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Sustaining biodiversity in forest and cocoa landscapes: Insights from pollinators, pesticides, and land use change in Ghana

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The thesis is submitted to University College Dublin in fulfilment of the requirement for the degree of Doctor of Philosophy in Agri-Environmental Sciences

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Abstract

Income from cocoa (*Theobroma cacao*) exports supports the economies of cocoa-producing countries, including many in West Africa. However, cocoa expansion has converted 10 million hectares of West African tropical rainforests to cocoa farmlands over the last 50 years with the most rapid loss occurring between the 1980s and the 2000s. Simultaneously, the extensive pesticide applications to safeguard yields may also pose risks to insect pollinators. This thesis therefore explored the effects of cocoa expansion on insect pollinators and pesticide residues in honeys. Three studies, each study addressing key components of the thesis, were conducted. In Chapter 2, I used a systematic literature approach to evaluate existing studies on the extent of honey contamination from plant protection products approved for cocoa cultivation. The findings showed that only 19% of existing studies were conducted in cocoa-producing countries. Organophosphates, organochlorines, and pyrethroids were the most detected pesticide classes in these countries, while globally, neonicotinoids were most prevalent. In Chapter 3, I analysed residues of key pesticides approved for cocoa cultivation in honey samples from cocoa and forest landscapes in Ghana. Honey samples from fullsun cocoa, agroforestry cocoa and natural forest contained pesticide residues, with imidacloprid being persistent in all honey samples, but concentrations did not exceed the specified EU MRLs. This highlights the pervasive presence of pesticides in the different landscapes. Chapter 4 assessed the influence of natural forest proportions on flower-visiting insects amidst cocoa expansion. Results indicated that proportions of natural forest influenced the abundance of Hymenoptera, Coleoptera, Hemiptera, and Diptera, altering bee community composition, though bee diversity metrics remained stable. To harmonise the economic benefits of cocoa cultivation with biodiversity conservation, I propose integrated pest management, preservation of natural forest patches, and the promotion of agroforestry systems. Such practices can mitigate pesticide impacts, support pollinator health, and ensure sustainable cocoa production.

Statement of Original Authorship

I hereby certify that the submitted work is my work, was completed while registered as a candidate for the degree stated on this title page and I have not obtained a degree elsewhere based on the research presented in this submitted work.

Collaborations

The studies included in this thesis are primary works of Richard Gyamfi Boakye (RGB) under the guidance and supervision of my primary supervisor, Dara A. Stanley (DAS) and co-supervisor, Blánaid White (BW) of Dublin City University.

- Chapter 2 This Chapter is entirely the work of RGB, with input from DAS and BW
- Chapter 3 This chapter is completely the work of RGB, with input from DAS and BW, except for the analysis of pesticide residues using the High-Performance Liquid Chromatography-Tandem Mass Spectrometry which was run by residues specialised expertise provided by MV due to technicalities involved, and also because it was specified by the School of Chemical Sciences of Dublin City University
- Chapter 4 This chapter is completely the work of RGB with input from DAS and BW except for collaboration with the University of Cape Coast for the use of the lab at the Department of Conservation Biology and Entomology for insect sorting and identification and the identification of bees to species level which was completed by RC. There was difficulty in transporting all specimens in alcohol from Ghana to UCD, Ireland for bee identification

RC= Rofela Combey, University of Cape Coast Ghana

MV= Mathavan Vickneswaran, Dublin City University

Publications from the thesis

1. One publication from Chapter Two [BOAKYE, R. G., STANLEY, D. A. & WHITE, B. 2023. Honey contamination from plant protection products approved for cocoa (*Theobroma cacao*) cultivation: A systematic review of existing research and methods. *PLoS One*, 18, e0280175].
2. A manuscript from Chapter Four titled “Relationships between flower-visiting insects and forest cover in cocoa-growing in landscapes in Ghana” has been submitted to the journal *Landscape Ecology* for consideration for publication.

Dedication

This thesis is dedicated to the loving memory of my mother, Margaret Amankwah for her encouragement when I started this academic journey. Although she passed on during my second year, her spirit and love continued to inspire me.

Also, to my wife, Pearl Gifty Ammah and our children Bernita, Bennett and Beryl for your constant encouragement and understanding which made this academic pursuit not only possible but immensely meaningful. Your patience and unwavering belief in me have been my pillars of strength. This achievement is as much yours as it is mine. Thank you for being my rock, my support system, and my source of joy throughout this academic venture.

With love and gratitude.

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My sincere appreciation goes to the University of Cape Coast for granting me access to the Museum of the Department of Conservation Biology and Entomology for sorting insect specimens and bee identification.

I look back and see that the collective support received from these diverse sources was the bedrock of my academic success, and for that, I am truly thankful.

Chapter 1 General Introduction

*“We glimpse the profound lessons of nature's ballet,
a symphony of life's beautiful diversity,
in the heart of Ghana's landscapes, where
pollinators hum, pesticides linger, and lands evolve.” RGB*

Chapter 1 General Introduction

1.1 Study context/overview

Within Ghana's landscape, a complex interaction between cocoa (*Theobroma cacao* L.) plantations and native forested lands takes place (Lewis, 2023). Cocoa serves as a cornerstone of Ghana's economy and culture and within cocoa landscapes, activities such as pesticide use, pollinator interactions, and land management interconnect (Hashmiu et al., 2022, Asare et al., 2014, Falcão et al., 2021). Amidst these interactions, the health of insect pollinators, responsible for pollinating 80-87.5 % of all pollinator-dependent plants and the general sanctity of ecosystems, becomes paramount (Ulyshen et al., 2023, Baxter-Gilbert et al., 2015, Ollerton et al., 2011, Klein et al., 2007). Cocoa cultivation is associated with intense pesticide application and deforestation (Okoffo et al., 2016, Somarriba and Lopez-Sampson, 2018). Despite their significance, pesticide exposure and habitat degradation from increased agricultural activity can pose a threat to these pollinators' health (Ali et al., 2021). There is a significant knowledge gap regarding how these factors affect pollinator populations in cocoa-growing regions, particularly in Ghana (Umeh et al., 2022). To retain ecological integrity and agricultural productivity, strategies for preserving land use changes on pollinator diversity, the dynamics of pesticide residues in honey, and the possibility for sustainable agriculture practices must all be understood. This provides a basis or need for a study which explores the relationship between pollinators, pesticides, and land use dynamics in Ghana forest and cocoa landscapes (Fig 1.1) in the context of bee pollinators. Such a study can help shed light on the difficulties and possibilities of preserving ecological balance in the face of changing land use, pest control, and the preservation of insect pollinators in the Ghanaian setting. The outcome of such a study portends the potential to contribute to the broader understanding of how these interconnections can inform sustainable agricultural and conservation strategies, considering the significant cultural and economic roles that cocoa plays in the region. To this end, this thesis aimed to explore these intricate interconnections.



Figure 1.1 Forest and cocoa landscapes in Bia West District of Ghana (BWD). A) Rising tree canopies of natural forest within Bia Conservation Area; B) Agroforestry cocoa where tree plantations are integrated into cocoa plantations and C) Understorey of mature fullsun cocoa monoculture at Adjoafua, a community within BWD.

