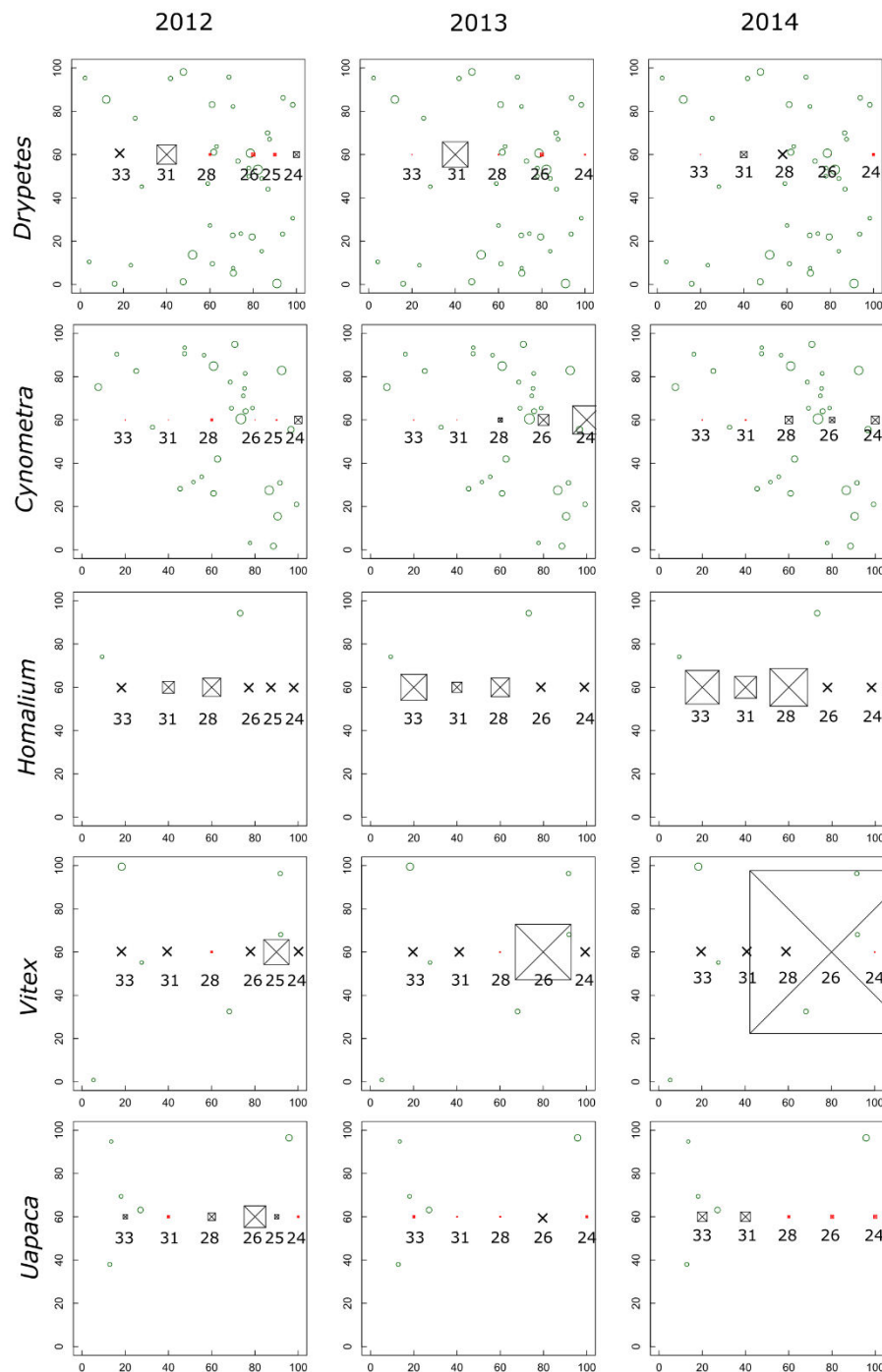
Figure 4.6: R-rel$_{(ind)}$ plots Ankasa. 100 x 100 m vegetation plot maps of Ankasa, for the five most abundant vegetation taxa also represented in the pollen assemblages, over the three years of sampling. Green circles indicate members of the taxon with DBH > 10 cm as labelled on the left of the plot, with size of circle scaled as in Fig.Figure 3. Symbols are scaled by R-rel$_{(ind)}$ value. Red symbols indicate R-rel$_{(ind)}$ <1, whereas black symbols indicate R-rel$_{(ind)}$ >1, black crosses indicate that no pollen of that taxon was recorded in that plot. Numbers underneath or to the left of traps indicate pollen trap number. *Homalium* symbols are square rooted, as this taxon's R-rel$_{(ind)}$ values were too large to fit on the plots.

4.5.1.1.1   Drypetes

Drypetes pollen was present in samples at percentages of between 0.0% (Trap 31, 2012 and Trap 28, 2014) to 73.3% (Trap 31, 2013) of the pollen sum. It was present, in 2012, at an average abundance of 15.9%, in 2013, 17.9% and in 2014, 5.0%. The trap with the lowest average abundance was Trap 33 (0.3%), and trap with the highest average abundance was Trap 31 (36.8%).

Drypetes was over-represented in four samples under-represented in 10 samples, and absent from two samples. The R-rel$_{(ind)}$ values of this taxon ranged from 0.01 (Trap 26 2014) to 7.64 (Trap 31 2013), with a sample average of 1.37. In 2012, the average R-rel$_{(ind)}$ was 1.65, in 2013, 1.87 and in 2014, 0.52. The trap with the lowest average R-rel$_{(ind)}$ value was Trap 33 (0.03) and the highest was in Trap 31 (5.12).

4.5.1.1.2   Cynometra

Cynometra pollen accounted for between 0.3% (Trap 31, 2013) and 68.8% (Trap 24, 2013) of the pollen sum. It was present in 2012 at an average abundance of 4.5%, in 2013, 21.2% and in 2014, 10.8%. Its lowest average abundance was in Trap 31 (0.7%), and its highest in Trap 24 (35.4%).

Cynometra was over-represented in seven samples, under-represented in nine samples and absent from none. The R-rel$_{(ind)}$ values of this taxon varied between 0.04 (Trap 31, 2013) and 8.48 (Trap 24, 2013), with a sample average of 1.44. In 2012 the average R-rel$_{(ind)}$ was 0.55, in 2013 it was 2.62 and in 2014 it was 1.33. Its lowest average R-rel$_{(ind)}$ was in Trap 31 (0.11) and the highest was in Trap 24 (4.36).

4.5.1.1.3   Homalium

Homalium was present at abundances between 0.0% (Traps 24 and 26 in all years, Trap 25 in 2012 and Trap 28 in 2013) and 49.8% (Trap 28, 2014). Its average abundance in 2012 was 2.8%, in 2013, 7.9% and in 2014, 21.2%. The lowest average abundance of this taxon was in Traps 24, 25 and 26, 0% and the highest was in Trap 28 (24.6%).

Homalium was over-represented in eight samples, and absent from the remaining eight samples. The R-rel$_{(ind)}$ values of *Homalium* ranged from 9.2 (Trap 31, 2013) to 127.92 (Trap 28, 2014), with a sample average of 26.05. In 2012, the average R-rel$_{(ind)}$ was 7.11, in 2013, 20.31 and in 2014, 54.52. The trap with the lowest average R-rel$_{(ind)}$ value was Trap 31 (21.5), and the highest was Trap 28 (63.09).

4.5.1.1.4   Vitex

Vitex was present in samples at abundances of between 0.0% (Traps 31 and 33 in all years, Trap 24 in 2012 and 2013 and Trap 28 in 2014) and 60.3% (Trap 26, 2014). Its average abundance in 2012 was 1.7%, in 2013 4.2% and in 2014 12.1%. Its its lowest abundance was 0.0%, in Traps 31 and 33 and its highest was 26.9%, in Trap 26

Vitex was over-represented in three samples, under-represented in three, and not present in 10. It had R-rel$_{(ind)}$ values from 0.26 (Trap 24, 2014) to 49.01 (Trap 26, 2014). Its average R-rel$_{(ind)}$ in 2012 was 1.35, in 2013 was 3.40, and in 2014, 9.85. The Trap with the lowest average R-rel$_{(ind)}$ value was Trap 24 (0.09) and the highest was in Trap 26 (21.90).

4.5.1.1.5   Uapaca

Uapaca was present in samples at abundances of between 0.0% (Trap 26, 2013) and 6.7% (Trap 26, 2012). Its average abundance in 2012 was 2.1%, in 2013 0.4%, and in 2014, 1.7%. Its lowest average trap abundance was 0.7% in Trap 24, and its highest average trap abundance was 2.5% in Trap 26.

Uapaca was over-represented in six traps, under-represented in nine, and absent from one trap. Its R-rel$_{(ind)}$ values ranged from 0.32 (Trap 31, 2013) to 6.51 (Trap 26, 2012). Its average R-rel$_{(ind)}$ in 2012 was 2.08, in 2013 was 0.38 and in 2014 was 2.89. The Trap with the lowest average R-rel$_{(ind)}$ was Trap 24 (0.69) and the trap with the highest R-rel$_{(ind)}$ value was Trap 26 (2.48).

4.5.1.1.6   Melastomataceae/Combretaceae

Melastomataceae/Combretaceae pollen was present in samples at abundances of between 0.3% (Trap 28, 2014) and 6.0% (Trap 28, 2012) of the pollen sum. Its average abundance in 2012 was 3.1%, in 2013 was 2.2% and in 2014, 0.9%. The lowest average trap abundance was 0.9% in Trap 31, and the highest average trap abundance was 2.9% in Trap 28.

Melastomataceae/Combretaceae was present, but under-represented in all samples. Its R-rel$_{(ind)}$ values ranged from 0.02 (Trap 28, 2014) to 0.45 (Trap 28, 2012). Its average R-rel$_{(ind)}$ in 2012 was 0.23, in 2013 was 0.16, and in 2014 was 0.07. The Trap with the lowest average R-rel$_{(ind)}$ was Trap 31 (0.07) and the highest average R-rel$_{(ind)}$ was 0.22 (Trap 28).

4.5.1.1.7   Moraceae

Moraceae pollen was present in samples at abundances of between 0.0% (Traps 24, 31 and 33 in 2012, and Trap 24, 2013) and 5.3% (Trap 26, 2012). Its average abundance in 2012 was 1.3%, in 2013 was 0.3% and in 2014 was 1.2% of the pollen sum. The lowest average abundance per trap was 0.4% (Trap 24) and the highest was 2.1% in Trap 26.

Moraceae type pollen was over-represented in 12 samples and absent from four. Its R-rel$_{(ind)}$ values ranged from 2.70 (Trap 31, 2012) to 43.72 (Trap 26, 2012). In 2012, its average R-rel$_{(ind)}$ value was 10.90, in 2013 was 2.26 and in 2014 was 9.75. The trap with the lowest average R-rel$_{(ind)}$ was Trap 24 (3.58), and the trap with the highest average R-rel$_{(ind)}$ was Trap 26 (17.71).
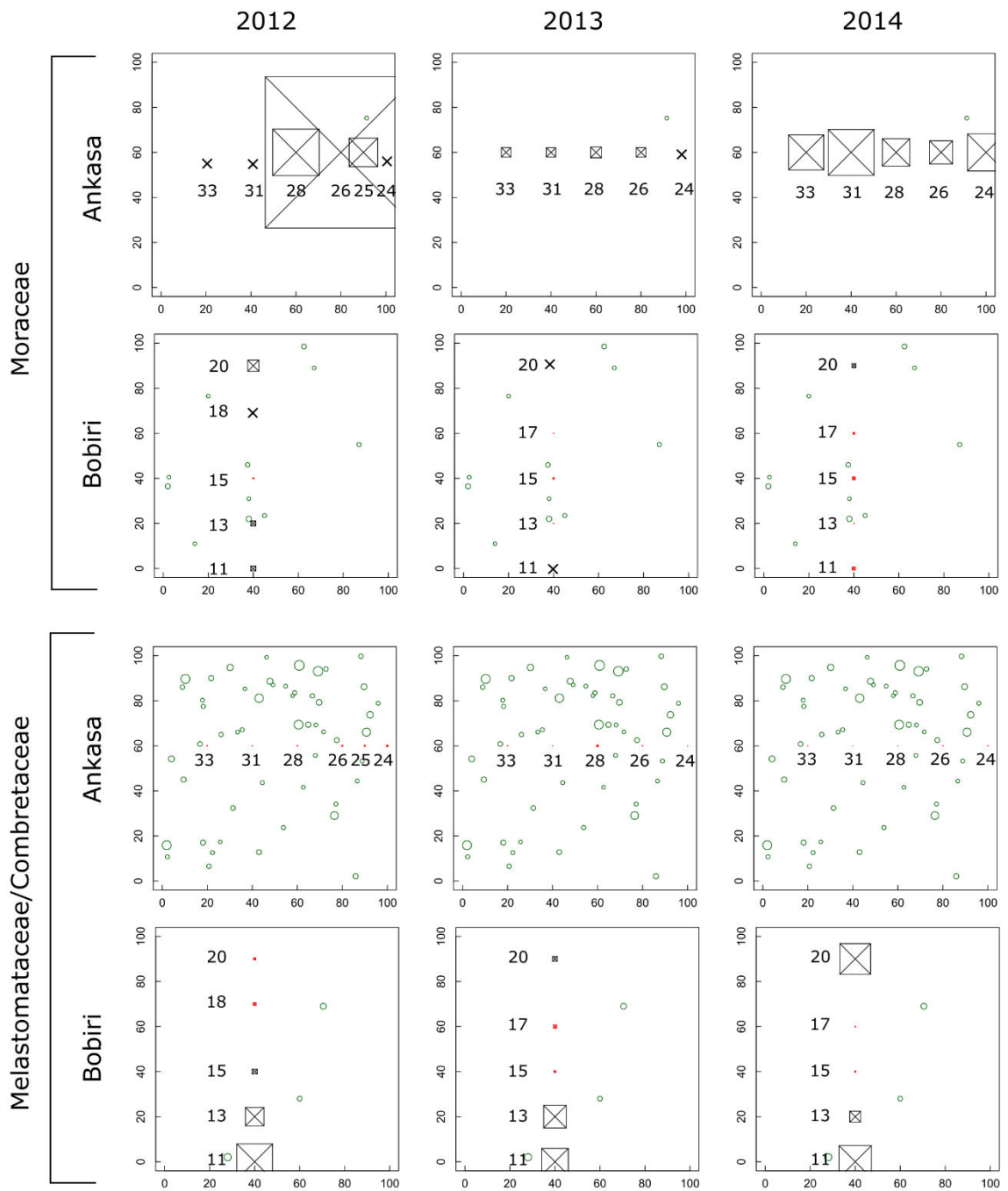
Figure 4.7: R-rel$_{(ind)}$ plots shared taxa 100 x 100 m vegetation plot map of Ankasa and Bobiri, for the shared taxa Melastomataceae/Combretaceae and Moraceae, over the three years of sampling. Green circles indicate members of the taxon with DBH > 10 cm as labelled on the left of the plot, with size of circle scaled as in Figure 3. Symbols are scaled by R-rel$_{(ind)}$ value. Red symbols indicate R-rel$_{(ind)}$ <1, whereas black symbols indicate R-rel$_{(ind)}$ >1, black crosses indicate that no pollen of that taxon was recorded in that plot. Numbers underneath or to the left of traps indicate pollen trap number.

4.5.1.2   Spatial and temporal separation of samples

Ordination of the samples from Ankasa illustrates that samples cluster by Trap (the same trap in different years e.g. Trap 24 in 2012, 2013 and 2014) rather than by year (e.g. Traps 24, 26

and 28 in 2012), this is illustrated by Figure 4.5, in which the hulls in a) demonstrate samples of the same trap over all three years, whereas b) shows the traps linked by Year of sampling.

Trap 24 samples fall between -0.5 and 0.0 on both NMDS axes. Traps 25 and 26 cluster together around 0.0 on both axes. Traps 28 and 31 do not occupy clearly separate areas of the ordination space, both falling around 0.5 on NMDS1 but being more widely spread along NMDS2. Trap 31 is more widely dispersed, with two years (2012 and 2013) falling at 0.5 on NMDS 2, but 2014 clustering more closely to the Trap 33 and 28 clusters. The traps therefore broadly show a gradient along NMDS axis 1.