1.2 The historical background of cocoa

The history of cocoa began in the fertile tropical environment of Mesoamerica, encompassing present-day regions such as Belize, Guatemala, Honduras, and Mexico (Rosenswig, 2015, Henderson et al., 2007). Historical documents and archaeological findings indicate that cocoa dates back more than 3,000 years to prehistoric cultures including the Olmecs, Mayans, and Aztecs (Fagan and Durrani, 2020, Evans and Webster, 2000, Weaver, 2019). Cocoa held a special place in these prehistoric societies, valued for its ceremonial and therapeutic uses. The Mayans, for example, consumed cocoa in a frothy, spiced beverage integral to their social interactions and ceremonies (Coe and Coe, 2013). In the Mayan culture, cocoa was frequently referred to as "kakaw," and it was essential to their social interactions and ceremonies (Dillinger et al., 2000, Vail, 2009). On the other side, the Aztecs drank a bitter, foamy beverage they named "xocoltl" that they thought had aphrodisiac properties (Presilla, 2009). Beyond ceremonial use, cocoa beans served as currency in Mesoamerican societies, underlining their economic significance (Norton, 2006, Richard and Giráldez, 2017). The beans were in high demand, and trade routes for cocoa connected many locations over a broad area (Presilla, 2009, Bergmann, 1969, Daymond and Bekele, 2022).

1.2.1 The spread of cocoa around the world

Until Christopher Columbus arrived in Mesoamerica in the late 15th century, cocoa was kept a well-guarded secret (History.com, 2023, Verna, 2013). Columbus's explorations of the New World brought cocoa to Europe, where it was initially a delicacy only available to the wealthy (Nunn and Qian, 2010). However, as its acclaim increased, so did its cultivation in tropical European colonies (Coe and Coe, 2013). The success of cocoa production in Sao Tomé and Príncipe in Central Africa showed that cocoa could be grown successfully outside of its natural home in Mesoamerica (Higgs, 2012, Heywood, 2017, Killingray, 2014). Plantations were established in the 19th century as cocoa planting spread from its original areas to tropical places all over the world (Ross, 2014, Cilas and Bastide, 2020). Through the ages, the cultivation of cocoa extended from Central and South America to other regions with favourable climates, such as West Africa, Southeast Asia, and even some Pacific islands (Leissle, 2018, Cilas and

Bastide, 2020) (Figure 1.2). Eventually, a cocoa powerhouse formed in West African countries including Ivory Coast, Ghana, Nigeria, and Cameroon, which has been instrumental in reshaping the sub-region's economy and landscapes (Miescher, 2022, Wiggins, 2018, Richards, 2023). Overall, cocoa is cultivated in 58 countries globally but West Africa alone accounts for 70% of the cocoa beans which are put on the world market (WPR, 2022, Asante et al., 2022) (Appendix A).

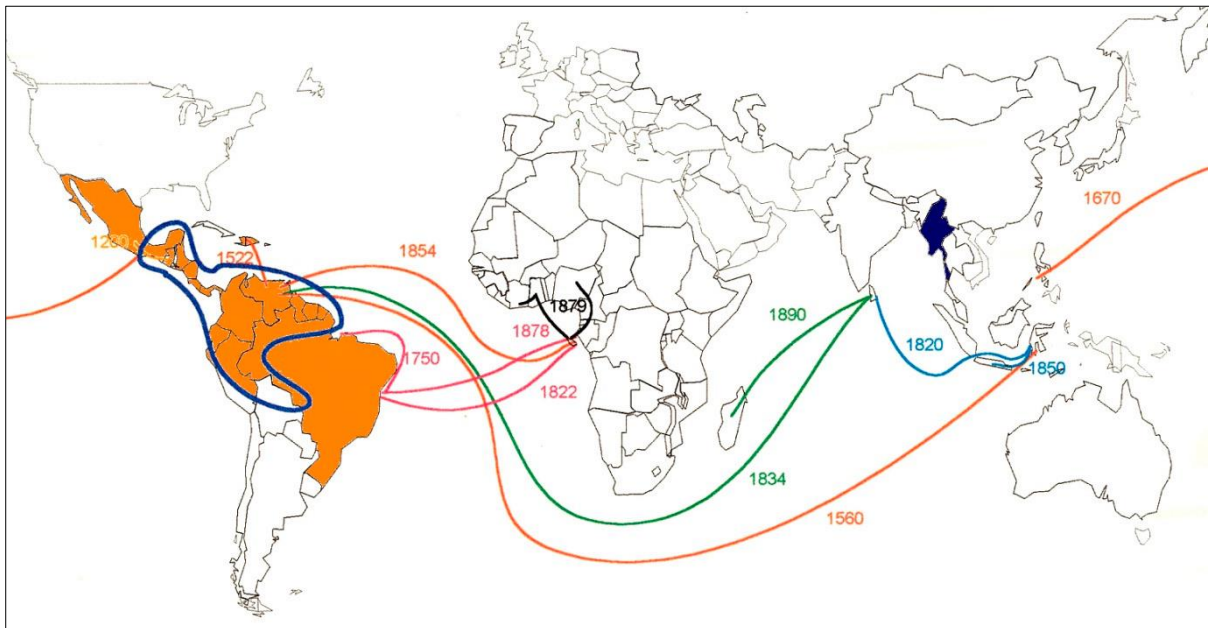


Figure 1.2 World map illustrating how cocoa cultivation has expanded from the origin of cultivation (The American Continent covering the orange-coloured areas) to the current places of cultivation. The lines indicate the period at which cocoa beans moved from their place of origin to a different geographical region. Map sourced from Cilas and Bastide (2020).

1.3 The global socioeconomic significance of cocoa

Today, cocoa is rated as one of 13 globally essential commercial commodities (Claus et al., 2018). Around the world, an estimated 14 million people work directly in the production of cocoa, which is grown on 7.5 - 10 million hectares of arable land (Adjaloo et al., 2012, Claus et al., 2018). In total, globally the cocoa industry provides 40 million people with a means of living, from cocoa cultivation to finished products and commercialization (Adjaloo et al., 2012). The majority of people employed in the sector directly participate in the planting, harvesting, and post-harvest processing

phases of cocoa production (Awafo and Owusu, 2022, Dawson et al., 2014, Amponsah-Doku et al., 2022). Rural populations, where entire livelihoods are inextricably related to cocoa farming, benefit economically from the production of cocoa (Achmad et al., 2022, Sostizzo, 2017). A complex network of actors, such as traders, cocoa processors, and chocolate producers, makes up the global cocoa trade and contributes to economic interdependence on a worldwide scale (Fold and Neilson, 2016, Neilson et al., 2020, Teye and Nikoi, 2022). More than 2,000 enterprises are involved in the chocolate and confectionery goods industry in the European Union and approximately 650 businesses make up the American cocoa and chocolate sector (Houston and Wyer, 2012).

Beyond planting, cocoa's economic importance also includes value-added through cocoa processing and chocolate production. Thomas (2017) reports that the global market for all chocolate products was in the region of US\$ 190 billion in 2012 with the market's value for chocolate alone reaching US\$ 105 billion, a startling 25% growth from 2007. Revenue generated from the cocoa sector in 2023 is estimated to be US\$ 12.8 billion and is projected to increase by 5.89% by 2028 (Statistica, 2023). In countries that import cocoa, the production of chocolate and other goods from cocoa beans creates significant economic value (Fowler and Coutel, 2017). The Netherlands is the biggest importer of cocoa beans followed by the United States (ISSD, 2019). The worldwide cocoa sector is a multi-billion-dollar global market.

The economic development of various Western African nations has benefited greatly from cocoa as a significant cash crop (Duguma et al., 2001). For instance, the economies of Ivory Coast and Ghana, two of the world's major cocoa producers, are strongly dependent on cocoa exports (Schroth et al., 2016). In 2016 alone, Ivory Coast, the largest exporter of cocoa generated US\$ 3.9 billion from cocoa exports with Ghana and Nigeria also generating US\$ 2.5 and US\$ 0.8 billion respectively (ISSD, 2019). Cocoa contributes roughly 12.5–14% to the foreign exchange earnings to Nigeria (Fadiji et al., 2021) and 25% in the case of Ghana (Group, 2018). In the 2021-2022 harvesting season, Ivory Coast alone produced 2.1 million metric tons (ICCO, 2022) (Figure 1.3). Cocoa's pivotal role in the economies of West African nations shines as a testament to its economic significance.

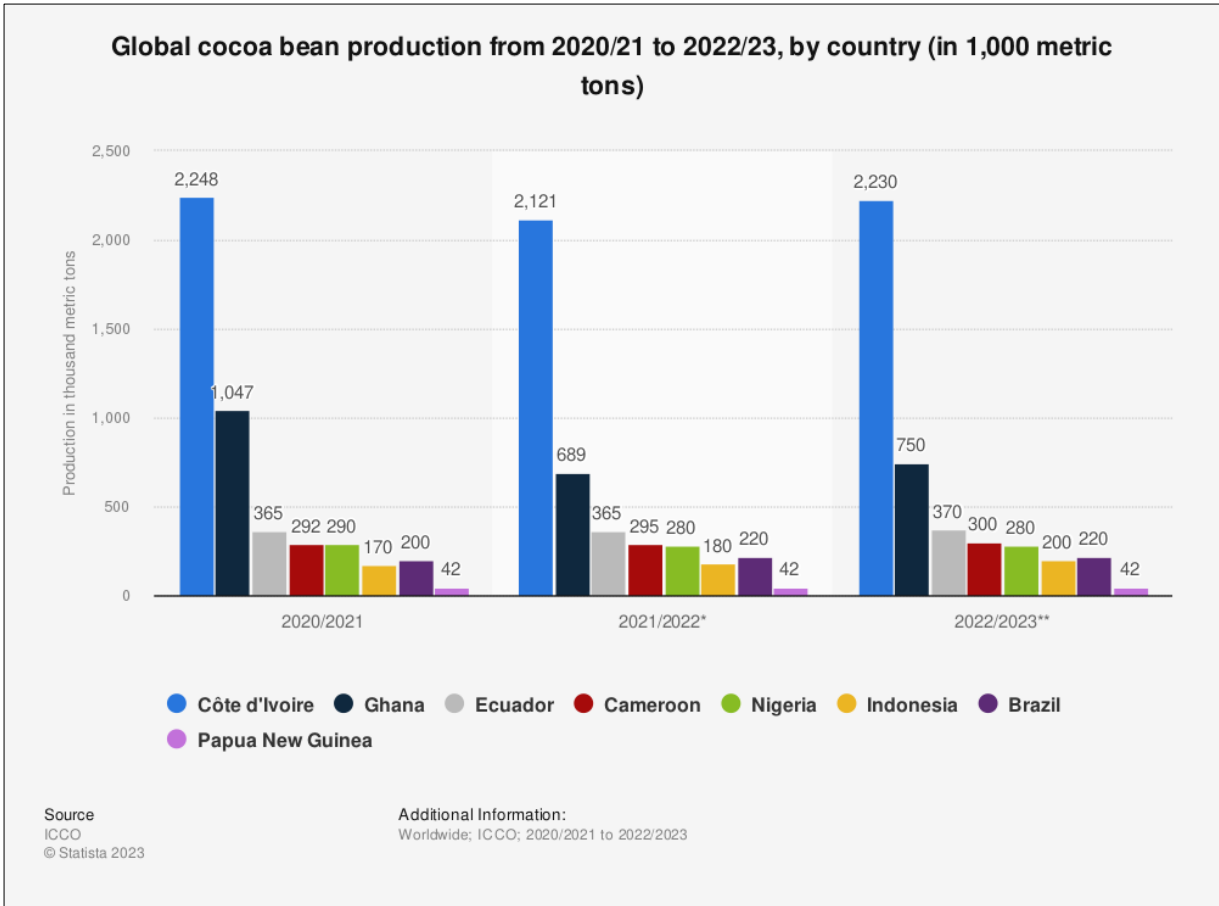


Figure 1.3 The annual production of cocoa beans from 2020–2021 to 2021–2022 per the eight leading producing countries with a projection for 2022–2023 [Source at (ICCO, 2022)].

1.3.1 Cocoa cultivation in Ghana

The origin of the commercial cultivation of cocoa in Ghana is attributed to Tetteh Quashie who travelled and returned from Fernando Po (now referred to as Biako) in present-day Equatorial Guinea in 1870 with cocoa seeds and started cocoa cultivation at Mampong-Akwapim in the Eastern Region of Ghana (Alhassan, 2021, Ayensah, 2019). However, evidence suggests that even before this, the Dutch had already experimented with its cultivation along the coastal belts of Gold Coast (present-day Ghana) as far back as 1815, albeit with little success (Kuusaana et al., 2021, Ayensah, 2019). Subsequently, the Swiss missionaries Lang and Marchand were thought to have made a great effort to introduce cocoa to present-day Ghana between 1857 and 1889 (Acquaah, 1999). Presently, the cultivation of cocoa in Ghana is concentrated in several regions, including Central, Western (comprising Western and Western North),