When year of sample is overlain as a hull on the NMDS, there is much overlap between the hulls. 2014 appears to be less widely dispersed across ordination space, but still overlaps with both 2013 and 2012, which also overlap with each other.

PERMANOVA tests indicate that the difference between samples when grouped by Trap is significant ($p = 0.002$), and that the difference between samples when grouped by year is not ($p = 0.217$).

4.5.2   Bobiri

Pollen assemblages from Bobiri samples were heavily dominated by one genus (*Celtis*), which was found in every sample and accounted for between 46.1% and 89.4% of the pollen sum. In addition to *Celtis*, taxa that were found in every trap were Pollen Type 46 (0.97 – 4.8%), Poaceae (0.1 – 1.5%), and Melastomataceae/Combretaceae (0.2 – 9.0%). Monolete and Trilete spores were present in abundances from 0.0% to 2.5% and 1.6% of the pollen sum respectively. The pollen influx varied from 97 grains/cm^2/month to 675 grains/cm^2/month, with an average of 462 grains/cm^2/month. A total of 104 pollen taxa were recorded, of which 43 were assigned botanical affinities. Count sizes for Bobiri ranged from 377 to 798 grains, with an overall count of 8295.

Figure 4.8: R-rel$_{(ind)}$ plots Bobiri 100 x 100 m vegetation plot maps of Bobiri, for the three most abundant vegetation taxa also represented in pollen assemblages, over the three years of sampling. Green circles indicate members of the taxon with DBH > 10 cm as labelled on the left of the plot, with size of circle scaled as in Fig.Figure 3. Symbols are scaled by R-rel$_{(ind)}$ value. Red symbols indicate R-rel$_{(ind)}$<1, whereas black symbols indicate R-rel$_{(ind)}$ >1, black crosses indicate that no pollen of that taxon was recorded in that plot. Numbers to the left of traps indicate pollen trap number.

4.5.2.1   Major Taxa

The five most abundant pollen taxa that were also present in the vegetation of the plot, ranked by their average percentage abundance per sample were *Celtis* (73.5%), *Triplochiton scleroxylon* (3.9%), Melastomataceae/Combretaceae (3.4%), Moraceae (1.1%) and *Ceiba* (0.3%). The R-rel$_{(ind)}$ values for Bobiri are illustrated in Figure 4.8, apart from Melastomataceae/Combretaceae and Moraceae, which are in Figure 4.7.

4.5.2.1.1   Celtis

Celtis was present in all samples at abundances of between 46.1% (Trap 20, 2014) and 89.4% (Trap 15, 2014). Its average abundance in 2012 was 73.8%, in 2013 was 73.9%, and in 2014 72.8%. The lowest average trap abundance exhibited by *Celtis* was 60.4% in Trap 20, and the highest was 86.1% in Trap 17.

Celtis was over-represented in all samples. Its R-rel$_{(ind)}$ values ranged from 2.28 (Trap 20, 2014) to 4.41 (Trap 15, 2014). Its average R-rel$_{(ind)}$ in 2012 was 3.65, in 2013 was also 3.65, and in 2014 was 3.59. The trap with the lowest average R-rel$_{(ind)}$ value was Trap 15 (2.98) and the highest average R-rel$_{(ind)}$ was recorded in Trap 17 (4.25).

4.5.2.1.2   Triplochiton scleroxylon

Triplochiton scleroxylon was present in samples at between 0.0% (Traps 18 and 20, 2012) and 20.3% (Trap 20, 2013) of the pollen sum. Its average abundance in 2012 was 0.2%, in 2013, 9.6%, and in 2014 1.9%. Its lowest average abundance in any trap was 0.0% in Trap 18, and its highest was 9.6%, in Trap 20.

Triplochiton scleroxylon was over-represented in four samples, under-represented in 10, and absent from two. Its R-rel$_{(ind)}$ values ranged from 0.04 (Trap 13, 2013) to 5.95 (Trap 20, 2013). The average R-rel$_{(ind)}$ of this taxon in 2012 was 0.07, in 2013 was 2.83, and in 2014 was 0.56. The trap with the lowest average R-rel$_{(ind)}$ value was Trap 18 (0.00), and the trap with the highest average R-rel$_{(ind)}$ value was Trap 20 (9.56).

4.5.2.1.3   Melastomataceae/Combretaceae

Melastomataceae/Combretace pollen was present in all samples at abundances of between 0.2% (Trap 17, 2014) and 9.1% (Trap 11, 2012). Its average abundance in 2012 was 2.3%, in 2013, 1.8% and in 2014, 3.4%. The lowest average trap abundance was 0.3% (Trap 17) and its highest was 7.8% (Trap 11).

Melastomataceae/Combretaceae pollen was over-represented in nine samples and under-represented in six. The R-rel$_{(ind)}$ values of Melastomataceae/Combretaceae in the plot ranged from 0.22 (Trap 17, 2014) to 10.31 (Trap 11, 2012). Its average R-rel$_{(ind)}$ in 2012 was 3.70, in 2013 it was 3.41 and in 2014, 4.37. The trap with the lowest average R-rel$_{(ind)}$ value was Trap 17 (0.57) and the trap with the highest average R-rel$_{(ind)}$ was Trap 11 (9.12).

4.5.2.1.4   Moraceae

Moraceae pollen was present in samples at abundances of between 0.0% (Trap 11, 2013 and Trap 18, 2012) and 5.3% (Trap 20, 2012). Its average abundance in 2012 was 2.1%, in 2013 was 0.2%, and in 2014 was 1.1%. The lowest average abundance per trap was 0.5% (Trap 17) and the highest was 2.4 (Trap 20).

Moraceae pollen was over-represented in four samples, under-represented in eight, and absent from three. The R-rel$_{(ind)}$ values of this taxon ranged from 0.09 (Trap 13, 2014) to 1.45 (Trap 11, 2012). The average R-rel$_{(ind)}$ value of this taxon in 2012 was 1.29, in 2013 was 0.12, and in 2014 was 0.69. The lowest average R-rel$_{(ind)}$ per trap was 0.29 (Trap 17) and the highest was 1.47 (Trap 20).

4.5.2.1.5   Ceiba

Ceiba was present in five samples, at abundances of between 0.1% (Trap 33, 2014) to 3.5% (Trap 20, 2014) of the pollen sum. Its average abundance in 2012 was 0.1%, in 2013, was 0.2%, and in 2014 was 0.7%. The lowest average abundance per trap of this taxon was 0.0% (Traps 11, 15 and 18), and the highest average abundance per trap was 1.1% (Trap 20).

Ceiba was over-represented in one sample, under-represented in four, and absent from 10 samples. Its R-rel$_{(ind)}$ values ranged from 0.06 (Trap 13, 2014) to 1.50 (Trap 20, 2014). The average R-rel$_{(ind)}$ value of this taxon in 2012 was 0.02, in 2013 was 0.09, and in 2014 was 0.31. The lowest average R-rel$_{(ind)}$ value per trap was 0.03 (Trap 17) and the highest was 0.5 (Trap 20).

4.5.2.2   Spatial and temporal separation of samples

Ordinations of the samples from Bobiri illustrate that there is clustering by both Trap and Year (Figure 4.5). When hulls outlining the same Trap in different years are overlain on the NMDS (Figure 4.5c), there is some overlap between traps Traps 11 and 17 show relatively wide dispersal in ordination space, Trap 20 samples cluster around 0.0 on NMDS axis 2 but are more widely spread on NMDS axis 1. Trap 13 samples are relatively constrained, although overlap with Traps 11 and 20. Trap 15 samples cluster separately in ordination space to the other traps, although it should be noted that B12T15 is more similar to the cluster of samples below it than to its sister traps.

When Year hulls are overlain (Figure 4.5d), there is overlap in the ordination space occupied by the three years of sampling. Samples from 2012 were the most tightly clustered, and 2014

showed the widest dispersal across ordination space. Samples from 2013 fell among those from 2012 and 2014, indicating a temporal gradient along NMDS axis 1. Although the hulls of 2012 and 2014 do not overlap, some samples show greater similar to samples from a different year than to those of their own. For instance, B14T13 is more similar to B12T18 than to its sister plots.

PERMANOVA tests indicate that difference between samples when grouped by Trap is significant (p = 0.017 and when grouped by Year, not significant (p = 0.326).

4.6   Discussion

4.6.1   Drivers of heterogeneity of pollen assemblages and characteristic taxa

Pollen assemblages from both plots, Ankasa and Bobiri, exhibit variation temporally and spatially. PERMANOVA tests indicate that there is a significant difference between traps, but not years, in both plots. Bobiri exhibits a greater temporal signal potentially driven by the response of *Triplochiton scleroxylon* to dry conditions in 2013. The samples from Bobiri also show a more homogenous signal, with samples containing upwards of 70% *Celtis* type pollen (Figure 4.4), whereas the most abundant taxon in Ankasa samples varies (Figure 4.3). Below, the most abundant pollen taxa from each plot are discussed, along with factors that are likely to have resulted in their observed pollen signals. In some taxa, vegetation structure is likely the driving force in the depositional pattern observed, whereas for others, temporal factors, reproductive strategy or pollination syndrome are identified as potential drivers. In reality, a complex interaction of all of these factors will have resulted in the results observed.

4.6.2   Taxa present in all samples from both plots

Alchornea and Poaceae are present in all samples from Ankasa and Bobiri except *Alchornea* is missing from one (Bobiri Trap 15, 2014), but neither taxa are present in the vegetation in the plot. *Alchornea* is present in Ankasa, but due to the fact that it is a small shrub whose recorded measurements are uniformly < 10 cm DBH (Hawthorne et al. 1998), it is not recorded in the vegetation surveys of our plots. In Bobiri, *Alchornea* is also not recorded in the vegetation survey, but is likely present as a shrub, as the genus contains several shrubby species that are widespread in tropical West Africa (Hawthorne & Jongkind 2006). As in Ankasa, it is likely that representatives of this genus were excluded from vegetation surveys, as they frequently possess stems <10 cm DBH. *Alchornea* is wind pollinated, however, and therefore is likely to produce abundant pollen, meaning that its presence in all samples may be due to transport from outside of the plots.

Poaceae is recorded in all samples but at very low abundances. The presence of Poaceae is somewhat surprising, as the understorey of the forest plots do not contain noticeable grasses, and so these grains likely represent longer-distance (the nearest open areas in which grasses could feasibly grow are not within 100 m of the plots) transportation of this wind-pollinated family. The likely presence of long distance transported pollen demonstrates that although the pollen assemblages within these plots are highly dominated by heterogeneous local pollen signals, a regional element may still be present, most notably evidenced by Poaceae grains, but potentially also by other pollen types without botanical parents within the plot.

4.6.3   Ankasa

A stronger spatial, rather than temporal signal is evident from the Ankasa samples. NMDS analysis shows that samples tend to cluster by Trap, and therefore, physical position in plot (Figure 4.5a) rather than by Year (Figure 4.5b). This higher spatial than temporal effect is contrary to some other studies of pollen rain (Kershaw & Strickland 1990; Haselhorst et al. 2013), which report higher variability between years of sampling. The two most abundant pollen taxa in Ankasa (*Cynometra* and *Drypetes*) are also the most common taxa recorded in the vegetation survey.

4.6.3.1   Drypetes

Drypetes exhibits some spatial and temporal heterogeneity in R-rel$_{(ind)}$ values. *Drypetes aylmeri*, the only species of *Drypetes* in this plot, accounts for 9.6% of the basal area, and has an average height of 18.3 m. Trap 31 shows larger R-rel$_{(ind)}$ values than the other traps in the plot, despite there being a higher density of *Drypetes* trees in the proximity of Traps 24-28. There is a lower density of all trees in the vicinity of Trap 31 (Figure 4.2a), because a very large canopy tree (the large buttressed tree visible in Figure 4.1a) died and left a gap in the canopy. The overall influx of pollen into Trap 31 is not higher than for the other traps, but the proportion of *Drypetes* pollen is higher. *Drypetes* is an entomophilous genus, but it is also dioecious, which may contribute to its pollen being dispersed by wind as well as by insects (ambophily), as described for other dioecious taxa (Bush & Rivera 1998).

4.6.3.2   Cynometra

Cynometra is a chiropterophilous taxon, in which flowers are borne on the trunk. In terms of pollen deposition, this may explain the taxon's abundance, as its pollen is released not into the canopy, but potentially the understorey of the forest, making it more likely to be deposited in traps placed near the forest floor. This taxon is both under- and over-represented in different

traps within the plot, indicating that pollen deposition is very local and originates from trees close to traps.

4.6.3.3   Homalium

Homalium exhibits the highest R-rel$_{(ind)}$ values of any taxon in this study, and is also highly spatially heterogenous, being either consistently over-represented or consistently absent in the same trap over all years (apart from Trap 33, from which it is absent in 2012 but over-represented in 2013 and 2014). *Homalium deweverei* De Wild. & T.Durand, the only *Homalium* species in this plot, is hermaphrodite but has open flowers, and produces flowers in large inflorescences, potentially accounting for the high levels of over-representation observed here. There is not a clear link between proximity to individuals and amount of pollen present. For instance, Trap 33 is nearest to one of the *Homalium* trees, but is not the Trap in which *Homalium* pollen is most abundant. This discrepancy could be due to smaller (under 10 cm DBH) *Homalium* plants producing pollen at a local scale within the plot.

4.6.3.4   Vitex

Vitex shows a very localised signal, being over-represented in Trap 25 and 26, but almost absent from the other traps in the plot. Members of the genus *Vitex* do not account for a large part of the vegetation (1.2% of the basal area). The degree of over-representation varied between years, being most over-represented in 2014. *Vitex micrantha* Gürke, the most abundant species of *Vitex* in the Ankasa plot, is generally a small tree, whose average height in the plot is 20 m. The height of the individual closest to Traps 25 and 26 is 15 m, meaning that pollen produced by this individual would be unlikely to travel far, due to a lack of air currents in the understory of the forest (Kuparinen et al. 2007).

4.6.3.5   Uapaca

Uapaca corbisieri De Wild., the only taxon of *Uapaca* in this plot, is dioecious and is most over-represented in 2012 but consistently under-represented in 2013. The majority of trees of this taxon are near Traps 33 and 31, but its pollen does not show a clear spatial pattern of representation, with Trap 26 showing the most over-representation in 2012, but Traps 33 and 31 in 2014. The sexes of the plants of *U. corbisieri* in this plot are not known, a factor that may contribute to the spatial discrepancy observed in its pollen signal.

4.6.4   Bobiri

Pollen assemblages from Bobiri are relatively more uniform, both temporally and spatially, than those from Ankasa, being largely dominated by the genus *Celtis*, which is the most abundant taxon in both the pollen and the vegetation.

4.6.4.1   Celtis

Celtis exhibits a relatively uniform distribution of R-rel$_{(ind)}$ values over both years and traps, and is consistently over-represented. It is an anemophilous taxon, meaning that over-production and wide dispersal of pollen is expected. This taxon is also the most abundant in the vegetation, accounting for 14.7% of the stems in the plot, so although it is over-represented in the pollen, the most abundant taxon in both vegetation and pollen is, in this case, the same. This taxon is one of the most evenly distributed, although how much of the signal is from outside the plot is not discernible.

4.6.4.2   Triplochiton scleroxylon

Tripochiton scleroxylon is most noticeably variable by year, being most abundant in 2013. Ghana experienced a rainfall deficit in summer 2013 (OCHA 2013), and therefore, this pattern of pollen dispersal is consistent with observations that this species tends to flower primarily during years in which the July – August rainfall is below average (Jones 1974). In 2012 and 2014 the pollen taxon was also present, but much less abundant. That pollen is observed in 2012 and 2014 as well as 2013 implies that *Triplochiton scleroxylon* may flower more frequently than herbarium records suggest (albeit with a lower frequency of individuals flowering, or trees producing fewer flowers). The nature of botanical collection is such that observing flowers in the canopy is often very difficult, and therefore, collection of specimens is most likely to occur when flowers are abundantly apparent. It is possible, therefore, that when few flowers are produced, they are less likely to be sampled for herbarium specimens (an effect that has been noted for other taxa e.g. Asteraceae (Schmidt-Lebuhn et al. 2013). This highlights a potential use for pollen traps in assessing phenology of tropical trees, as direct observations of these taxa is often impractical or impossible.