Ashanti, Eastern, Brong-Ahafo (now divided into Bono, Bono East, and Ahafo regions), Central, and Volta (Yahaya et al., 2015, Antwi-Agyakwa et al., 2015). Cocoa cultivation is propagated from cocoa seeds which are usually nursed and planted as seedlings in carefully managed environments (Asare, 2005, Sena Gomes et al., 2015). The life span of cocoa averages between 25 to 40 years, but with proper care, they may bear fruit for over half a century (Wessel and Quist-Wessel, 2015, Fowler and Coutel, 2017). The cocoa trees require a stable climate, with temperatures between 21°C and 32°C and consistent rainfall throughout the year (Wessel and Quist-Wessel, 2015). Ghana practices both fullsun and agroforestry cocoa systems (Abdulai et al., 2018). In fullsun systems, cocoa is grown in open fields without shade. This is the most popular cocoa-growing system globally, but is associated with biodiversity loss, soil fertility depletion, and soil quality degradation (Tondoh et al., 2015, Yoshida, 2008). The lack of shade trees in these systems may lead to a reduction in habitat complexity, which may in turn adversely affect various species that rely on these habitats. Besides, the exposure of soil to direct sunlight can also increase evaporation rates and reduce soil moisture, leading to erosion and nutrient loss (Lin, 2010, Klocke et al., 2009). In agroforestry cocoa systems, cocoa is cultivated with other trees and crops, in what is viewed as a more environmentally sustainable practice. In both systems, however, pest management is crucial, with integrated strategies involving regular monitoring, selective pesticide use, and the promotion of natural predators to maintain a healthy ecosystem (Afrane and Ntiamoah, 2011). The Cocoa Research Institute of Ghana (CRIG) plays a pivotal role in the advancement of Ghana's cocoa industry (Baah and Anchirinah, 2011). This includes research and development to enhance cocoa quality and yield, the development of disease-resistant cocoa varieties, and the promotion of sustainable farming practices (Obuobisa-Darko, 2015, Dormon et al., 2004). CRIG also collaborates with national and international partners, providing valuable data and advisory services to support cocoa farmers while working towards environmental sustainability and social responsibility in the industry. The bodies of research which are conducted by CRIG provide valuable insights and innovations that can be applied by cocoa farmers, and COCOBOD implements policies and programs based on this research to support the cocoa industry as a whole. Overall, the Ghana Cocoa Board (COCOBOD) remains the national authority responsible for regulating and promoting cocoa production in the country (Darkwah and Verter, 2014).

1.3.2 Impact of cocoa on the development of Ghana

Cocoa production is essential to Ghana's economy (Grohs et al., 2023, Alho et al., 2021), and is a key factor in Ghana's economic vibrancy (Kyeremeh, 2022). It was a crucial cash crop for the British colony during colonial times which influenced the economic development of Ghana (Enu, 2014, Kolavalli and Vigneri, 2011, Fountain and Hütz-Adams, 2020). Ghana's export profits are mostly based on cocoa sales (Wongnaa and Babu, 2020, GCB, 2020) and the sales of cocoa create foreign currency that helps the country's balance of payments and stabilises the currency (Quarmine et al., 2014, Boysen et al., 2023). This inflow of foreign currency is essential to sustaining Ghana's economy and meeting its import requirements. In Ghana, 800,000 farm families are engaged in cocoa farming in 7 out of the 16 regions of the country and this accrues an estimated annual income of US\$ 2.5 billion in foreign exchange (ISSD, 2019). This represents about 25% annual contribution of foreign exchange to Ghana's economy. Collectively, cocoa's deep-rooted importance in Ghana's economy and the significant foreign currency it generates underscore its pivotal role.

The economic impact of cocoa extends beyond agricultural pursuits (GCB, 2020). Rural development has been aided by the creation of cocoa cooperatives, processing facilities, and transportation networks (Garnevska et al., 2017, Thompson et al., 2022). These infrastructure upgrades improve connectivity and raise living standards in areas that produce cocoa (Attipoe et al., 2021, Kehinde and Ogundeji, 2022). A major place at the world trade table has been made available to Ghana as a result of its position as a renowned cocoa grower (Ahoa et al., 2020, Roldan et al., 2013). The country has a significant impact on the development of international cocoa policies because of its active involvement in these organisations and agreements. Notwithstanding the advantages that cocoa production has afforded Ghana, challenges related to market volatility, climate change, and sustainability issues remain pressing concerns (Ofori-Boateng and Insah, 2014, Ruf et al., 2015, Ariza-Salamanca et al., 2023). Concerns about the sustainability of cocoa farming have increased as the scale of cocoa plantations has grown, as discussed below.

1.3.3 The interplay between cocoa expansion and deforestation

Rising global demand for chocolate products has driven a significant expansion in cocoa farming, leading to the encroachment of tropical rainforests in countries like the Ivory Coast and Ghana (Nasser et al., 2020, Kalischek et al., 2023). In the last five decades, cocoa expansion has led to the disappearance of 14–15 million hectares (ha) of tropical forests globally (Somarriba and Lopez-Sampson, 2018). Cocoa farming is the main cause of more than 37% of forest loss in Côte d'Ivoire's protected areas and more than 13% in Ghana (Kalischek et al., 2023). In Ivory Coast, the forest cover of the classified forest of Haut-Sassandra (CFHS) decreased from 93% historically to 28% in 2015 with cocoa cultivation implicated as the key cause of deforestation. This has resulted in the loss of 40% of the plant species endemic primarily to the Ivory Coast (Barima et al., 2016). Available data suggest that the importation of cocoa beans to the European Market makes it one of the commodities with the largest imported deforestation footprint (WWF, 2021) (Figure 1.4). Due to its perceived profitability, smallholder farmers who frequently have limited access to alternate sources of income choose to cultivate cocoa (Aneani et al., 2011). The rapidly increasing cultivation of cocoa farming has posed a severe threat to tropical rainforests, causing substantial global forest loss and endangering vital ecosystems in countries like the Ivory Coast and Ghana.