4.6.4.3   Ceiba

Ceiba is a pan-tropical genus that has been identified, in very low abundances (< 1.0% of pollen rain), in the Lake Bosumtwi record (Maley & Livingstone 1983), although it was not found in more recent, detailed studies of the Bosumtwi record (Miller & Gosling 2014). The taxon is chiropterophilous, with pollen being distributed long distances by bats (20km) (Dick et al.

2007), a factor that may explain its scarcity in the fossil record, as pollen from bat pollinated flowers tends to be larger, and therefore may be less likely to be borne on air currents and deposited in sediments (Stroo 2000). Here, a consistent presence of *Ceiba* pollen was observed in all years in one trap, Trap 13, which is close to an individual of *Ceiba pentandra* (L.) Gaertn. in the plot. Trap 20 in 2014 exhibits an over-representation of *Ceiba* pollen relative to the vegetation of the plot, although this may result from extra-plot pollen rain, as Trap 20 is relatively close to the edge of the surveyed area. The presence of *Ceiba* indicates that small amounts of its pollen are transported on air currents, potentially also accounting for its presence in the fossil record.

4.6.5   Shared taxa (Melastomataceae/Combretaceae and Moraceae)

Melastomataceae/Combretaceae pollen was present in both plots, although tended to be over-represented in Bobiri and under-represented in Ankasa. This taxon accounted for under 10% of the pollen sum in all samples at both plots, but accounted for a much larger proportion of the number of trees (52 stems) and basal area of Ankasa (13.2%) than in Bobiri (three stems and 0.9%).

Moraceae pollen was found in both plots at relatively low abundances (not above 5.5% in any sample), in Ankasa being heavily over-represented, whilst in Bobiri being under-represented. This is due to the differing number of plants from the Moraceae in each plot (one in Ankasa, and 11 in Bobiri, accounting for 0.12% and 1.64% of the vegetation respectively), but could also be accounted for by pollen from outside of the plot contributing to the signal in Ankasa. This finding differs from that in the Neotropics (Gosling et al. 2009) in which Moraceae pollen is found to be over-produced in a variety of ecosystems.

Melastomataceae/Combretaceae and Moraceae have been shown to over-produce pollen relative to their vegetative abundance in other modern pollen studies (Bhattacharya et al. 2011; Urrego et al. 2011). Here, however, it is shown that there is disparity in the representation of these taxa at a local level, and that pollen production by these taxa does not consistently over- or under-represent their vegetative abundance. The more even distribution of the pollen of Melastomataceae/Combretaceae and Moraceae could indicate that these taxa disperse pollen higher up in the canopy, so that it is dispersed further and is rained into traps in a relatively more even manner, potentially giving a regional vegetation signal, but one that is not necessarily representative of the local vegetation.
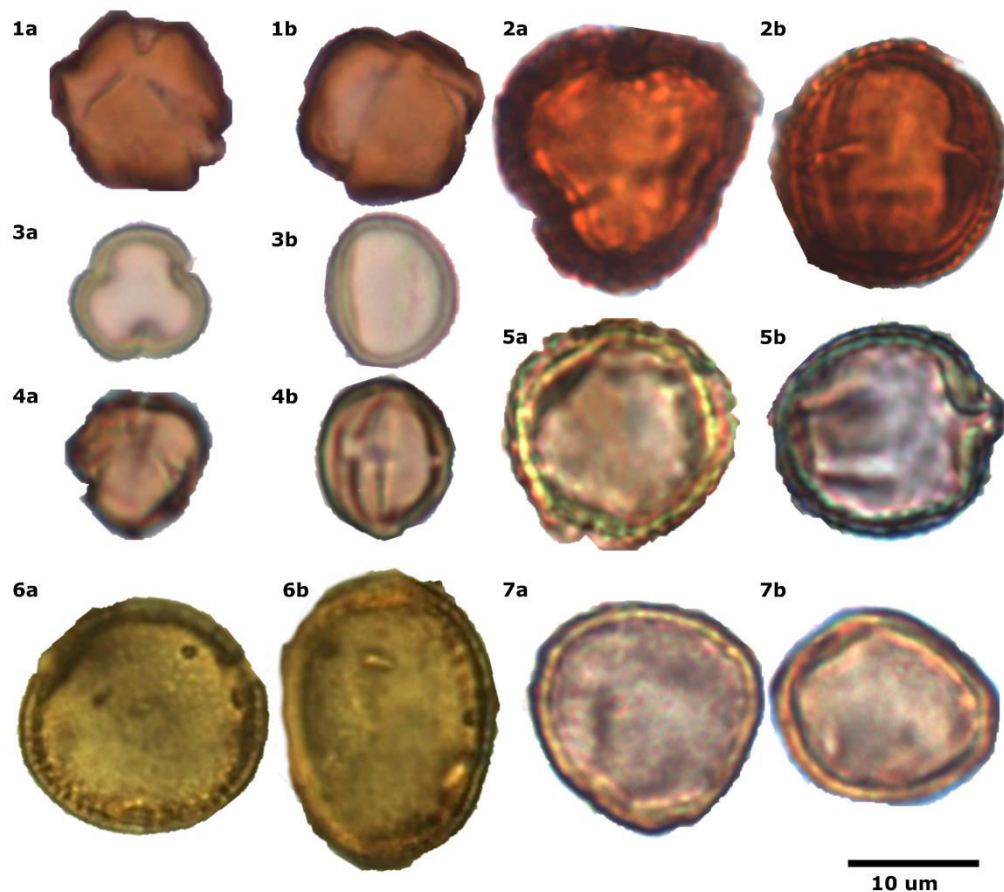
Figure 4.9: Pollen plate showing most abundant taxa for Ankasa and Bobiri. Numbers indicate taxa, a= polar view b= equatorial view. 1. *Cynometra*, 2. *Drypetes*, 3. *Vitex*, 4. *Homalium*, 5. *Uapaca*, 6. *Triplochiton scleroxylon*, 7. *Celtis*

4.6.6   Comments on movement of pollen

4.6.6.1   Implications for models of pollen dispersal

The movement of pollen within ecosystems has long been recognised as a complex phenomenon (Davis 1963; Tauber 1965). Factors such as wind speed and direction, canopy structure and pollen weight and morphology can all influence how pollen grains move (Prentice 1985). Here, it has been demonstrated that both spatial and temporal factors influence how pollen generated by plants in tropical West African forests moves and is deposited in ecosystems, and that pollen does not appear to be produced by plants in these plots in a consistent manner.

Some models assume an isotropic pollen dispersal curve (Sugita 1994; Miller 2016), that is, that pollen is equally likely to be distributed all around the source. In our data, however, there are many instances where a taxon is over-represented in one trap, and under-represented or absent from one of the traps adjacent to it. This is likely due to the structure of the forest impeding pollen movement (Tauber 1967), so although in theory pollen distribution may be

isotropic, in this example, it appears to be strongly anisotropic. Langrangian stochastic dispersal models of pollen dispersal (Kuparinen et al. 2007; Theuerkauf et al. 2013), which have now been integrated into the REVEALS model of quantitative vegetation reconstruction (Theuerkauf et al. 2016) may help to account for this discrepancy by modelling the movement of individual grains, although these models have yet to be applied to complex tropical systems.

Pollen sources of the same taxon in models of pollen distribution are often assumed to produce the same amounts of pollen, with pollen productivity values being generated for taxa using empirical data (Theuerkauf et al. 2016). Our data show that this does not appear to be the case: not only does the amount of pollen arriving in traps vary between traps within the same plot, even when there is a relatively uniform distribution of the parent vegetation within the plot (e.g. *Drypetes*) but it also varies absolutely between years (e.g. *Triplochiton* and *Vitex*), indicating that pollen production from the same ecosystem may be very variable between years and sampling locations. Melastomataceae/Combretaceae and Moraceae R-rel$_{(ind)}$ values (Figure 4.8) demonstrate that in different ecosystems, estimates of pollen productivity for the same taxon can be very different, and therefore that generating reliable pollen productivity estimates for tropical taxa could be problematic.

4.6.6.2   Implications for artificial pollen trapping studies

At Ankasa, a high level of spatial consistency of pollen rain between years was observed, likely due to low wind speeds under the canopy of the systems studied (Kuparinen et al. 2007). This leads to very different relationships between pollen and vegetation being observed within very small spatial scales. For instance, if Trap 33 alone had been sampled across the three years of the study, *Drypetes, Cynometra* (two of the most abundant tree species in the plot) and *Vitex,* would have been severely under-represented or entirely absent from the resultant pollen assemblages, whilst 20m away, Trap 31 exhibits very high levels of *Drypetes*. Based on the levels of heterogeneity observed, it is likely the case that, had more pollen traps been counted, a higher level of spatial heterogeneity would have been revealed.

At Bobiri, although there was separation of traps and years, a temporal change was clearer than at Ankasa. The three years over which pollen was collected in this study were not highly heterogenous, climatologically; 2013 was relatively dryer than 2012 and 2014, but this signal was only reflected noticeably in one taxon, *Triplochiton scleroxylon.* As only three years of data collection was possible, it is possible that a stronger temporal signal would be observed had more years of sampling been undertaken.

These disparities between artificial pollen trap samples from within one vegetation plots has implications for the design of future pollen-trapping studies, as high spatial resolution sampling is not the norm in such studies. Conversely, however, it is also possible that estimates of the number of years needed for pollen trapping studies in the tropics of at least three (Bush & Rivera 1998) or at least 10 (Haselhorst et al. 2013) in order to obtain representative samples may be unnecessarily high, and a good level of representation may be possible by increasing the spatial resolution of sampling within years, particularly in rainforest settings. In sampling pollen produced by ecosystems, artificial pollen traps may perform relatively well compared to soil surface and moss polster samples in terms of inherent bias (Lisitsyna et al. 2012). However, using assemblages from traps to directly inform models of pollen dispersal in the tropics may be inadvisable due to the high levels of structural heterogeneity observed in tropical systems, which may result in very local pollen signals being recorded.

Although the most abundant trees and pollen taxa are the same for both plots, some of the most abundant pollen taxa (such as *Alchornea* and *Macaranga*) are not recorded in the vegetation. This is, largely, a vegetation survey design problem, however, in that the vegetation surveys undertaken exclude trees < 10 cm DBH, and it is therefore recommended that vegetation surveys for palynological purposes include samples of shrubby and herbaceous taxa so that R-rel$_{(ind)}$ values can be obtained for taxa such as these.

4.7 Conclusions

The pollen assemblages recovered from Ankasa capture the dominant vegetation taxa relatively well, with the two most abundant vegetation taxa (*Cynometra* and *Drypetes*) also being the most abundant taxa in the pollen assemblages. In Bobiri, *Celtis* is the most abundant vegetation and pollen taxon. There are, however, many vegetation taxa in both plots that are not represented in the pollen assemblages, which is likely due to the dominance of entomophilous taxa.

Pollen production and dispersal are not homogenous in the plots studied, and there is considerable heterogeneity between pollen assemblages recovered from artificial pollen traps placed within the same plot. This finding is likely due to the high spatial resolution of our traps within the ecosystems studied (5 per 100 x 100 m plot), the structure of the vegetation and the phenology and pollen dispersal mechanisms of the vegetation taxa within our plots.

Although there is not a statistically significant difference between the samples when grouped by year in either Ankasa or Bobiri, at Bobiri, Figure 4.5 indicates that there is a temporal gradient along NMDS axis 1. This is likely due to 2013 being a drier year than 2012 and 2014,

although the data are noisy and as only three years of data are available, it is difficult to determine the extent to which pollen assemblages may vary on a year-to-year basis.

5   Chapter 5: Modern pollen rain across sites from savannah to rainforest in tropical West Africa (Ghana)

Note on format: This chapter presents an overview of the pollen-vegetation relationships aspect of this thesis, and has therefore been formatted for submission to a journal focussed towards a palaeoecological audience, whose primary interest in the subject matter is likely to be based upon its relevance to the interpretation of fossil records. Results are, therefore, presented as bulk sums from the ecosystems in order to give a broad over-view of the pollen signals from the range of vegetation types sampled. Some taxa that were split in Chapter 2 to provide fine scale detail (such as *Terminalia* out of the Melastomataceae/Combretaceae and *Milicia* and *Ficus* out of the Moraceae) are here lumped, to reflect common practice in palaeoecological studies and enhance the study's applicability to that field.

5.1   Abstract

An overview of pollen-vegetation relationships across an ecological gradient in Ghana is presented, with a view to informing the interpretation of the fossil record of Lake Bosumtwi and other fossil records from the region, and thereby improving the understanding of past vegetation in tropical West Africa. Artificial pollen traps (n=46) were deployed across a range of ecosystems; three plots were from within a forest-savannah mosaic landscape (Kogyae), one plot from a moist semi-deciduous forest (Bobiri) and one from a wet evergreen rainforest (Ankasa). The sites studied can be distinguished using pollen diagrams and multivariate methods, and characteristic taxa for each site were identified. Poaceae and Melastomataceae/Combretaceae pollen were characteristic of the forest-savannah mosaic plots. *Celtis* and *Triplochiton* typified the moist semi-deciduous forest plot, and *Cynometra* and *Drypetes* pollen were most abundant in the wet evergreen rainforest plot. The most abundant taxa in the vegetation of the semi deciduous forest and wet evergreen forest were the same as the most abundant pollen. Taxa that were identified in all or most of the plots and therefore classified as non-informative were: *Alchornea*, Amaranthaceae, *Eugenia*, Moraceae and Trema. It was therefore concluded that, due to these non-informative taxa and the high percentages of Poaceae, *Celtis*, and Melastomatacee/Combretaceae present in the forest sections of the Bosumtwi fossil pollen record, these forests were likely more similar to the moist semi-deciduous forest or wooded savannah, rather than wet evergreen rainforest of the present day.

5.2   Introduction

The climate of Africa is changing at an unprecedented rate (Diffenbaugh et al. 2017), and this change is already affecting ecosystems and the people who depend on them (Collier et al. 2008; Niang et al. 2014). The continent is susceptible to changes in precipitation and temperature, as well as in the frequency and severity of extreme climate events such as droughts and flooding, which will have knock-on effects on vegetation and ecosystems (Hély et al. 2006; James et al. 2013; Niang et al. 2014). In order to understand the effects that these changes will have on vegetation and ecosystem dynamics, it is necessary to understand how vegetation has responded to changes in the past. Information on past environmental change can be obtained from fossil pollen records, together with carbon-isotope nitrogen-isotope, and oxygen-isotope data, non-pollen palynomorphs and fossilised plants, can be used to reconstruct past vegetation (Ficken et al. 2002; Birks & Birks 2006; Willis et al. 2013).

The interpretation of the fossil pollen record relies upon an understanding of how plants in modern ecosystems produce pollen (Davis 1963; Jackson & Lyford 1999; Farrell et al. 2016). In temperate regions, this issue has received much attention, with modern pollen rain studies having been carried out extensively for the past century, and the Pollen Monitoring Programme (Hicks et al. 2001) being established to standardise collection techniques (for a thorough review of the history of pollen trapping in Europe, see Giesecke et al. (2010), and for a meta-analysis of North American assemblages see Goring (Goring et al. 2013)). Pollen assemblages recovered from artificial traps represent local vegetation (Chapter 4, Haselhorst et al. 2013), and consequently they are not directly comparable to those recovered from large lakes. The larger the lake, the higher the proportion of regional and anemophilous, taxa it is likely to contain (Janssen 1966; Jacobson & Bradshaw 1981), whereas pollen traps tend to record local signals, meaning that entomophilous and locally abundant taxa can be relatively over-represented. Modern pollen studies can be useful in identifying taxa that, when identified at lower abundances in the fossil record, might be indicative of certain ecosystems (e.g. Fabaceae (Watrin et al. 2007)), and may also allow the identification of taxa that are not useful in differentiating between ecosystems, or those that must be treated with caution, such as Poaceae (Bush 2002).