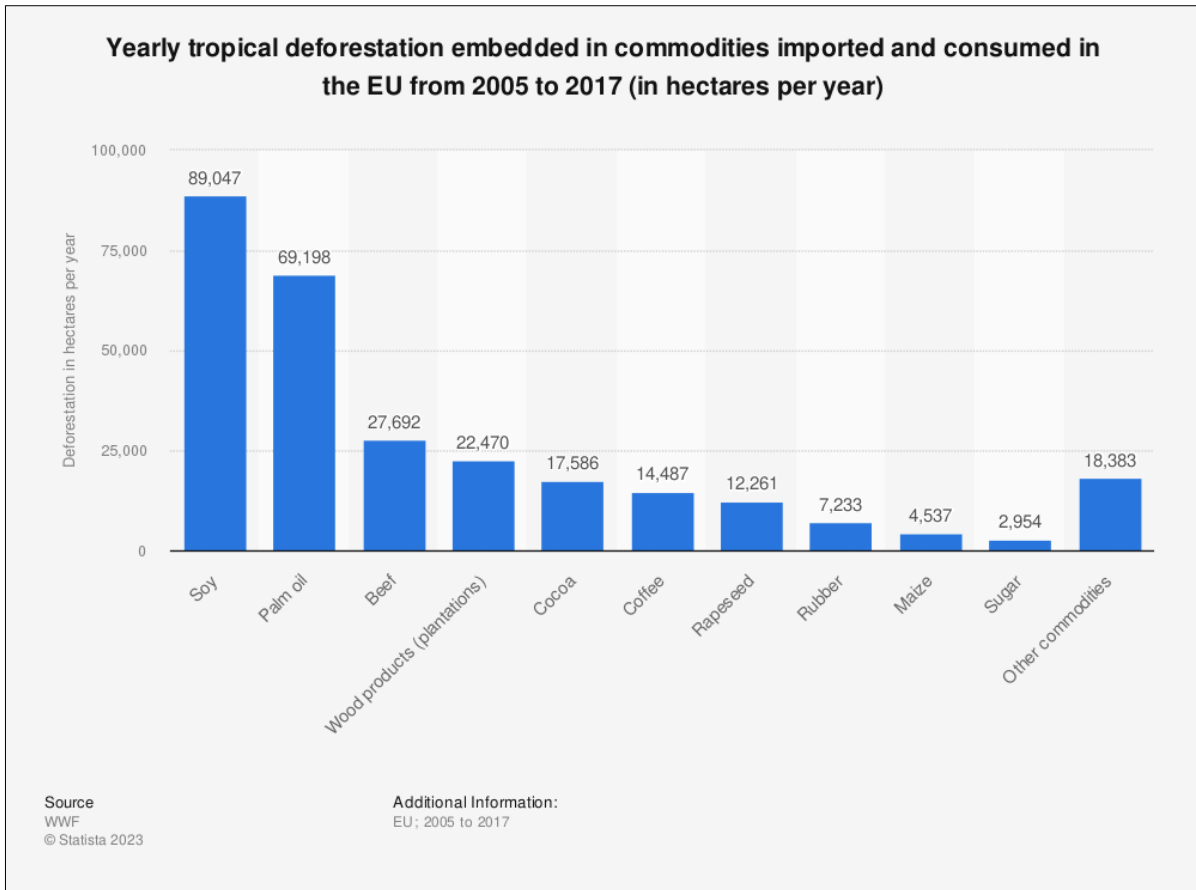


Figure 1.4 Between 2005 and 2017, 17,586 hectares of tropical forest were cleared to produce cocoa beans to meet demand in the European Union [Sourced at (WWF, 2021)].

1.3.4 Sustainability in cocoa farming

Besides deforestation, unsustainable cocoa farming methods also pose a serious risk since they contribute to the slow degradation of the soil, which may reduce the fertility of the land leading to long-term loss of productivity (Tittonell et al., 2007). Again, faced with a lack of assurance of land ownership, some farmers adopt a short-term perspective and concentrate on maximising short-term gains (Kyere-Boateng and Marek, 2021) and in the process resort to environmentally unsustainable cocoa farming techniques including overusing pesticides and fertilisers and over-cultivation, and deforestation (Tittonell et al., 2007). It is imperative for farmers to embrace techniques that increase cocoa production while at the same time contributing to environmental sustainability. One method to attain this balance is to implement agroforestry systems, such as shade-grown cocoa, which resemble natural forest environments and support biodiversity (Rice and Greenberg, 2000). Sustainable

cocoa production has been promoted by certification programmes like Fair Trade and the Rainforest Alliance (Moinina et al., 2023). The social and economic facets of sustainability are addressed by these programmes, which give farmers incentives to follow environmentally friendly practices while also assuring equitable salaries and working conditions (Rametsteiner and Simula, 2003). Ensuring sustainability comes with its challenges. Additionally, farmers face considerable financial risk due to shifting cocoa prices on the international market (Mulangu et al., 2017). These issues are made more difficult by climate change, which affects cocoa production through rising temperatures and erratic weather patterns (Ojo and Sadiq, 2010). Achieving a balance between social justice, environmental accountability, and economic prosperity in cocoa production will require determined action and approach.

1.4 Pollinators and Ecosystem Services

All life on Earth depends on ecosystem services, which are fundamental advantages gained from the environment (Balvanera et al., 2016, Bakure et al., 2022). These services manifest in various forms, encompassing cultural, regulatory, provisioning, and supporting activities (MA, 2005, Burkhard et al., 2012, Thomaz, 2023). Pollinators play a key part in each of these, demonstrating their importance in the context of ecosystem services (Ashman et al., 2004). Pollinators primarily comprise an array of insects and other animals which facilitate the transfer of pollen, a male plant's reproductive material, from the anthers (the male reproductive structures) to the stigma (the female reproductive structures) of flowers which leads to fertilisation and subsequently the reproduction of seeds and fruits (Ollerton et al., 2011, Papa et al., 2022, Ingram et al., 1996, Potts et al., 2010). Some plants can self-pollinate, but extensive self-pollination can result in in-breeding (Ollerton et al., 2011). Pollinators therefore play a crucial role in maintaining plant diversity and abundance because most angiosperms depend on them for reproduction (Ollerton, 2017). An estimated 299,200 (87.5%) of the approximately 352,000 species of blooming plants (Paton et al., 2008) benefit from biotic pollination (Ollerton et al., 2011). Among insect groups, bees, notably honeybees, bumble bees, and solitary bees, are the most recognised and essential pollinators (Klein et al., 2007). Klein et al. (2007) found that bees visit 107 different crop varieties such as apple, coconut, orange, and mango, among others, and are responsible for 90% of all pollination visits. For fruit production and

yield enhancement in several crops, pollinators are necessary (Basualdo et al., 2022, Roubik, 1995). Others such as butterflies, moths, birds (hummingbirds), beetles, wasps, bats, ants (Rader et al., 2016, Klein et al., 2007), wind and water (Culley et al., 2002, Tanda, 2022) are also able to facilitate pollination. It is worth noting that virtually all the vital elements such as calcium, folic acid, vitamins A and C, and fluoride required for healthy diets are found in pollinator-dependent plants, such as fruits and vegetables (Hein, 2009). Pollination services are essential not only to plant life but also to human nutrition and the broader ecosystem.

1.4.1 Plant-pollinator interaction

Plant-pollinator relationships, which are regarded as one of the most ecologically critical plant-animal interactions, increase plant diversity, and abundance, and protect from pollinator losses (Kearns et al., 1998). Even though plant-pollinator interactions have been the subject of in-depth study over the past 200 years (Nabhan and Buchmann, 1996, Leather, 2018, Potts et al., 2010), it is insightful to note that this relationship has an astonishing 170-million-year history (Ollerton, 2017). As the importance of plant-pollinator relationships for the preservation of biodiversity and food production became clearer, more research on pollination gathered impetus. However, human actions simultaneously put these linkages in jeopardy (Antiqueira et al., 2020, Cane and Tepedino, 2001). The extent of our understanding of these interactions, however, remains a matter of debate despite significant research efforts (Ollerton, 2017). Although a great deal of research has illuminated these interactions, persistent problems resulting from human activities stress the need for additional research to fully comprehend and protect these important ecological relationships.

1.4.2 The economic importance of pollinators

The valuable services which are rendered by pollinators necessitate concerted efforts for their protection not least because of the economic contribution of pollination services. Overall, the estimated contribution of pollinators to global crop production is estimated in the region of US\$ 235 billion to US\$ 577 billion annually (Potts et al., 2016, Gallai et al., 2009). Fruits and vegetables that depend on pollinators command a significant worth of €100 billion (Alebachew, 2018). The US economy benefits from agricultural pollination by bees to the tune of US\$10 to US\$ 14.6 billion, demonstrating

the economic importance of pollination (Morse and Calderone, 2000, Dar et al., 2013). Similar to the US, the UK receives projected pollination services worth £202 million, dwarfing the £15.7 million in revenue from honey production produced by 200,000 honey bee colonies (Carreck and Williams, 1998, Hanley et al., 2015). This clearly illustrates how pollination services by honeybees have higher economic rewards than just honey production. In the absence of pollination services, the global agricultural sector could experience losses of 5% to 8% (Aizen et al., 2009). Raising pollination research to a critical level is fundamental for both biodiversity preservation and guaranteeing food security (Rodger et al., 2004).