In the tropics, and particularly in Africa, less work has been carried out on modern pollen-vegetation relationships than in temperate regions; Lézine et al. (2009) synthesised modern pollen studies in sub-Saharan and returned 452 modern samples from the whole region. There have, however, been some wide-ranging African studies that have shown that modern pollen assemblages, drawn mainly from surface samples, represent their parent vegetation types

relatively well (Gajewski et al. 2002; Watrin et al. 2007; Lebamba et al. 2009). Consequently, reconstructing vegetation, with a view to informing interpretations of the fossil record and predictions of the effects of future climate change is a possibility (Hély et al. 2006; Blois et al. 2013). Neotropical pollen studies also show that there are clear changes in pollen assemblages along savannah to forest transitions, with shifts from herbaceous and grass dominated assemblages to arboreal dominated forest assemblages (Gosling et al. 2009; Alejandra et al. 2013).

The sediments in Lake Bosumtwi, a meteor-impact crater lake in Ghana, provide a 540,000 year pollen record tracking vegetation and climate change in tropical West Africa (Miller & Gosling 2014; Miller et al. 2016). It is therefore important to characterise modern pollen-vegetation relationships of the modern day ecosystems around Lake Bosumtwi, in order to better understand the fossil pollen record. In Ghana and its neighbouring tropical West African countries, there have been few recent studies published on modern pollen-vegetation relationships (Ybert 1975; Lézine & Hooghiemstra 1990; Lézine & Edorh 1991; Lézine et al. 2009). Furthermore, tropical West African modern pollen studies have used different sampling media (predominantly soil samples), meaning that comparing across studies and ecosystems is difficult. Soil type has, for instance, been shown to have an effect on the pollen assemblages recovered, with water logged and well-drained soils producing different pollen assemblages (see Chapter 4 for more detail on bias in modern pollen sampling) (Elenga et al. 2000).

Here, artificial pollen traps are used to recover modern pollen assemblages from plots within a forest-savannah mosaic, moist deciduous forest and wet evergreen rainforest in Ghana (See Chapters 2 and 4 for detailed interpretations of pollen spectra from these vegetation types). The modern pollen rain data are then compared to establish if it is possible to differentiate between these ecosystems based on their pollen assemblages. Modern pollen data are then used to re-examine interpretations of the fossil record of tropical West Africa, particularly that of Lake Bosumtwi.

Pollen assemblages are presented as sums across traps and years, as well as, in the pollen diagram, on a per-trap basis, providing a bulk signal for the ecosystems over the three years, without considering issues of phenology or small-scale variation. This has been done with the intention of allowing the data to be more applicable to the fossil record. In previous chapters, pollen data were averaged across traps (Chapter 2), or treated individually (Chapter 4).

5.3   Site Descriptions

Study sites are located in Ghana, tropical West Africa, across an ecological gradient from Poaceae-dominated savannah in the north to wet evergreen rainforest in the south (Figure 1.4). 46 traps from five vegetation plots were counted (Table 5.1): three plots were in the Kogyae Strict Nature Reserve, here referred to as 'Kogyae Savannah' (7°18'04"N, 1°09'53"W), 'Kogyae Transition' (7°18'07"N, 1°10'50"W) and 'Kogyae Forest' (7°15'41"N, 1°09'00"W), one in the Bobiri Forest Reserve, which is referred to as 'Bobiri' (6°42'15 "N 1°19'06 "W) and one in the Ankasa Conservation Area, 'Ankasa' (5°16'06"N 2°41'38"W). When classifying the vegetation types, vegetation descriptions by White (1983), Hall & Swaine (1981) and Bongers et al. (2004) are used as appropriate (see Section 1.4.4). Although the plots cannot represent their 'biomes' perfectly, they do not contain large numbers of species that would be considered abnormal for their latitude or soil type, and have been protected from extreme modern anthropogenic influence by dint of being located in protected areas. It is, however, important to note that it is unlikely for any tropical African forest to be truly free of human interference (Malhi et al. 2013).

In Ghana, there is a rainfall gradient of decreasing precipitation from south to north, which is a primary driver of vegetation cover (Maharjan et al. 2011). The plot-specific rainfall data reflect the large scale precipitation gradient, the average yearly rainfall over the years of this study are: Kogyae: 1000 mm, Bobiri: 1219 mm, and Ankasa: 1926 mm (see Section 1.4.2 for further information on climate of Ghana).

Vegetation surveys were conducted at the sites by researchers from the University of Oxford and FORIG (see Section 1.4.4). Trees > 10 cm Diameter at Breast Height (DBH) were measured and identified to species level; vegetation data is available at www.forestplots.net (Lopez-Gonzalez et al. 2011). The descriptions below are summaries of the main features of the vegetation at the plots; more detailed descriptions can be found in Sections 2.3.3.1-3 for Kogyae, Section 4.3.1 for Ankasa and 4.3.2 for Bobiri.

Table 5.1: Details of sites and samples taken from each site

Site	Plot	Latitude and Longitude	Vegetation type following White (1983) and Bongers et al. (2004)	Number of samples counted	Years Deployed
Kogyae	Kogyae Savannah (KOG05)	7°18'04"N, 1°09'53"W	Sudanian woodland/Guineo-Congolian secondary grassland and wooded grassland	5	2013-2014
Kogyae	Kogyae Transition (KOG04)	7°18'07"N, 1°10'50"W	Guineo-Congolian Transition Woodland	5	2013-2014
Kogyae	Kogyae Forest (KOG02)	7°15'41"N, 1°09'00"W,	Drier peripheral semi-evergreen rainforest/Guineo-Congolian transition woodland	5	2013-2014
Bobiri	Bobiri (BOB01)	6°42'15 "N 1°19'06 "W	Moist semi-deciduous forest/Drier peripheral semi-evergreen Guineo-Congolian rainforest	15	2011-2014 (yearly collections)
Ankasa	Ankasa (ANK02)	5°16'06"N 2°41'38"W	Wet evergreen rainforest/Hygrophilous coastal evergreen Guineo-Congolian rainforest	16	2011-2014 (yearly collections)

5.3.1   Kogyae

The Kogyae Strict Nature Reserve is located in the forest-savannah mosaic transitional region of Ghana, which lies between the Guineo-Congolian region to the south and the Sudanian region to the north. Although the flora of the three plots comprises a mixture of taxa from these regions (See Figure 1.4), in terms of openness and understorey structure, there is a gradient from the Savannah plot, which has a grass-dominated understorey and is relatively open, to the Forest plot, which contains more woody biomass (basal area covered by trees > 10 cm DBH is 300 m^2, against 270 m^2 in the Transition plot and 230 m^2 in the Savannah plot) and has an understorey dominated by non-Poaceae monocotyledonous taxa. The term 'Savannah' is used to describe a range of ecosystems that are grass-dominated with sparser tree cover than 'Forests' (Ratnam et al. 2011), the Savannah plot in this study does still contain many trees (see Figure 2.2), which themselves are primarily 'wooded grassland' taxa (Section 5.3.1.1), thus making it open woodland with a grass-dominated understorey, as opposed to grass-dominated with isolated trees.

5.3.1.1   Kogyae Savannah (KOG05)

The three most abundant taxa of the Kogyae Savannah plot, by percentage of stems, are *Bridelia ferruginea* Benth (27.7%), *Pterocarpus erinaceus* Poir (10.7%) and *Uapaca togoensis* Pax (8.1%) (Table 5.2). The vegetation of this plot is most closely aligned with Sudanian Woodland, or Guineo-Congolian secondary grassland and wooded grassland (White et al. 1983). The understorey is dominated by tall Poaceae, with some Asteraceae. The diversity (Shannon index) of the Kogyae Savannah plot is 2.56.

Table 5.2: Kogyae Savannah most abundant vegetation taxa

Family	Species	Stems (%)	Basal area (%)	Flower structure	Pollination syndrome
Phyllanthaceae	*Bridelia ferruginea* Benth.	27.7	16.8	Monoecious	Entomophilous
Fabaceae	*Pterocarpus erinaceus* Poir.	10.7	16.7	Hermaphrodite	Entomophilous
Phyllanthaceae	*Uapaca togoensis* Pax	8.1	5.4	Dioecious	Unknown
Combretaceae	*Terminalia glaucescens* Planch. ex Benth.	6.4	11.1	Hermaphrodite	Entomophilous
Combretaceae	*Anogeissus leiocarpa* (DC.) Guill. & Perr.	6.0	10.3	Hermaphrodite	Unknown
Meliaceae	*Trichilia emetica* Vahl	6.0	3.1	Hermaphrodite	Entomophilous
Sapotaceae	*Vitellaria paradoxa* C.F.Gaertn.	5.4	7.9	Hermaphrodite	Entomophilous
Anacardiaceae	*Lannea velutina* A. Rich.	3.3	3.8	Monoecious	Entomophilous

5.3.1.2   Kogyae Transition (KOG04)

The three most abundant taxa in the Kogyae Transition plot, by percentage of stems, are *Sterculia tragacantha* Lindl. (26.9%), *Pterocarpus erinaceus* (10.7%) and *Maranthes polyandra* (Benth.) Prance (6.8%) (Table 5.3). There are 34 species recorded at >10 cm DBH in this plot. The vegetation type of this plot most broadly correlates with Guineo-Congolian Transition woodland (White et al. 1983). The average tree height is 12.1m. The diversity (Shannon's index) of the Kogyae Transition plot is 2.82.

Table 5.3: Kogyae Transition most abundant vegetation taxa

Family	Species	Stems (%)	Basal area (%)	Flower structure	Pollination syndrome
Malvaceae	*Sterculia tragacantha* Lindl.	26.9	18.6	Hermaphrodite	Entomophilous
Fabaceae	*Pterocarpus erinaceus* Poir.	10.7	12.8	Hermaphrodite	Entomophilous
Chrysobalanaceae	*Maranthes polyandra* (Benth.) Prance	6.8	5.9	Hermaphrodite	Chiropterophilous
Combretaceae	*Terminalia glaucescens* Planch. ex Benth.	6.4	6.0	Hermaphrodite	Entomophilous
Sapotaceae	*Manilkara obovata* (Sabine & G.Don) J.H.Hemsl.	6.0	7.8	Hermaphrodite	Entomophilous
Phyllanthaceae	*Bridelia ferruginea* Benth.	3.8	2.3	Monoecious	Entomophilous
Fabaceae	*Pericopsis laxiflora* (Baker) Meeuwen	3.8	2.9	Hermaphrodite	Unknown
Combretaceae	*Terminalia avicennioides* Guill. & Perr.	3.8	3.7	Hermaphrodite	Entomophilous
Anacardiaceae	*Lannea velutina* A.Rich.	3.4	5.4	Monoecious	Entomophilous

5.3.1.3   Kogyae Forest (KOG02)

The three most abundant taxa in the Kogyae Forest plot, by percentage of stems, are *Cola gigantea* A.Chev (12.3%), *Sterculia tragacantha* (10.7%) and *Dacryodes klaineana* (Pierre) H.J.Lam (9.6%). (Table 5.4). Understorey, herbaceous taxa include members of the Zingiberaceae, Costaceae and Marantaceae. This plot falls between 'Drier peripheral semi-evergreen rainforest and 'Guineo-Congolian transition woodland', in terms of its taxonomic composition (White et al. 1983). The diversity (Shannon index) of the Kogyae Forest plot is 3.10.

Table 5.4: Kogyae Forest most abundant vegetation taxa

Family	Species	Stems (%)	Basal area (%)	Flower structure	Pollination syndrome
Malvaceae	*Cola gigantea* A. Chev	12.3	13.3	Monoecious	Entomophilous
Malvaceae	*Sterculia tragacantha* Lindl.	10.7	5.7	Hermaphrodite	Entomophilous
Burseraceae	*Dacryodes klaineana* (Pierre) H.J.Lam	9.6	13.7	Monoecious	Entomophilous
Sapotaceae	*Pouteria alnifolia* (Baker) Roberty	8.6	5.4	Hermaphrodite	Entomophilous
Bignoniaceae	*Spathodea campanulata* P.Beauv.	8.0	4.9	Hermaphrodite	Zoophilous
Malvaceae	*Ceiba pentandra* (L.) Gaertn.	4.8	5.4	Hermaphrodite	Zoophilous
Fabaceae	*Erythrophleum suaveolens* (Guill. & Perr.) Brenan	4.8	4.4	Hermaphrodite	Entomophilous
Fabaceae	*Afzelia africana* Pers.	3.7	5.8	Hermaphrodite	Entomophilous
Arecaceae	*Elaeis guineensis* Jacq.	3.2	3.0	Monoecious	Entomophilous

5.3.2   Bobiri (BOB01)

The vegetation type of the Bobiri plot is moist semi-deciduous rainforest (Hall & Swaine 1981), and fits into the category of 'Drier peripheral semi-evergreen Guineo-Congolian rain forest' by White (1983). The three most abundant taxa in this plot, by percentage of stems, are *Celtis mildbraedii* Engl. (14.7%), *Funtumia elastica* (Preuss) Stapf (5.0%) and *Nesogordonia papaverifera* (A. Chev.) Capuron ex N. Hallé (4.8%) (Table 5.5). Herbaceous taxa include Marantaceae and Verbenaceae (White et al. 1983). The diversity of Bobiri (Shannon index) is 3.8.

Table 5.5: Bobiri main vegetation taxa

Family	Species	Stems (%)	Basal area (%)	Flower structure	Pollination syndrome
Cannabaceae	*Celtis mildbraedii* Engl.	14.7	17.3	Monoecious	Anemophilous
Apocynaceae	*Funtumia elastica* (Preuss) Stapf	5.0	3.4	Hermaphrodite	Entomophilous (p)
Malvaceae	*Nesogordonia papaverifera* (A. Chev.) Capuron ex N. Hallé	4.8	6.5	Hermaphrodite	Entomophilous (p)
Malvaceae	*Cola caricifolia* (G.Don) K.Schum.	4.1	3.6	Monoecious	Entomophilous (p)
Fabaceae	*Hymenostegia afzelii* (Oliv.) Harms	4.1	3.6	Hermaphrodite	Zoophilous
Simaroubaceae	*Hannoa klaineana* Pierre & Engl.	3.9	5.2	Hermaphrodite	Entomophilous (p)
Fabaceae	*Albizia zygia* (DC.) J.F.Macbr.	3.1	2.5	Hermaphrodite	Entomophilous
Fabaceae	*Baphia nitida* Lodd.	2.7	1.6	Hermaphrodite	Entomophilous
Malvaceae	*Cola gigantea* A.Chev.	2.7	3.1	Hermaphrodite	Entomophilous
Olacaceae	*Strombosia glaucescens* Engl.	2.7	1.9	Hermaphrodite	Entomophilous (p)

5.3.3   Ankasa (ANK02)

The vegetation of the Ankasa plot is wet evergreen rainforest (Bongers et al. 2004), and conforms to White's 'Hygrophilous coastal evergreen Guineo-Congolian rainforest' (White et al. 1983). The three most abundant taxa in this plot, by percentage of stems, are *Drypetes aylmeri* Hutch. & Dalziel (8.9%), *Cynometra ananta* Hutch. & Dalziel (7.1%) and *Strephonema pseudocola* A.Chev. (5.6%) (Table 5.6). Herbaceous plants likely include taxa such as *Psychotria*, members of the Zingiberaceae, Orchidaceae and Commelinaceae, and a variety of fern species (Hawthorne et al. 1998). The vegetation diversity of the Ankasa plot is 4.0 (Shannon Index).

Table 5.6: Ankasa most abundant vegetation taxa

Family	Species	Stems (%)	Basal area (%)	Flower structure	Pollination syndrome
Putranjivaceae	*Drypetes aylmeri* Hutch. & Dalziel	8.9	9.2	Dioecious	Entomophilous
Fabaceae	*Cynometra ananta* Hutch. & Dalziel	7.1	8.1	Hermaphrodite	Chiropterophilous
Combretaceae	*Strephonema pseudocola* A.Chev.	5.6	5.8	Hermaphrodite	Zoophilous
Olacaceae	*Strombosia pustulata* Oliv.	4.9	4.5	Hermaphrodite	Entomophilous (p)
Melastomataceae	*Memecylon lateriflorum* (G. Don) Bremek.	3.3	3	Hermaphrodite	Entomphilous
Rhizophoraceae	*Cassipourea hiotou* Aubrév. & Pellegr.	3.1	3.8	Hermaphrodite	Entomophilous
Ebenaceae	*Diospyros sanza-minika* A. Chev.	2.9	2.7	Monoecious	Entomophilous
Clusiaceae	*Pentadesma butyracea* Sabine	2.9	2.3	Hermaphrodite	Zoophilous
Malvaceae	*Heritiera utilis* (Sprague) Sprague	2.7	3.1	Monoecious	Entomophilous (p)
Dichapetalaceae	*Tapura ivorensis* Breteler	2.2	2.5	Hermaphrodite	Entomophilous

5.4   Methods

The methods for the chapter are the same as those outlined in Sections 2.4 and 4.4 and explained in the Introduction (Section 1.5).

5.4.1   Field Methods

Pollen traps were deployed in the field for a duration of one year at a time. Traps at Ankasa and Bobiri were deployed between 2011 and 2014, while traps from Kogyae were placed in the field for one year, from 2013-2014 (see Chapters 2 and 4 for further details of sampling).

Artificial pollen traps were designed in accordance with Gosling (2003) and comprised a plastic funnel with filter paper at the bottom fixed with bathroom sealant. Filter funnels were filled with cotton wool and then topped with plastic mesh (for video outlining trap construction see Jardine (2014)). Traps were placed at approximately 50 cm aboveground level to avoid inundation and were replaced each year in October.

5.4.2   Laboratory Methods

Traps were processed following the method in Gosling et al. (2003), with acetolysis being used to destroy cotton wool and HF to remove filter paper. *Lycopodium* tablets were added (University of Lund, batch number 124961, containing 12542 +/- 931 spores per tablet) to enable influx calculations (Stockmarr 1971). Samples were sieved at 180 μm, washed with potassium hydroxide and sodium pyrophosphate and mounted in glycerol. Model 1 of Keen et al. (Keen et al. 2014) was used to calculate count sizes. Samples were counted at x 400 magnification using a Nikon 50i microscope. Pollen was identified by reference to literature on tropical West Africa (van Campo 1974; Ybert 1979; Riollet & Bonnefille 1980; Gosling et al. 2013), an offline version of the African Pollen Database (Vincens et al. 2007) and the reference collections at The Open University and the University of Amsterdam.

5.4.3   Statistical Methods

Pollen data were visualised in C2 (Juggins 2007) and the R package rioja (Juggins 2015). Other statistical analyses and plots were undertaken in R statistical software, using R studio (RStudio 2012). The package vegan (Oksanen et al. 2015) was used to generate Non-Metric Multidimensional Scaling (NMDS) ordinations. Wisconsin Double Standardisation, which standardises both column and row totals, hence down-weighting hyper-abundant taxa, was used, and singletons were removed (taxa that only occur in one sample and which therefore do not provide any useful classification information). R-rel$_{(sum)}$ values were calculated by dividing the percentage of a pollen taxon by the percentage basal area of its corresponding vegetation taxon. The Shannon index was used to calculate diversity metrics (Shannon 1948).

5.5   Results

The pollen assemblages recovered in this study varied dependent on their parent vegetation type, see Sections 2.5 and 4.5 for detailed information. Melastomataceae/Combretaceae and Poaceae dominated the most northern, savannah-mosaic plots (Kogyae), *Celtis* was the most abundant pollen at the moist semi-deciduous plot (Bobiri), and the wet evergreen rainforest samples showed a variable signal, but with high abundances of *Cynometra* and *Drypetes* pollen. Here, the most abundant taxa from each plot are presented, with values summed within plots and between years (e.g. the percentage abundance of *Celtis* in Bobiri is the percentage of this taxon from samples from all traps and all three years, as a percentage of the total pollen counted for Bobiri). In total, 46 pollen traps were counted from the five different plots; five each from the three Kogyae plots, 15 from Bobiri, and 16 from Ankasa. These are the same counts as in Chapters 2 and 4, but re-interpreted in a manner that facilitates comparisons to the fossil record
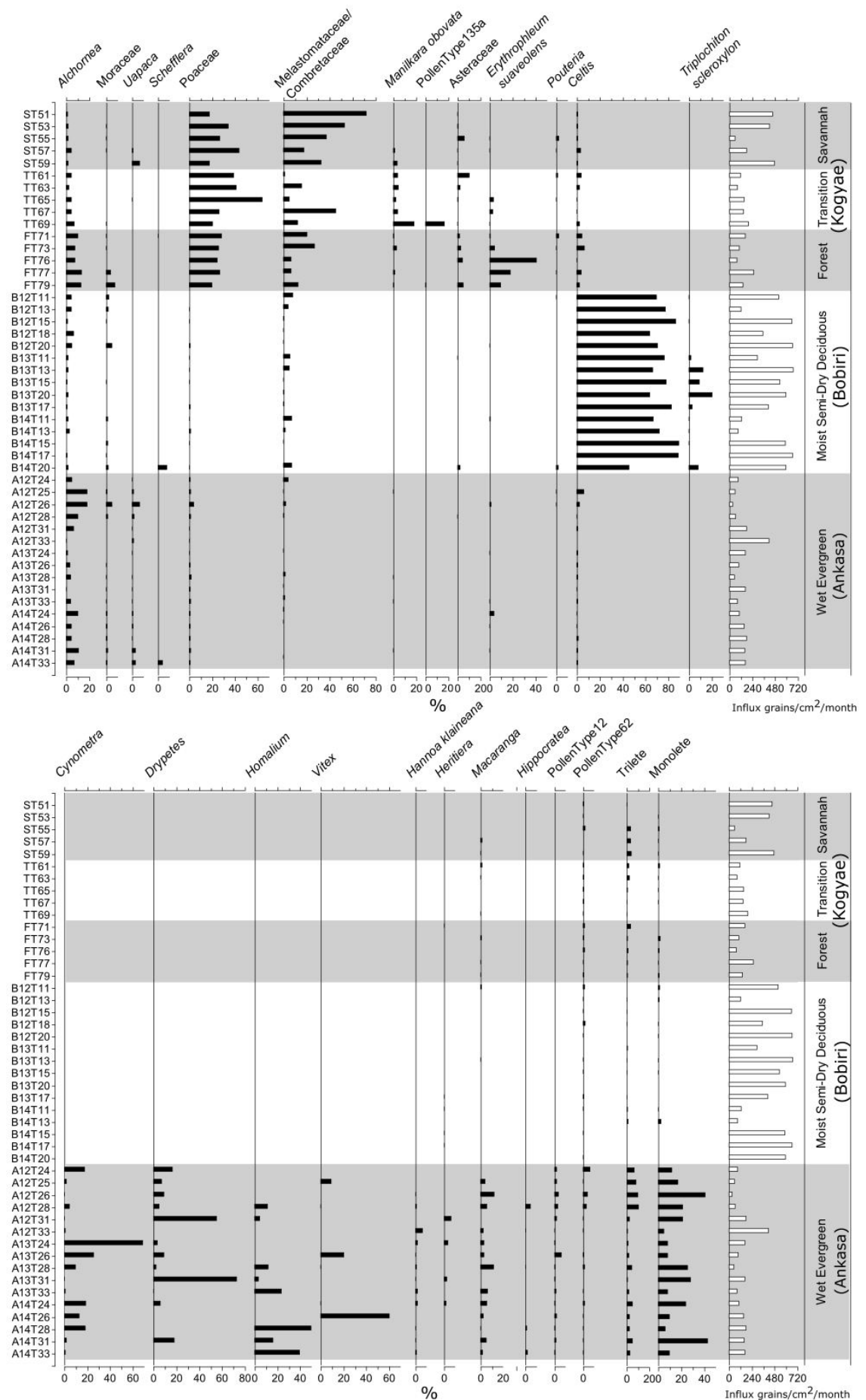
Figure 5.1: Pollen diagram showing most abundant taxa from each site, in percentage. Zones are used to denote sites. Y axis shows individual traps, grouped by year i.e.'A14T33'= Ankasa 2014, Trap 33.

5.5.1   Kogyae

The five most abundant pollen taxa for all three plots at Kogyae, together, were Poaceae (30.9%), Melastomataceae/Combretaceae (21.0%), *Terminalia* (7.2%), *Alchornea* (5.6%), and *Erythrophleum suaveolens* (5.5%). The most abundant taxa by influx were Poaceae (884 grains/cm^2/month), Melastomataceae/Combretaceae (760 grains/cm^2/month), *Terminalia* (281 grains/cm^2/month), *Alchornea* (165 grains/cm^2/month), and *Erythrophleum suaveolens* (118 grains/cm^2/month). The overall diversity was 2.6. There were 121 pollen taxa counted from the sites at Kogyae, of which 37 were assigned botanical affinities, and of those 37, 11 were also found in vegetation surveys.

5.5.1.1   *Kogyae Savannah*

The five most abundant pollen taxa, by overall percentage, from the Savannah plot were Melastomataceae/Combretaceae (35.3%), Poaceae (26.6%), *Terminalia* (13.8%), PollenType134 (3.9%) and *Alchornea* (2.3%).

Table 5.7: Kogyae Savannah pollen-vegetation relationships

Taxon	Pollen abundance (%)	Basal area (% of plot area)	R-rel$_{(sum)}$	Flower structure	Pollination Syndrome
Melastomataceae/Combretaceae	49.5	25.9	3.53	Hermaphrodite	Entomophilous
Moraceae	0.2	0.2	1.00	Monoecious/ Dioecious	Anemophilous/ Entomophilous
Pterocarpus	3.9	16.9	0.23	Hermaphrodite	Entomophilous
Uapaca	1.6	5.6	0.28	Dioecious	Entomophilous

The most abundant taxa by influx were Melastomataceae/Combretaceae (574 grains/cm^2/month), Poaceae (415 grains/cm^2/month), *Terminalia* (226 grains/cm^2/month), Pollen Type 134 (84 grains/cm^2/month), and Pollen Type 181 (38 grains/cm^2/month). The diversity of the Kogyae Savannah plot pollen was 2.1. Four taxa were present in both pollen and vegetation (>10 cm DBH, grass and herbaceous taxa not included) (Table 5.7). Of these, one was over-represented (Melastomataceae/Combretaceae), two were under-represented (*Uapaca* and *Pterocarpus*), and one had a R-rel$_{(sum)}$ value of 1 (Moraceae).

5.5.1.2   Kogyae Transition

The five most abundant pollen taxa, by overall percentage, from the Transition plot, were

Poaceae (40.2%), Melastomataceae/Combretaceae (12.5%), *Manilkara obovata* (7.4%),

Alchornea (5.2%), and Pollen Type 135a (4.3%). The most abundant taxa by influx were

Poaceae (271 grains/cm^2/month), Melastomataceae/Combretaceae (98 grains/cm^2/month),

Manilkara obovata (58 grains/cm^2/month), *Alchornea* (39 grains/cm^2/month) and Pollen Type

135a (35 grains/cm^2/month). The diversity of the Kogyae Transition plot pollen was 2.5.

Table 5.8: Kogyae Transition pollen-vegetation relationships

Taxon	Pollen abundance (%)	Basal area (% of plot area)	R-rel$_{(sum)}$	Flower structure	Pollination Syndrome
Afzelia africana	0.2	3.4	0.06	Hermaphrodite	Entomophilous
Ceiba	0.2	2.4	0.07	Hermaphrodite	Zoophilous
Erythrophleum suavolens	1.7	4.1	0.42	Hermaphrodite	Entomophilous
Lannea	0.3	5.8	0.05	Monoecious	Entomophilous
Manilkara obovata	7.4	7.9	0.94	Hermaphrodite	Entomophilous
Melastomataceae /Combretaceae	16.0	12.0	1.33	Hermaphrodite	Entomophilous
Moraceae	0.1	0.5	0.16	Monoecious/ Dioecious	Anemophilous/ Entomophilous

Seven taxa were present in both pollen and vegetation of the Kogyae Transition plot. Of these,

one was over-represented (Melastomataceae/Combretaceae), and six were under-

represented (Table 5.8 and Section 2.8).

5.5.1.3   Kogyae Forest

The five most abundant pollen taxa, by overall percentage, from the Forest plot, were Poaceae (25.5%), *Erythrophleum suaveolens* (17.5%), Melastomataceae/Combretaceae (11.6%), *Alchornea* (10.6%), and *Celtis* (3.5%). The most abundant taxa by influx were Poaceae (199 grains/cm^2/month), *Erythrophleum suaveolens* (105 grains/cm^2/month), *Alchornea* (88 grains/cm^2/month), Melastomataceae/Combretaceae (87 grains/cm^2/month) and *Celtis* (31 grains/cm^2/month). The diversity of the Kogyae Forest plot pollen assemblage was 2.7.

Eight taxa were present in both pollen and vegetation of the Kogyae Forest plot (Table 5.9 and Section 2.9). Of these, three were over-represented, and five were under-represented.

Table 5.9: Kogyae Forest pollen-vegetation relationships table

Taxon	Pollen abundance (%)	Basal area (% of plot area)	R-rel$_{(sum)}$	Flower structure	Pollination Syndrome
Afzelia africana	0.1	5.8	0.02	Hermaphrodite	Entomophilous
Bombax	0.1	2.6	0.02	Hermaphrodite	Zoophilous
Celtis	3.5	1.6	2.21	Monoecious	Anemophilous
Erythrophleum suaveolens	17.5	4.4	3.96	Hermaphrodite	Entomophilous
Lannea	0.1	2.2	0.05	Monoecious	Entomophilous
Manilkara obovata	1.3	2.0	0.61	Hermaphrodite	Entomophilous
Melastomataceae /Combretaceae	14.5	1.7	8.71	Hermaphrodite	Entomophilous
Moraceae	2.5	3.7	0.74	Monoecious/ Dioecious	Anemophilous/ Entomophilous

5.5.2 Bobiri

The five most abundant pollen taxa at Bobiri, by overall percentage, were *Celtis* (73.5%), *Triplochiton scleroxylon* (3.5%), Pollen Type 46 (2.8%), *Alchornea* (2.7%), and Melastomataceae/Combretaceae (3.8%). The most abundant taxa by influx were *Celtis* (5120 grains/cm^2/month), *Triplochiton scleroxylon* (339 grains/cm^2/month), Pollen Type 46 (213 grains/cm^2/month), *Alchornea* (168 grains/cm^2/month) and Melastomataceae/Combretaceae (157 grains/cm^2/month). The diversity of the Bobiri pollen assemblage was 1.5.

Taxon	Pollen abundance (%)	Basal area (% of plot area)	R-rel$_{(sum)}$	Flower structure	Pollination Syndrome
Bombax	0.2	0.8	0.27	Hermaphrodite	Zoophilous
Ceiba	0.4	2.3	0.15	Hermaphrodite	Zoophilous
Celtis	73.5	20.3	3.60	Monoecious	Anemophilous
Heritiera	0.2	0.6	0.26	Monoecious	Entomophilous
Irvingia	0.1	0.1	0.96	Hermaphrodite	Entomophilous
Lannea	0.1	0.8	0.12	Monoecious	Entomophilous
Melastomataceae /Combretaceae	3.8	0.9	4.27	Hermaphrodite	Entomophilous
Moraceae	1.2	1.6	0.70	Monoecious/ Dioecious	Anemophilous/ Entomophilous
Musanga	0.1	1.0	0.06	Dioecious	Anemophilous
Nesogordonia papaverifera	0.3	6.5	0.04	Hermaphrodite	Entomophilous (p)
Petersianthus	0.02	3.7	0.01	Hermaphrodite	Entomophilous
Pycnanthus	0.2	1.7	0.14	Monoecious/ Dioecious	Entomophilous
Triplochiton scleroxylon	3.5	3.4	1.04	Hermaphrodite	Entomophilous

Table 5.10: Bobiri pollen-vegetation relationships

Of the 104 pollen taxa recorded at Bobiri, 43 were assigned botanical affinities. Thirteen taxa were identified in both pollen and vegetation from Bobiri (Table 5.10 and Chapter 4). Of these, three were over-represented and ten were under-represented.

5.5.3   Ankasa

The five most abundant pollen taxa at Ankasa, by overall percentage, were *Cynometra* (11.5%), *Drypetes* (11.5%), *Homalium* (10.4%), Pollen Type 89, and *Vitex* (9.6%). There was also high abundances of monolete spores at Ankasa, with an overall abundance of 18.3% (not included in pollen sum). The most abundant taxa by influx were Pollen Type 89 (404 grains/cm^2/month), *Drypetes* (316 grains/cm^2/month), *Cynometra* (260 grains/cm^2/month), *Homalium* (239 grains/cm^2/month) and *Vitex* (125 grains/cm^2/month). The diversity of the Ankasa pollen assemblage was 3.3.