Over the past 50 years, the demand for pollination services has doubled globally (Potts et al., 2016a). Due to the high costs of keeping controlled colonies, small-scale farmers in many poor countries heavily rely on wild pollinators for pollination (Kasina et al., 2009, Tanda, 2019). Studies on equally important biological control services for global crop production have been disproportionately focused on large-scale agriculture in temperate regions, which highlights the need for a more balanced approach in ecosystem-services research to address food security concerns in smallholder-farmed landscapes, particularly in Africa and continental Asia (Steward et al., 2014). In addition, the protection of pollinators frequently takes a backseat in global agricultural objectives, which leads to an inadequate appreciation of their influence on decision-making (Dar et al., 2013). Neglecting the protection of pollinators not only jeopardizes crop yields but also has far-reaching consequences on global food consumption patterns and costs (Aizen et al., 2009).

1.4.3 Other benefits of pollinators to humans

Apart from contributing to biodiversity conservation and agricultural productivity, pollinators are important to humans in many unique and diverse ways. Plants which require animals for pollination provide many essential materials used for building, biofuels and musical instruments (Potts et al., 2016a, Rehel et al., 2009). The practice of beekeeping for honey and other bee products provides jobs and income generation which supports many rural families in different parts of the world (Hilmi et al., 2010, Dietemann et al., 2009, Gallai et al., 2009) (Figure 1.5). Beekeeping contributes to social cohesion through the formation of beekeeping associations which creates a

platform for information sharing and collective actions (Garibaldi et al., 2016). Home gardens, which are a source of recreation and can facilitate social relations, are dependent on pollinators for sustenance (Calvet-Mir et al., 2012). Bee products namely honey, pollen, propolis and wax have been used by many indigenous people as traditional medicines and the anti-bacterial, anti-diabetic agents and anti-fungal agents derived from honey have been used to bring about health improvement (Jull et al., 2015). Bees have inspired many cultures, societies, religions, songs, literary works, and technological advancements all over the world (Smith et al., 2015, Potts et al., 2016a, CBD, 2018). Pollinator-dependent plants also contribute to carbon sequestration (Christmann, 2019). Given the value of pollinators to human existence and the general ecosystem, declines in the population of pollinators are likely to have multiple implications for human existence.



Figure 1.5 Beekeeping enterprise at Bia Conservation Area. A) Beehives being constructed for residents near BCA for honey production; B) A beehives mounted to bait for bee colonies; C) Rural dwellers near Bia undergoing training in beekeeping practices and D) Beekeepers being trained in honey harvesting (Photos: RB).

1.4.4 Drivers of pollinator declines

Pollinator loss, which affects bees, butterflies, and other important insect pollinators, is a serious ecological issue with far-reaching effects on ecosystems and human food systems (Rhodes, 2019, Ellman et al., 2023). Habitat loss and fragmentation brought on by urbanization, agricultural growth, and deforestation tend to affect insect pollinators in several ways. The availability of pollinator-friendly habitats is impacted by specific land use practices, such as the removal of hedgerows and wildflower meadows (Tonietto and Larkin, 2018, Lynch et al., 2021). In a meta-analysis, Winfree et al. (2009) found that the abundance and species richness of wild unmanaged bees are adversely affected by disturbances, chiefly habitat loss and fragmentation. Landscape intensity has been found to alter both the biotic and abiotic habitat parameters in areas inhabited by the bee and wasp communities (Klein et al., 2002). Kremen et al. (2003) indicated that agricultural intensification reduces pollination services by native bee communities because it causes a reduction in diversity and abundance and declines in abundance and diversity in native bees. Alterations in land use may disrupt the co-evolved relationships between plants and their pollinators, potentially leading to constraints on plant reproduction due to insufficient pollen supply (Bennett et al., 2020). The complex web of consequences stemming from pollinator loss, driven by habitat alterations and land use practices, underscores the urgency of safeguarding ecosystems for the well-being of both wildlife and human food systems.

Pollinator declines can also be traced to climate change which impacts populations of insect pollinators in a variety of ways. Temperature and weather variations can cause delays in plant flowering and pollinator emergence, which can restrict foraging and cause food scarcity for pollinators (Bartomeus et al., 2011). Specific temperatures and photoperiods are required by butterflies and bees to start their migrations and therefore rising global temperatures may reduce reproductive success and cause population reductions which may result from this being out of sync with their natural environments (Ovaskainen et al., 2013). Again, increasing CO₂ levels, combined with drought conditions, can influence floral traits which may cause plants to produce smaller, less attractive flowers with reduced nectar and pollen rewards (Glenny et al., 2018).

Pesticides have also been found to contribute to pollinator declines (Potts et al., 2010). For example, exposure of bees to neonicotinoids was shown to adversely impact their foraging behaviour, navigation and learning process (Stanley et al., 2015a) and caused the impairment of their immune systems leading to induced vulnerability to diseases and parasites (Alkassab et al., 2023). In another study, when bees were subjected to prolonged and dietary exposure to neonicotinoids, the resultant effect was a 50% decline in the overall reproduction of offspring and a skewed sex ratio towards males in a controlled experiment (Sandrock et al., 2014).

Additionally, pollinator populations are weakened and made more vulnerable to disease and parasites such as *Nosema apis* and *Varroa destructor* mites (Ullah et al., 2021, Guzmán-Novoa et al., 2010). The Small Hive Beetle (*Aethina tumida*), which has severely harmed honeybee colonies all over the world (Hood, 2004), is an example of how globalization can foster the spread of diseases and pests that are damaging to pollinators (Vanbergen and Initiative, 2013, Rodríguez-Flores et al., 2022). The introduction of invasive plant species Japanese knotweed (*Fallopia japonica*), purple loosestrife (*Lythrum salicaria*), and giant hogweed (*Heracleum mantegazzianum*), exacerbates this issue further by out-competing native plants, reducing sources of nectar and pollen, and perhaps causing pollinator malnutrition (Vanbergen et al., 2018, Kovács-Hostyánszki et al., 2022, Memmott et al., 2007, Middleton, 2019, Barney et al., 2013). Conservation efforts, sustainable farming methods, decreased pesticide use, and climate change mitigation must all be included in a holistic plan to combat pollinator reductions (Kleijn et al., 2015, Russo et al., 2020).