Table 5.11: Ankasa pollen-vegetation relationships

Taxon	Pollen abundance (%)	Basal area (% of plot area)	R-rel$_{(sum)}$	Flower structure	Pollination Syndrome
Cola	0.5	0.8	0.60	Monoecious	Entomophilous
Cynometra	11.5	8.1	1.42	Hermaphrodite	Chiropterophilous
Drypetes	11.5	9.6	1.19	Dioecious	Entomophilous
Heritiera	0.9	3.1	0.28	Monoecious	Entomophilous
Homalium	10.4	0.4	26.7	Hermaphrodite	Entomophilous
Manilkara	0.1	2.3	0.05	Hermaphrodite	Entomophilous
Melastomataceae /Combretaceae	2.0	13.2	0.15	Hermaphrodite	Entomophilous
Moraceae	0.93	0.12	7.74	Monoecious/ Dioecious	Anemophilous/ Entomophilous
Parkia	0.2	0.6	0.32	Hermaphrodite	Zoophilous
Pycnanthus	0.04	0.3	0.14	Monoecious/ Dioecious	Entomophilous
Scytopetalum	0.1	1.4	0.06	Hermaphrodite	Entomophilous
Tabernaemontana	0.1	0.6	0.09	Hermaphrodite	Entomophilous
Uapaca	1.4	1.0	1.39	Dioecious	Entomophilous
Vitex	9.6	1.2	7.7	Monoecious	Entomophilous

In total, 49 of the 144 pollen taxa recorded at Ankasa were assigned botanical affinities.

Fourteen taxa were identified in both pollen and vegetation (Table 5.11 and Chapter 4). Of these, five were over-represented and nine were under-represented.

5.5.4   Ordination
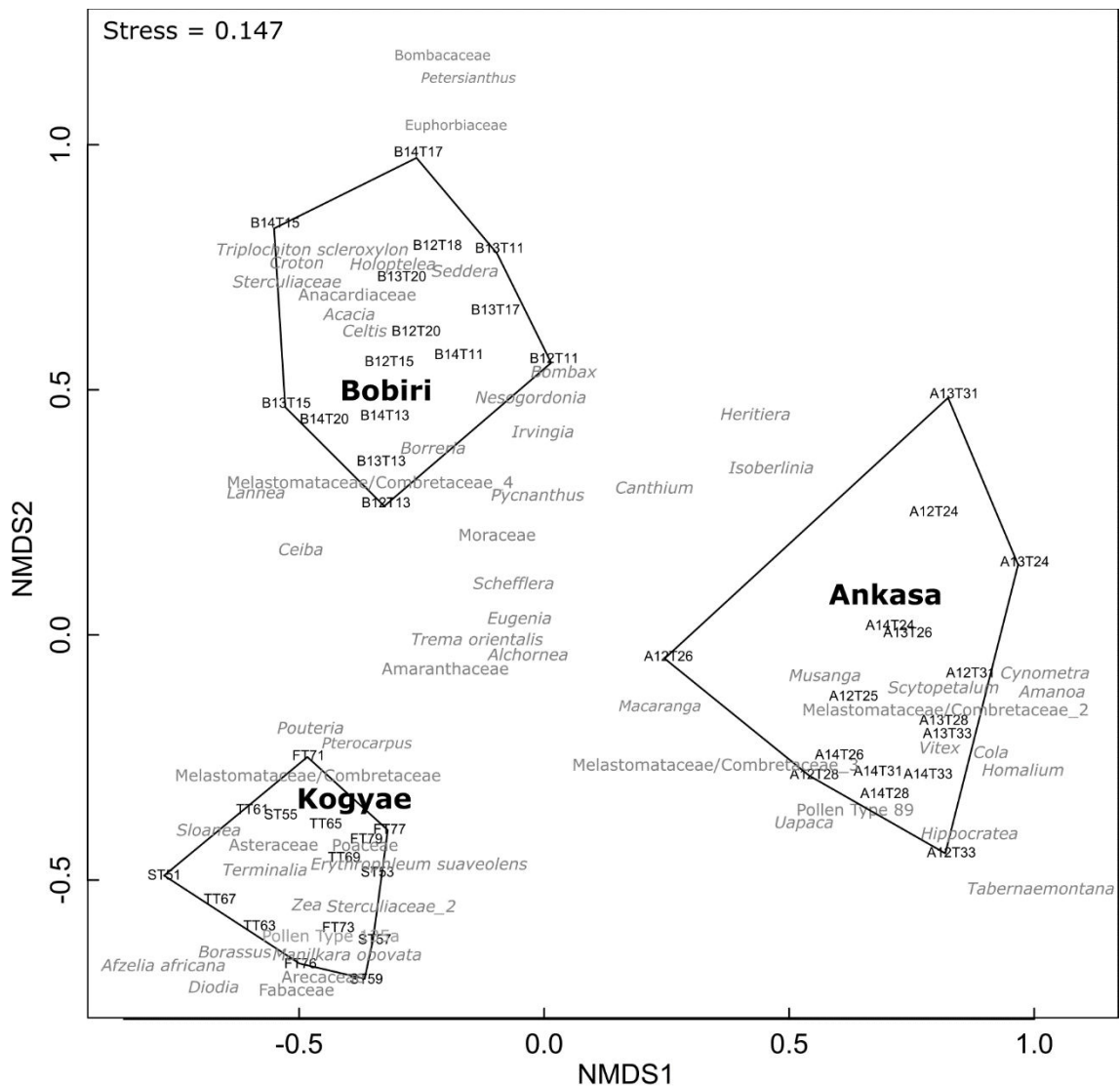
Figure 5.2: NMDS plot showing named taxa and plots. Kogyae= Kogyae Savannah, Transition and Forest, represents a mosaic landscape, Bobiri= a moist semi-deciduous forest, Ankasa = wet evergreen rainforest. Labels in grey are taxa, labels in black are individual samples (e.g. B14T15 indicates Trap 15 from Bobiri, collected in 2014).

The samples separated according to their locations (Kogyae, Bobiri and Ankasa), and there was no overlap in ordination space between sites (Figure 5.2). The most abundant taxa from each plot (Sections 5.1 – 5.3) were associated with their parent plots (e.g. *Celtis* was located in the centre of the cluster of Bobiri samples, whereas Poaceae was clustered within the Kogyae samples). The samples from Kogyae were the most tightly clustered in ordination space, with samples dispersed over less than 0.5 on both axes, meaning they displayed the least variable pollen assemblages of all three sites. The Bobiri samples exhibited more spread in ordination space, with samples spread over around 0.6 on NMDS1 and 0.7 on NMDS2. Samples from Ankasa were most dispersed, showing a spread of around 0.7 on NMDS1 and 0.9 on NMDS2. There are six taxa with botanical affinities that do not cluster with samples of one plot, but

appear within 0.25 of the centre the ordination on both axes; *Alchornea,* Amaranthaceae, *Eugenia*, Moraceae, *Schefflera* and *Trema*.

5.6   Discussion

This study demonstrates that it is possible to distinguish between wet evergreen rainforest, moist semi-deciduous forest and forest-savannah mosaic environments based upon their pollen rain (Figure 5.1). The pollen assemblages recovered from these plots vary in their most abundant taxa, their diversity and their influx rates. Here, key taxa from each ecosystem, pollen-vegetation relationships in the plots, non-informative taxa and diversity are discussed. It is then considered how these findings may influence interpretations of the fossil pollen record of Lake Bosumtwi.

5.6.1   Informative taxa

Here, taxa are considered that distinguish the sites from one another in their modern pollen rain and may therefore be considered informative. These taxa may differ from the most abundant taxa for each plot as presented in the results (Section 6.5), if the most abundant taxa are present across many different plots.

The three Kogyae plots, which together represent a forest-savannah mosaic and whose vegetation comprises Guineo-Congolian and Sudanian taxa, are characterised by high proportions of Poaceae and Melastomataceae/Combretaceae. Within this mosaic, *Manilkara obovata* distinguishes the pollen of the Transition plot (7.4% of the pollen sum), being also one of the most abundant taxa in the vegetation of that plot (7.8% of the basal area of the plot). *Erythrophleum suaveolens* is characteristic of the Forest plot pollen assemblages (17.5% of the pollen), and accounts for 4.4% of the basal area of the vegetation plot.

Bobiri, the moist semi-deciduous forest plot, is characterised by very high abundances of *Celtis* pollen (> 70%), an anemophilous taxon that is highly productive (Miller & Gosling 2014). *Celtis* is also the most abundant taxon in the vegetation of Bobiri (20.3% of basal area). *Triplochiton scleroxylon* is also a major component of the pollen assemblage of Bobiri, although this signal varies between years due to the phenology of *Triplochiton* (Jones 1974), and *Triplochiton* accounts for 3.4% of the vegetation of the plot.

Pollen assemblages from the wet, evergreen rainforest plot, Ankasa, are not dominated by any one taxon, instead showing relatively similar percentages (around 10%) of *Cynometra*, *Drypetes*, *Homalium* and *Vitex*. *Drypetes* and *Cynometra* are the two most abundant vegetation taxa in the Ankasa plot, together accounting for 17.3% of the basal area of the plot, whereas *Homalium* and *Vitex* are less abundant components of the vegetation, account for 0.4% and 1.2% of the basal area of the plot respectively.

5.6.2   Pollen-vegetation relationships

The majority of taxa in all of the vegetation plots are zoophilous or entomophilous and either palynologically silent or under-represented in the pollen assemblages (Tables 6-11). Widespread under-representation of the majority of taxa is a well-known phenomenon in the tropics, where most taxa are not anemophilous and are therefore under-represented in fossil records (Bush & Rivera 2001; Behling & Negrelle 2006). Widespread zoophily also makes tropical taxa difficult to incorporate into models of pollen distribution (Duffin & Bunting 2008). In this study, the anemophilous taxa (*Alchornea*, *Celtis*, Melastomataceae/Combretaceae and Moraceae) were either ubiquitous (that is, present in all traps), over-represented or both.

Celtis, Melastomataceae/Combretaceae and Moraceae vary in their representation between plots. *Celtis* is over-represented in Bobiri and Kogyae Forest, and is present in pollen assemblages but not vegetation at Ankasa, and Kogyae Savannah and Transition plots. Melastomataceae/Combretaceae pollen is over-represented relative to its abundance in the vegetation in all plots except Ankasa, in which it is under-represented. Moraceae, a taxon found to over-produce in the Neotropics (Weng et al. 2004; Gosling et al. 2009), is over-represented in Ankasa, but under-represented in the other plots. The observed variation in the representation of these taxa is likely due to pollen landing in traps from outside of the plot (an occurrence made more likely by the anemophilous nature of the taxa) and therefore not representing the vegetation composition of the plots.

The high variation in pollen signal between traps in Ankasa (Figure 5.1, and Section 4.5.1) suggests that the pollen produced within this vegetation type does not travel far. This finding is consistent with other modern pollen-vegetation relationship studies of tropical lowland ecosystems, and is likely a product of the closed canopy and zoophilous nature of the taxa (Elenga et al. 2000; Haselhorst et al. 2013).

The pathways that pollen takes to be deposited in a lake, such as Lake Bosumtwi, and a pollen trap are very different. The pollen deposited in a large Lake, such as Bosumtwi is likely to comprise largely of a regional, anemophilous dominated signal (Prentice, 1985). This is reflected in the record observed in Bosumtwi, with high percentages of grass, even in 'forested' zones. The way pollen moves in tropical ecosystems is has not been extensively investigated, although it has been assumed that trunk-space dispersal is minimal. This is due to the high density of vegetation and low wind-speeds of the understorey in tropical rainforests, which may mean that large amounts of pollen becomes trapped on leaves and twigs (Tauber 1967).

There are several pathways by which pollen is dispersed in tropical ecosystems:

- Gravity

- Convection currents

- Rain (either washing pollen out of the air or off vegetation)

- Wind (both under the canopy in the trunk space and above the canopy)

Gravity is likely to be important beneath the canopy, along with convection currents and rainfall (Lowman & Rinker 2004). These are the factors that will result in the majority of the pollen deposition into pollen traps placed beneath the canopy. Due to the low wind-speeds observed in tropical forests, it is unlikely that wind within the trunk-space will contribute to much deposition into pollen traps (illustrated by the high levels of spatial heterogeneity in this study). The proportion of horizontal (wind transferred) pollen deposited into traps on the forest floor could be tested as in Tauber (1967) with traps including a small roof. Above-canopy dispersal is likely to constitute the main mechanism of transport that results in pollen being dispersed over long distances and deposited into lakes like Bosumtwi. The proportion of the total pollen produced that is dispersed in this way, however, is not yet known, but could be tested using above-canopy traps and comparing these to below-canopy traps.

These considerations are important, as they affect the use of pollen traps to interpret pollen assemblages from the sedimentary record; until experiments such as those outlined above are conducted in the tropics, it will not be possible to calibrate models of pollen-vegetation relationships and pollen dispersal in the tropics.

5.6.3   Diversity

The highest pollen diversities are recovered from Ankasa, and the lowest from the Kogyae Savannah plot, with a broad gradient between (Figure 5.3), although Bobiri has relatively lower pollen diversity compared to the other plots, which is likely due to its high abundance of *Celtis* pollen. The vegetation diversities exhibit the same trend (decreasing from Ankasa through to Kogyae Savannah) although without the lower diversity at Bobiri. There is not a clear correlation between pollen and vegetation diversity (Figure 5.3). In temperate regions, the relationship between pollen diversity and vegetation has been relatively well quantified, with the number of pollen taxa in a sample of 10 grains and the Shannon index of diversity showing positive correlation (Matthias et al. 2015b). The relationship between tropical vegetation and pollen diversity is, however, more complex due to the high levels of diversity in tropical systems, and depends on a variety of factors including vegetation structure, phenology, and sampling medium (Jantz et al. 2013, Gosling et al., in press). It is likely that, in this case, both vegetation and pollen diversity values are under-estimations of the complete diversity of both systems. In the vegetation this under-estimation is due to only individuals of > 10 cm DBH

being recorded in the vegetation surveys, and in the pollen, due to palynologically silent taxa, which may be entomophilous or may have not flowered at all during the time over which pollen was collected for this study.
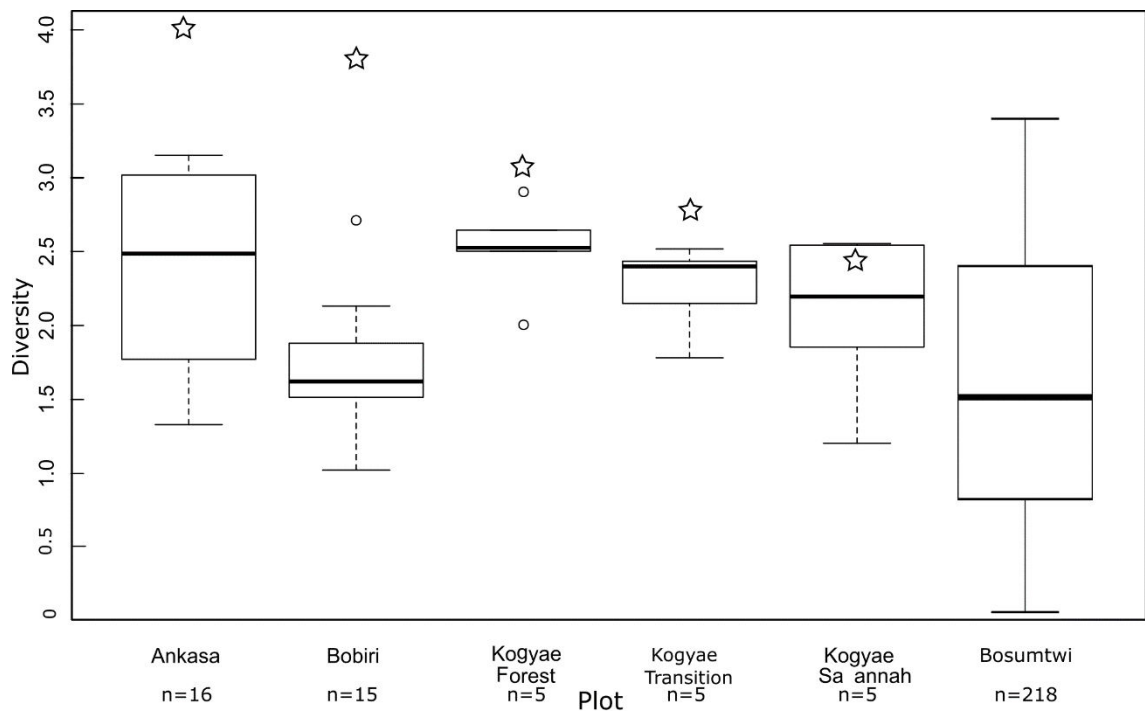
Figure 5.3: Diversity (Shannon Index) of the pollen assemblages (box and whisker plots) and the vegetation diversity (> 10 cm DBH) of the plots (star symbols). The last column is of the pollen assemblages of Lake Bosumtwi. Boxes show 25th and 75th percentiles of data, bars near the middle of the boxes show the median value, and ends of whiskers show the extremes of the data, as long as those are not more than 1.5 inter-quartile ranges from the 25th and 75th percentiles. For 'Kogyae Forest' the diversity data are not very variable, leading to its whiskers being short, and its outlying values not being joined by a whisker.