1.4.5 Cocoa pollinators and other visitors

Cocoa yields benefit from insect pollination (Toledo-Hernández et al., 2017, Kofi et al., 2014) and pollination services can therefore be a limiting factor in cocoa production. Insects of the order Hymenoptera, Diptera, Orthoptera and Coleoptera visit the cocoa tree with the Dipterans and the Hymenopterans being the more regular visitors (Adjaloo and Oduro, 2013). Given the different assemblage of visitors to the cocoa plants, Toledo-Hernández et al. (2017) posited that cocoa productivity could be increased by the pollination services of many other arthropod groups including ants,

bees and parasitic wasps. Adjaloo and Oduro (2013) are of the opinion that pollinator abundance does not mean a high rate of pollination success in cocoa, and they go ahead to suggest that the floral structure of the cocoa inhibits access to many cocoa flower visitors, and this prevents pollination success. This is further supported by O'Doherty and Zoll (2012) who assert that the evolutionary structure of cocoa flowers makes it unsuitable for many insects to pollinate it. For instance, Adjaloo and Oduro (2013) found that 40% of insects visiting the cocoa trees were crawling insects which belonged to the orders Coleoptera and Hymenoptera but none of the crawling insects ever carried pollen to the stigma. O'Doherty and Zoll (2012) also made similar observations when they observed no evidence of pollen on aphids and ants' visitors to cocoa which were either captured or photographed. However, Toledo-Hernández et al. (2017) are of the opinion that the pollinating role of many cocoa visitors has not sufficiently been explored yet. The number of studies undertaken on cocoa pollination have largely recorded pollination success by the ceratopogonid (family Ceratopogonidae) midges in cocoa. Adjaloo and Oduro (2013) found that only the ceratopogonid midges showed pollination importance in terms of visitation rates and pollen deposition on the stigma. Diptera and Hymenoptera have been suggested as potential pollinators with the suggestion that thrips found on the petal punch and inside the flowers are an indication of being possible to pollinate cocoa (Schawe et al., 2016). O'Doherty and Zoll (2012) established that ceratopogonid midge, *Forcipomyia hardyi*, visits the cocoa flowers to pick pollen and therefore act as pollinators, providing the first observed evidence of the role of native midges as a pollinator of cocoa in Hawaii. Schawe et al. (2016) also found that only the Encyrtidae carried pollen but their study did not establish the actual pollinators of cocoa as it compared visitors to both wild and cultivated cocoa. However, in an experiment conducted to determine tropical pollinators of cocoa, Adjaloo and Oduro (2013) found that, the ceratopogonid midges accounted for 97% of insects' visitations to cocoa flowers and showed pollination importance but none of the crawling insects or other insects carried pollen to the stigma. The midges are known to have limited foraging ranges. They have been found to usually swarm and forage flowers in a 5>6 m radius during the day (Toledo-Hernández et al., 2017) and they primarily act as generalists and are likely to pollinate different plants during foraging (Young, 1986). Unfortunately, little is known about the role of non-native species in cocoa pollination (Klein et al., 2008). By and large, as an

insect-pollinated crop, cocoa relies chiefly on the ceratopogonid midges (*Forcipomyia*) as its main pollinator (Winder, 1978, Tschardt et al., 2012a, Young, 1982).

Understanding midges' habitat preferences and how these relate to cocoa pollination is important. According to Tschardt et al. (2012b), cocoa farms frequently have a variety of agroecosystems, including trees providing shade and varied degrees of agroforestry techniques. These agroecosystems, especially the shade trees, are crucial for providing midges with optimal breeding and feeding settings. Klein et al. (2003) have suggested that shade trees offer microclimates with the ideal temperature and humidity levels for midge activity. Because midges need particular substrates for the development of their larvae, the presence of organic matter in these shady habitats, such as decomposing leaf litter, provides breeding grounds for them (Young, 1982). Additionally, the presence of a variety of plant species in cocoa agroforests aids in the provision of floral resources needed by midges for nutritional needs for efficient pollination (Tschardt et al., 2012b). The limited foraging ranges of midges are such that they are highly dependent on the localised resources within cocoa habitats (Toledo-Hernández et al., 2017). These habitats act as centres of midge activity and influence their foraging patterns. Proper management of cocoa habitats, including the maintenance of shade trees and habitat diversity, can therefore enhance midge populations and, consequently, cocoa yield (Kofi et al., 2014).

1.5 Pesticides

Pesticides have been essential to agriculture owing to their effectiveness in combating several pests which pose threats to crops, livestock and public health (Tudi et al., 2021). These chemical substances are specifically formulated to control, repel, or eliminate unwanted organisms, which include insects, weeds, fungi, bacteria, rodents, and more (Casida and Quistad, 1998). Pesticides can broadly be categorised either as organic or inorganic compounds (Frimpong et al., 2013). Organochlorines, neonicotinoids, organophosphates, carbamates and synthetic pyrethroids pesticides comprise the broad categories of organic pesticides (Patnaik, 2007). Previously organochlorines gained prominent use because of their effectiveness in combating a broad spectrum of insect pests, including malaria-causing mosquitoes, especially

during the mid-1900s (Turusov et al., 2002). Because of their persistence in the environment, their use has either been phased out or restricted (Stoichev et al., 2005). Neonicotinoids are a class of synthetic insecticides which share similar properties to nicotine and were developed as a safer and more effective alternative to previous insecticide classes such as organochlorines (Jeschke et al., 2011). They are the most widely used class of insecticides globally (Bonmatin et al., 2015, Simon-Delso et al., 2015). The nicotinic acetylcholine receptor located in the central nervous system of insects is impacted by neonicotinoids at certain concentrations when insects come in contact with it (Matsuda et al., 2001). The organophosphates such as malathion and chlorpyrifos, alter the proper functioning of the acetylcholinesterase, an enzyme required for neurotransmission, resulting in the death of insect pests (Khan & Shahzad, 2008). The carbamates, such as carbaryl, act similarly to the organophosphates on insect pests but differ in having shorter environmental half-life therefore providing safer alternatives to other chemical classes (Casida and Quistad, 1998, Gupta et al., 2017). The naturally occurring pyrethrin compounds present in chrysanthemum serve as the key component of the synthetic pyrethroids which include permethrin and cypermethrin and are used in different formulations for insect control in agriculture, public health, and household pest management (Borowik et al., 2023, Zerba, 1988, Ahamad and Kumar, 2023).

Pesticides can be further categorised based on their uses. Insecticides are those designed to specifically target and regulate the population of insect pests (Ware and Whitacre, 2004). Herbicides on the other hand are employed to control or eradicate undesirable plants, primarily focusing on weeds, and are classified into various groups such as glyphosate-based herbicides, triazine herbicides, and acetolactate synthase inhibitors (Gianessi, 2013, Gianessi and Reigner, 2007). Fungicides are utilised to combat fungal pathogens and belong to diverse chemical groups such as azoles, strobilurins, and anilinopyrimidines (Russell, 1995). Bactericides are instrumental in managing bacterial infections and exhibit diverse chemical structures (Sundin et al., 2016). Nematicides are specifically designed to target parasitic nematodes and are classified based on their chemical properties (Rich et al., 2004). They are particularly applied in agriculture to effectively manage crop damage caused by nematodes. Furthermore, mites are controlled by acaricides and others such as rodenticides are utilised to control rodents such as rats and mice (Frimpong et al., 2012a).