5.6.4   Non-informative taxa
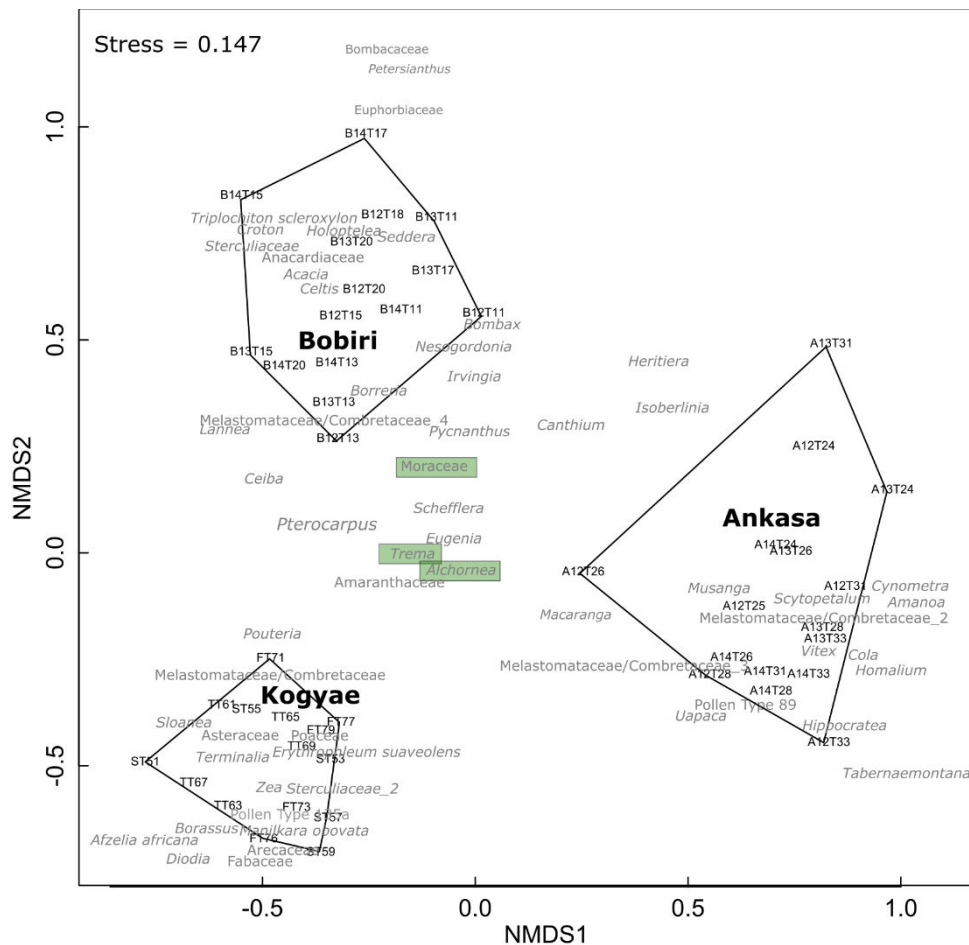
Figure 5.4: NMDS plot showing named taxa and plots. Kogyae= Kogyae Savannah, Transition and Forest, represents a mosaic landscape, Bobiri= a moist semi-deciduous forest, Ankasa = wet evergreen rainforest. Labels in grey are taxa, labels in black are individual samples (e.g. B14T15 indicates Trap 15 from Bobiri, collected in 2014). Taxa highlighted in green indicate those taxa used in the Bosumtwi record to indicate moist forest but which, here, are found at all sites.

Alchornea, Amaranthaceae, *Eugenia*, Moraceae, *Schefflera* and *Trema* do not contribute to the differentiation of the three sites, being present at all plots (albeit with differing abundance and R-rel$_{(sum)}$ values), and not clustering with samples of one site in Figure 5.4. Amaranthaceae, *Eugenia*, *Schefflera* and *Trema* abundances were generally low or inconsistent across the plots, meaning that although these taxa are not indicative of one vegetation type here, this could be an issue of sampling, as NMDS analysis down-weights hyper-abundant and up-weights very rare taxa. It is important to note, however, that these taxa do occur across the wide ecological gradient investigated here, and so care should be taken in using these taxa as indicative of a certain ecosystem, especially at low the abundances often observed in tropical pollen work (Lézine & Edorh 1991)

The presence of *Alchornea* pollen in all plots, but not in the vegetation of any, is probably due to individuals being under 10 cm DBH (Chapter 4), a phenomenon also observed in the Neotropics (Gosling et al. 2009). *Alchornea* is, however, widely distributed across tropical

Africa (GBIF Secretariat 2016), and its pollen has been shown to be spread outside of the climatic range of its parent plant (Watrin et al. 2007). Therefore, although *Alchornea* has a distinct pollen morphotype which is easily identifiable to genus, it should not be used as an indicator taxon of any one ecosystem.

Moraceae is recognised at a widespread taxon, whose pollen is difficult to identify to below family level (although this is possible (Burn & Mayle 2008)). That Moraceae is most abundant palynologically in the Kogyae Forest plot, where it is under-represented (R-rel$_{(sum)}$ = 0.74), but is most over-represented at Ankasa, where although it accounts for less of the pollen sum, its R-rel$_{(sum)}$ value is 7.74 indicates that, when treated at family level this taxon is not likely to provide reliable ecological differentiation between plots.

5.6.5   Implications for the fossil record

In the fossil record of Lake Bosumtwi, changes in the fossil pollen assemblages over time have been interpreted to reflect vegetation change in response to climate, including glacial-interglacial transitions, and orbital forcing on shorter timescales (Miller et al. 2016). The fossil pollen record from Lake Bosumtwi provides a unique insight into the terrestrial vegetation response to climate change in tropical West Africa. The Lake was initially cored in 1976, returning a 27,500 year record (Talbot et al. 1984), but a longer core was recovered spanning the past 1 ma years in 2004 (Shanahan et al. 2006). Bosumtwi Forest Zones were defined for the past 540,000 years by Miller and Gosling (2014), and these are interpreted to be equivalent to warm interglacial Marine Isotope Stages. Here, modern pollen assemblages presented are compared to those from the Bosumtwi Forest Zones.

5.6.5.1   *Non-informative taxa in the fossil record*

Miller and Gosling (2014) use taxa such as *Alchornea*, *Celtis*, *Macaranga*, Moraceae, *Uapaca*, *Trema* and, coupled with low (< 40%) abundances of Poaceae, to define a wet, lowland forest vegetation, such as 'Western Guinean Lowland Forest' and 'Nigerian Lowland Forest'. *Alchornea*, Moraceae and *Trema* occur in most samples of this study, from an open, Poaceae-dominated savannah (Kogyae Savannah) through to the wet evergreen rainforest of Ankasa, and it is therefore unlikely that these taxa are diagnostic of one particular ecosystem.

Macaranga is most abundant in this study in Ankasa, but is also present in the pollen assemblages of all three sites, in low abundances. This taxon has, also, however, when present alongside *Celtis*, been used to indicate a dry, semi-deciduous forest (Elenga et al. 1994). *Uapaca*, which groups with Ankasa in the NMDS, is present in the Kogyae Forest plot, suggesting that this taxon should not be taken as a reliable indicator of a moist, broadleaf

forest. *Macaranga* and *Celtis* are present throughout the Bosumtwi record, apart from in the period between BF3 and BF2, during which they are present in only very low abundances.

The abundance of Poaceae in the Lake Bosumtwi record is very high (percentages reach 90% and above) for much of the record. Due to its size, up to 90% of the pollen signal of Lake Bosumtwi is likely to be regional (from more than 100s m away from the lake), potentially accounting for the high influx of Poaceae into the lake, an estimate arrived at by consulting Jacobson and Bradshaw (1981). However, even within the wettest forest zones of the Bosumtwi record (BF1), Poaceae abundance still reaches 20% of the pollen sum, which is in line with percentages of Poaceae recovered in this study from the Kogyae plots, and could therefore indicate a forest-savannah mosaic landscape. In some sections of the Bosumtwi record, Poaceae is present at abundances of >90%, and the counts of other taxa are very low (less than 50 grains in many cases). Poaceae may, therefore, be masking more subtle signals from other taxa that may be indicative of the vegetation around the lake (Bush 2002). Future studies, therefore, may benefit from removing Poaceae from the pollen sums. The identification of Poaceae grains in the record may also help to determine the sort of environment around the lake (Chapter 3 and Julier et al. (2016)).

5.6.5.2   Re-interpretation of forest zone vegetation

In Chapter 2, it was concluded that the threshold of Poaceae abundance in the Lake Bosumtwi record (55%) may have been too high, and that a more realistic value may be 40% (Figure 5.5). This threshold, when applied to the record, does not alter timing or length of the Forest Zones in a significant way, except potentially in the case of Bosumtwi Forest Zone 3, which is equivalent to Marine Isotope Stage 7 (MIS7), which would no longer meet the criterion of possessing three consecutive samples in which the Poaceae percentage was lower than the threshold. This stage is an unusual interglacial, in that it may not have experienced full de-glaciation and its magnitude and characteristics are debated (Levis et al. 1999; Past Interglacials Working Group of PAGES 2016; Tzedakis et al. 2017). It is therefore possible that this has been captured in Bosumtwi Forest Zone 3 as a more savannah-like pollen signal, with higher Poaceae abundances, than in other interglacials.
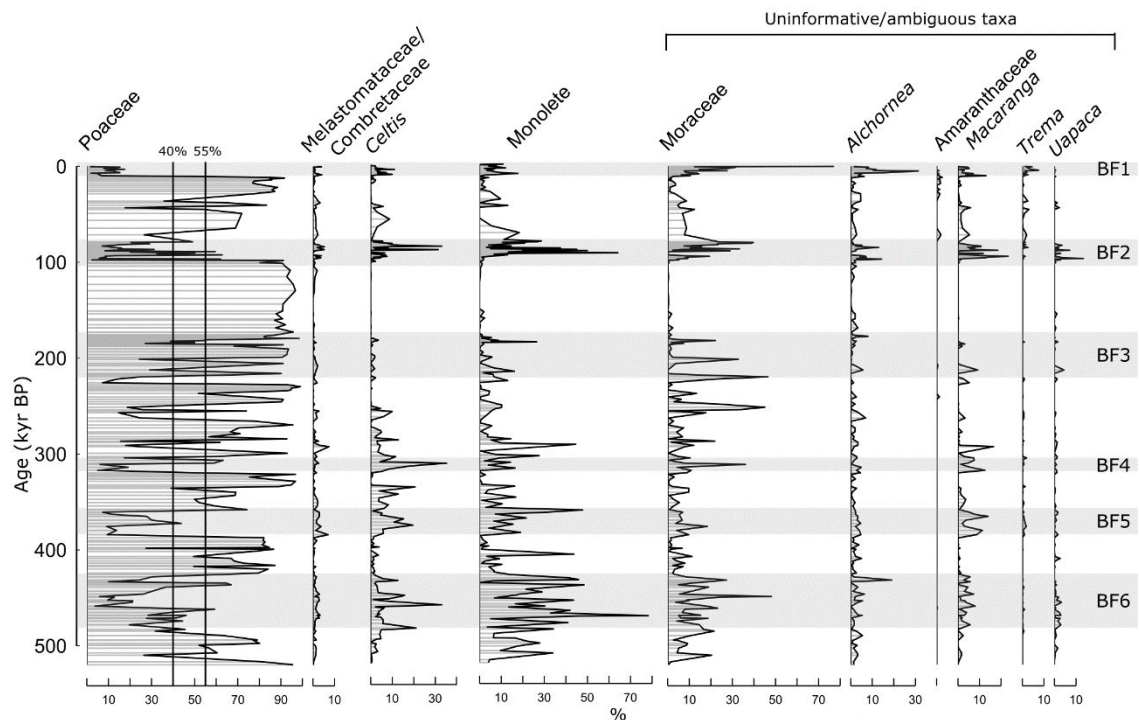
Figure 5.5: Figure showing pollen record from Lake Bosumtwi (Miller and Gosling, 2014) re-plotted to highlight new putative Poaceae percentage above which a 'savannah' landscape shoul be inferred (40%, as opposed to the 55% value previously used), and with uninformative taxa as identified in this thesis highlighted. BF= 'Bosumtwi Forest Zone' as designated in Miller and Gosling (2014).

Melastomataceae/Combretaceae pollen is not a dominant taxon in any section of the Lake Bosumtwi record, although it is present at abundances from 0 - 7.5% throughout the whole core. Melastomataceae/Combretaceae pollen has been used as an indicator of Sudanian ecosystems in this tropical West Africa in the past (Lézine & Hooghiemstra 1990). This interpretation is supported by the data presented here by Melastomtaceae/Combretaceae pollen's highest occurrence in the Kogyae plots, although morphotypes of Melastomataceae/Combretaceae are present in all vegetation types of this study, but at much lower percentages. Therefore, the presence of Melastomataceae/Combretaceae pollen in the forest zones of Bosumtwi may also indicate that the vegetation around the lake during forested intervals was relatively more open.

None of the most abundant pollen taxa identified from the wet evergreen rainforest site of this study (Ankasa) were identified in the fossil record of Lake Bosumtwi. Monolete spores were, however, found in abundance in the Bosumtwi record, at some points reaching 70% of the pollen sum. The monolete spore spikes sometimes coincide with spikes of other taxa identified at Ankasa, such as *Macaranga* and *Uapaca*, but also co-occur with *Celtis* and relatively high levels of Poaceae, meaning that the signal is not clear-cut. The lack of a clear signal from a wet evergreen rainforest in the Bosumtwi record may be due to the local deposition of pollen from this ecosystem. It may also indicate that the wet evergreen

rainforest biome is a new in Ghana since the onset of the Holocene, which would support the finding that the Holocene has brought the wettest conditions of the past 540,000 years to tropical West Africa (Miller & Gosling 2014).

Pollen concentrations in the Neotropics have been shown to be higher in closed canopy ecosystems (Gosling et al. 2009), and high pollen concentrations are also used to indicate a wet forest environment in Bosumtwi forest zones. Our data suggest that high pollen concentrations may be indicative of moist semi-deciduous forest, but that wet, evergreen rainforest such as that found at Ankasa exhibit low pollen concentrations. There is potential bias in our results due to the closed-canopy nature of the forest and the placement of the traps near ground level; further studies should explore the production of pollen from higher up in the canopy in African systems, in addition to ground level traps, as was undertaken by Haselhorst et al. (2013) on Barro Colarado island, Panama.

The diversity over time in the Bosumtwi core fluctuated, but did not always show a noticeable increase within Forest Zones. Our data show that the diversity of the pollen assemblages from Bobiri is lower than those from the Kogyae plots, although the ranges of these values overlap (Figure 5.3). Similar diversities across forest-grassland transitions in the Bosumtwi record may therefore indicate a relatively drier forest vegetation type.

5.6.5.3   Comparison to other fossil pollen records in tropical West Africa
When compared to other pollen records from the region, the Lake Bosumtwi core is consistently the most northern example of 'Tropical and sub-tropical moist broadleaf forest' during interglacials (Olson et al. 2001; Miller & Gosling 2014). Marine records from further north, off the western coast of West Africa show a signal consistent with 'Tropical and Subtropical Grasslands, Savannahs and Shrublands' in odd Marine Isotope Stages 13 through 5e (Dupont et al. 1989; Lézine 1991; Dupont & Agwu 1992). One terrestrial record to the north-east of Lake Bosumtwi, from Lake Tilla, Nigeria, exhibits a 'Tropical and Subtropical Grasslands, Savannahs and Shrublands' signal for the Holocene interglacial (Salzmann et al. 2002).

Marine records to the south of Lake Bosumtwi, in the Gulf of Guinea, exhibit pollen assemblages characteristic of 'Tropical and Subtropical Moist Broadleaf Forests' or Mangrove swamps during interglacials (Sowunmi 1981; Lézine & Vergnaud-Grazzini 1993; Frédoux 1994; Dupont & Weinelt 1996; Jahns et al. 1998). Lake Marombi-Mbo, to the south of Lake Bosumtwi exhibits a 'Tropical and Subtropical Moist Broadleaf Forest' signal in the Holocene interglacial (Maley & Brenac 1998). It therefore appears that in interglacial periods, Lake Bosumtwi likely lay at the northern extent of the forested region of tropical West Africa. The high percentages

of Poaceae pollen are probably testament to its position within what was probably the forest-savannah transitional mosaic. It is likely that the present day extent of moist semi-deciduous forest in Ghana is the most northern that this vegetation type has extended within the past 540,000 years.

5.6.5.4 CO_2 and no-analogue assemblages

Low CO_2 levels during glacial maxima (as low as 180-190 ppm) may contribute to decreasing tropical forest cover, which has knock-on effects on albedo, transpiration and therefore precipitation (Levis et al. 1999; Harrison & Prentice 2003). In interglacial periods CO_2 levels were around 280 ppm, well below today's levels (400 ppm), which may support the idea that a vegetation assemblage more strongly resembling the forest-savannah mosaic than wet evergreen rainforest would be more likely in tropical West Africa. CO_2 has also been shown to have an effect on pollen production and length of pollen season (Ziska & Caulfield 2000; García-Mozo et al. 2010), potentially increasing pollen production under elevated CO_2 conditions. Conversely, certain taxa may produce less pollen under low CO_2 conditions. This could mean that attempting to use modern-day pollen assemblages as a proxy for past assemblages could be inherently flawed, considering the currently high levels of CO_2 present in the atmosphere, and that the physiological reaction to elevated CO_2 is likely to vary between plant taxa and on an ecosystem scale.

Lower CO_2 levels combined with different weather patterns, precipitation and temperatures may also have resulted, in previous interglacials, in no-analogue assemblages. No analogue assemblages are assemblages of taxa that are observed in the fossil record, but not in modern ecosystems (Jackson & Overpeck 2000; Williams & Jackson 2007; Correa-Metrio et al. 2012). That pollen assemblages similar to those from Ankasa have not been recovered from the Lake Bosumtwi core is not conclusive evidence that wet evergreen rainforest did not exist around the lake at any point in the past. It is possible that it is, instead, a product of the bias inherent in pollen trapping, in that the small surface area of the trap will not capture the same regional signal as the large surface area of Lake Bosumtwi.

 The plots sampled here represent snapshots of five different vegetation types, and cannot be considered representative of the hugely diverse and gradual gradient from rainforest to savannah that exists in Ghana (Section 1.4.2). Nor do the traps themselves necessarily capture the full diversity of the vegetation, or even an equivalent assemblage to that observed in a lake sediment (Jackson 2012). Rather, the key points are that there are taxa which had previously been used to indicate wet environments in the Lake Bosumtwi core which here are identified

across the range of ecosystems sampled, and whose use as indicators should, therefore, be reconsidered.

5.7   Conclusions

- The results presented here show that pollen assemblages from across a wide environmental gradient in Ghana are clearly distinguishable from one another and are able to differentiate forest-savannah mosaic, moist semi-deciduous forest and wet evergreen rainforest.

- The forest-savannah mosaic assemblages were characterised by high levels of Poaceae and Melastomataceae/Combretaceae, the most abundant pollen taxa at the moist semi-deciduous plot were *Celtis* and *Triplochiton*, and the wet evergreen rainforest plot was typified by *Cynometra* and *Drypetes* pollen.

- There were several taxa whose presence across the whole gradient indicates that they are not likely to be useful indicator taxa of any one vegetation type, these include *Alchornea* and Moraceae, two taxa commonly identified in fossil pollen records.

- These findings indicate that interpretations of the interglacial vegetation around Lake Bosumtwi from the past 540,000 years may have been more similar to the forest-savannah mosaic vegetation or moist semi-deciduous forest than to the wet evergreen rainforest.

- Future work should integrate fossil and modern data sets to test similarities between the fossil record of Lake Bosumtwi and modern assemblages from Ghana. This would further improve interpretations of this important record of climate change in tropical West Africa.

6   Chapter 6: Conclusions

This thesis has dealt with modern pollen-vegetation relationships across a wide ecological gradient, from wet evergreen rainforest to savannah in Ghana, and has explored a new method for the identification of Poaceae pollen grains. This work has implications for the interpretation of fossil pollen records, in terms of assemblage composition and taxonomic resolution of pollen identification.

In this chapter, the thesis aims (Chapter 1, Section 1.2) are addressed, with reference to their relevant chapters. Limitations, and potential future directions of this work are then considered.

6.1   Aims

6.1.1   To determine how tropical West African ecosystems, including a forest-savannah mosaic, moist semi-deciduous forest and wet evergreen rainforest are represented by their modern pollen rain (Chapter 5)

The three main vegetation types explored in this thesis have been shown, in Chapters 2, 4 and 5 to produce unique pollen assemblages that allow them to be differentiated based upon the pollen taxa and the proportions of those taxa present in their assemblages. The forest-savannah mosaic as a whole (three plots) is distinguished by the dominance of Poaceae and Melastomataceae/Combretaceae pollen, with the former accounting for up to 60% and the latter up to 68% of the pollen signal from these plots. The moist semi-deciduous forest is characterised by very high abundances of *Celtis* pollen, which accounts for up to 89% of the pollen signal. The pollen signal of the wet evergreen rainforest site was not dominated by any one pollen taxon, but showed high heterogeneity between traps, with taxa such as *Cynometra*, *Drypetes*, *Homalium*, and *Vitex* being dominant in individual traps. It has also, however, been shown that there are several taxa, such as *Alchornea* and Moraceae, whose presence is not useful in distinguishing between the different vegetation types.

The most abundant taxa identified in the vegetation are also found in the pollen, but the majority of tree taxa are either palynologically silent, or produced pollen that was unidentified and present at low abundances (<3%). The ecosystems are, in general, not well represented by their pollen rain in terms of the number of taxa that are shared between pollen and vegetation. Generally, pollen assemblages did not provide a taxonomically fidelitous or complete reflection of their parent vegetation types.

The discrepancy between pollen and vegetation assemblages is hypothesised to be due to pollination syndrome and vegetation structure. Pollination syndrome is an important factor determining which taxa are dominant in pollen assemblages due to the majority of tropical plant taxa being entomophilous, and only a few exhibiting anemophily (notably Poaceae, *Celtis* and *Alchornea*). Vegetation structure is considered to be important, particularly in the closed canopy of the wet evergreen site, where a high level of variability is observed in pollen assemblages between traps, likely due to the lack of wind currents in the understorey dispersing pollen far from its parent tree.

6.1.2   Whether ecosystems within a forest-savannah mosaic landscape can be distinguished from one another using their modern pollen spectra (Chapter 2)

In Chapter 2, the three plots from the forest-savannah mosaic (Kogyae Forest, Kogyae Transition and Kogyae Savannah) are shown to produce distinct pollen assemblages. The assemblages are distinguishable based upon their absolute values of Poaceae pollen (which decrease from Savannah to Transition to Forest) and their relative proportions of Melastomataceae/Combretaceae. The percentage values of Poaceae do not reflect the gradient of forest to savannah, being highest in the Transition plot, due to very high influx of Melastomatacee/Combretaceae in the Savannah plot. In addition to Poaceae and Melastomataceae/Combretacee, various taxa are also characteristic of the different plots; the Forest plot was characterised by *Erythrophleum suaveolens*, the Transition plot by *Manilkara obovata*, and the Savannah by *Terminalia*.

The results of this chapter suggest that the percentage of Poaceae pollen that is taken as indicative of a savannah-type vegetation in the Lake Bosumtwi record (55%) may be too high, as the majority of traps in these plots exhibit Poaceae abundances below that, and would therefore be categorised as forest by the metric used by Miller and Gosling (2014) and Miller (2016). It is suggest that this threshold might be better placed at 40%.

6.1.3   How modern pollen assemblages represent high-resolution vegetation patterns within closed-canopy moist semi-deciduous forest and wet evergreen rainforest (Chapter 4)

The data presented in Chapter 4 demonstrate that the two forest plots considered in this thesis, moist semi-deciduous forest (Bobiri) and wet evergreen rainforest (Ankasa) give rise to pollen assemblages that differ considerably from one another, but that capture the most abundant taxa in the vegetation. In the moist semi-deciduous forest, *Celtis* is consistently over-

represented, whereas *Triplochiton scleroxylon* is over-represented in one year but under-represented in other years, due to weather conditions favouring flowering of the taxon in 2013. The most abundant pollen taxa from the wet evergreen site vary in their representation, sometimes very widely (e.g. the R-rel of *Homalium* varies between 9.20 and 127.92 in traps in which it is present, but is completely absent from 50% of traps).

The most striking conclusion from this chapter is the differences between R-rel values generated from pollen traps within the same plot, Ankasa, in the same year. This conclusion highlights the need for high spatial resolution in pollen trapping studies, especially in vegetation types that possess closed canopies and high vegetative diversities.

6.2 Whether FTIR spectroscopy can be used to improve the identification of Poaceae pollen taxa (Chapter 3)

The results of Chapter 3 show that, using FTIR spectroscopy on a selection of taxa from tropical West Africa, it is possible to distinguish between different pollen taxa within the Poaceae, with up to 80% successful identifications at sub-family level. Potential differences in the composition of sporopollenin between taxa that allow this differentiation include aliphatic chain length and saturation. The signal was considered to be taxonomic rather than environmental due to the increase in identification success observed between grains from one individual to grains of one species (Section 3.6).

As sporopollenin preserves well on geological timescales (Fraser et al. 2012), it is likely that a taxonomic signal could be recovered from the fossil pollen record, potentially improving interpretations of vegetation change, particularly of transitional or ambiguous sections rich in Poaceae grains, such as those from Marine Isotope Stage 5 in the Lake Bosumtwi record.

6.3 How an improved understanding of modern pollen-vegetation relationships contributes to the interpretation of the tropical West African fossil pollen record, particularly that of Lake Bosumtwi.

The pollen-vegetation relationship aspect of this thesis, as outlined in Chapters 2, 4 and 5 suggests that previous interpretations of conditions around Lake Bosumtwi may imply that the regional vegetation type at the lake during interglacials prior to the Holocene was likely more similar to a forest-savannah transitional mosaic than to moist semi deciduous or wet evergreen rainforest type. It has also been demonstrated (Chapter 5) that certain taxa previously used to indicate forested conditions, such as Moraceae and *Alchornea* may not be

of use in distinguishing between vegetation types, due to their ubiquitous occurrence in the modern pollen rain across the gradient of sites used in this study. The use of FTIR to investigate the taxonomic composition of previously cryptic taxa such as the Poaceae would also help to interpret past vegetation types by distinguishing wet adapted Poaceae taxa from those that grow in drier conditions. The results presented here have implications in the reconstruction of past ecosystems, both around Lake Bosumtwi and in tropical West Africa more widely, but also in predictions of how vegetation may change in the future.

6.4   Future work

6.4.1   Modern pollen monitoring

In order to develop a more complete picture of pollen-vegetation relationships in tropical West Africa, long term trapping experiments should be established in the region, with facilities and capacity at West African universities and government institutions developed to undertake palynological analyses. Preferably, seven years or more of pollen traps would be collected from plots, to produce reliable modern pollen assemblages (Haselhorst et al. 2013). Pollen production can vary widely on a yearly basis depending upon weather conditions or the phenology of the plants within the ecosystems, meaning that the more years are sampled, the better represented the vegetation is likely to be in cumulative pollen rain. Establishing modern pollen rain monitoring schemes in the region may also be of use to the medical and timber industries, in allergen monitoring and studies of phenology.

6.4.2   Improvements to trapping protocol

The higher than expected spatial heterogeneity observed in Chapter 4 raised the need for high spatial resolution pollen trapping studies in the tropics, with many pollen traps being deployed and counted within well-botanically understood vegetation plots. As pollen traps contained a large volume of cotton wool, which had to be processed in its entirety, in future studies it would be useful to compare traps set out in the field with different amounts of trapping medium (cotton wool) to determine if this volume could be reduced and therefore ease processing time burdens, allowing more samples to be examined. The possibilities of automated pollen identification opened up by Chapter 3 could also aid in reducing the amount of time required to generate pollen counts.

6.4.3   Vegetation data

The vegetation data for this thesis were collected by researchers from the University of Oxford and the Forestry Research Institute of Ghana, in order to monitor carbon storage and sequestration. Therefore, trees of < 10 cm DBH were not surveyed, and no surveys of herbaceous taxa or epiphytes were conducted as these did not fall under the scope of the carbon storage project. These issues mean that it is difficult to determine which pollen taxa were deposited in traps from outside of the plots, and which originated from within plots but from vegetation taxa that were not recorded.

In order to generate modern pollen assemblages that are comparable to others from the literature, and applicable to the fossil record, a standardised vegetation survey technique is necessary, such as that suggested by Bunting et al. (2013). Undertaking this type of vegetation survey in the tropics would require considerable time, resources and expertise, but should be attempted and/or adapted for tropical regions if models of pollen-vegetation relationships are to be developed that are applicable in the tropics. Comparing the 100m x 100m > 10 cm DBH vegetation surveys used in this study to the methods outlined in Bunting et al. (2013) would test the feasibility of modern pollen studies being 'attached' to standard vegetation plots of the type used in this thesis, and widely throughout the tropics (Lopez-Gonzalez et al. 2011).

6.4.4   Pollen taxonomy

An expansion of the number of taxa scanned from West Africa, and from other geographical regions would improve the taxonomic resolution of sampling and therefore enhance the applicability of FTIR as a means of pollen identification and allow it to be tested on a wider scale, with potential relevance to a wider range of fossil records. Another potential line of investigation would be to apply this technique to other typically cryptic pollen taxa, such as *Celtis*, the Moraceae, and the Melastomataceae/Combretaceae, to improve the taxonomic resolution of interpretations of fossil pollen assemblages.

6.4.5   The Lake Bosumtwi record

A next step for this work would be to integrate the pollen data from the Lake Bosumtwi record and the modern pollen samples analysed here to allow a quantitative comparison of the datasets. Integration of the modern and fossil datasets would, for instance, allow ordinations and diversity metrics to be generated which would enable comparisons to be drawn between the forest zones of the Bosumtwi record and modern ecosystems. This would of allow the

investigation of whether previous interglacial vegetation types were more similar to moist semi-deciduous or forest-savannah mosaics to be undertaken more quantitatively.

High resolution sampling of sections of the Lake Bosumtwi record would allow the extreme fluctuations in Poaceae abundances observed at some points in the record, particularly in MIS 5, to be resolved in greater detail both temporally (due to sampling resolution increase) and taxonomically, by using FTIR to investigate which grass taxa were present. This would enable higher resolution vegetation reconstructions to be developed, and the effects on vegetation of the switch from glacial to interglacials and vice versa to be understood in more detail.

6.5   Concluding comments

This thesis has demonstrated that it is possible to distinguish between several different vegetation types in Ghana, from savannah to rainforest, using their modern pollen assemblages as captured by artificial pollen traps (Chapters 2, 4, and 5). It is the first modern pollen rain study from Ghana, and therefore may inform future interpretations of fossil pollen records in the region. The feasibility of using FTIR to identify grass pollen grains to below family level has been demonstrated (Chapter 3), and represents a step forward in techniques to resolve taxonomic details of pollen assemblages with fast, cheap identification of cryptic pollen taxa to below family level. The results presented in this thesis should inform research on fossil pollen records from Africa and the tropics more widely, enabling researchers to form a clearer understanding of our planet's past, so that we might better prepare for its future.

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