1.5.1 Pesticides and agricultural efficacy

Pesticides have been crucial in boosting agricultural productivity and guaranteeing food security for expanding populations by successfully minimising the damage caused by pests (Rajak et al., 2023, Headley, 1968, Oludoye et al., 2023). Around the world, approximately 4.12 million tonnes of pesticides are used annually for crop production (FAO, 2022) (Figure 1.6). According to a meta-analysis by Oerke (2006), the use of pesticides can lead to a 39% reduction in yield losses globally. Although pesticides play a role in increasing agricultural productivity, it is important to balance their use with potential drawbacks. Pesticide use may bring about undesired results such as environmental damage, harm to non-target species, and the emergence of pests that are resistant to them (Critchley et al., 2022, Sargent et al., 2023). Therefore, pesticides must be used sparingly and responsibly together with integrated pest management (IPM) techniques to maximise agricultural productivity and reduce negative impacts (Bottrell and Schoenly, 2018, Jacobsen, 1997). The purpose of IPM is to use a friendly approach for pest control with minimal effect on the environment (Kogan, 1998). Therefore, to keep pest populations under control while protecting the environment and public health, IPM integrates cultural practices, biological controls, and targeted pesticide application (Afrane and Ntiamoah, 2011). Though the purpose of IPM is intended to be compatible with biological control, Stenberg (2017), has been mooted for an extended approach of Integrated Pest and Pollinator Management (IPPM) on the grounds that some IPM actions can have detrimental effects on pollinators (Egan et al., 2020). IPPM, a concept propounded by Biddinger and Rajotte (2015), is aimed at reducing pollinator exposure to conventional insecticides.

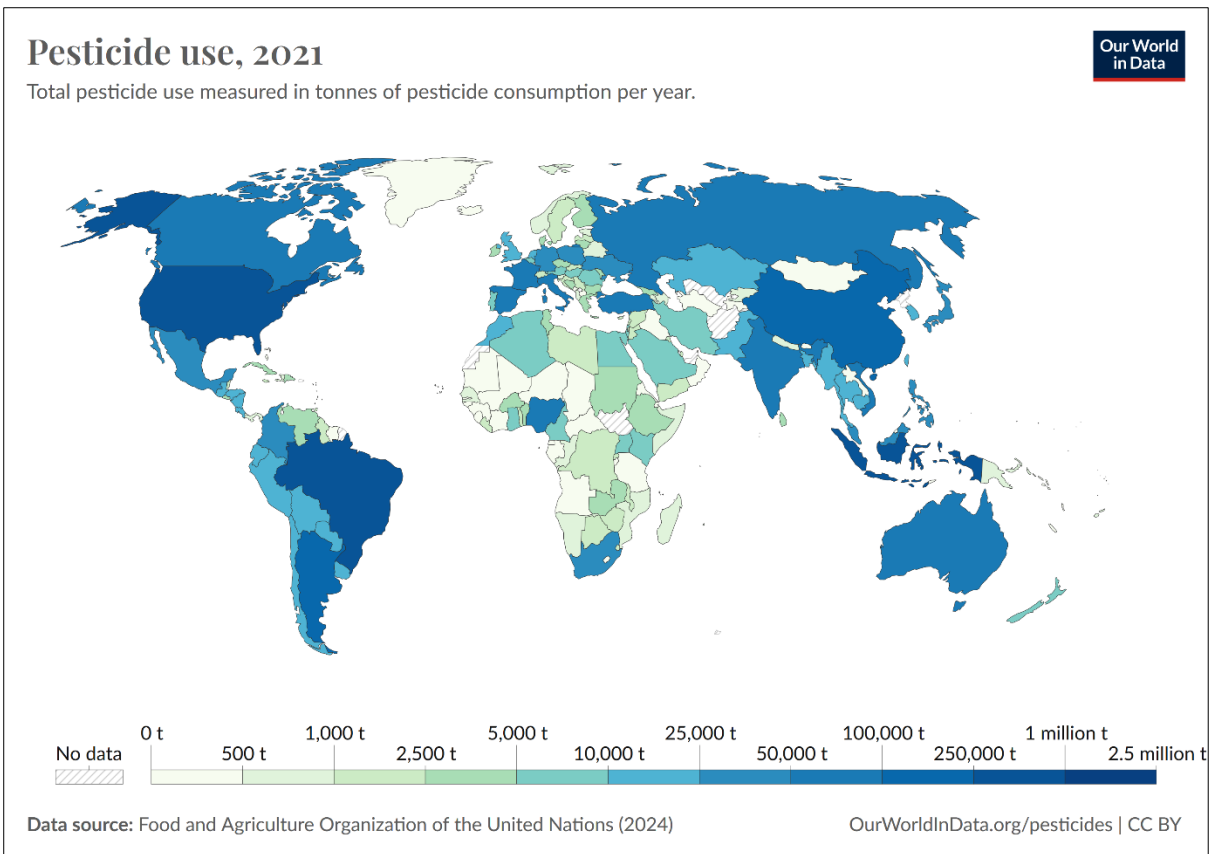


Figure 1.6 Total pesticides (in tonnes of active ingredients) used globally from 1990 to 2021, including mineral oils, insecticides, fungicides, and bactericides (including seed treatments), as well as herbicides, plant growth regulators, rodenticides, and other chemicals [Taken from Hannah Ritchie et al. (2022)].

1.5.2 Pesticide and pest control in cocoa cultivation

Due to the high susceptibility of cocoa to disease and pest infestations, the cultivation of cocoa is exceedingly challenging and may result in significant financial losses (Bateman, 2015, Asare et al., 2014). Generally, farmers use a variety of management techniques, such as fungicides, insecticides, pruning, and good agricultural practices, to address these vulnerabilities (Wessel and Quist-Wessel, 2015, Adu-Acheampong et al., 2015). Globally it is estimated that 30% to 40% of cocoa produced is lost (Lanaud et al., 2009) owing to its vulnerability to pest and disease attacks (Ntiamoah and Afrane, 2008). These include the mealybug-transmitted cocoa swollen shoot virus (CSSV), which results in swollen shoots and tree mortality (Fargette et al., 2006); the fungus-transmitted Black Pod disease (Adomako et al., 2017); the virus-transmitted

Witches' Broom Disease and the bacterium-transmitted Frosty Pod Disease (Meinhardt et al., 2008). The cocoa pod borer (*Conopomorpha cramerella*), which bores into pods (Ndubuaku and Asogwa, 2021, Saripah and Alias, 2016), soil-borne pathogens that cause wilt illnesses (Bailey et al., 2005) and insects like capsids (*Distantiella theobroma* and *Sahlbergella singularis*) and mirids (*Helopeltis spp.*) that feed on pods and spread disease are just a few examples. It is projected that in the absence of preventive measures, disease attacks alone could lead to losses ranging from 70% to 100% (Ndoumbe-Nkeng et al., 2004). In order to prevent losses and to meet global demands around the world, pesticides are applied for cocoa production (Okoffo et al., 2016) (Figure 1.7). In Nigeria alone, for instance, 125,000 to 130,000 metric tons of pesticides are applied for cocoa cultivation to ensure satisfactory production levels (Asogwa and Dongo, 2009). A total of 31% of all pesticides imported into Nigeria were used to produce cocoa (Oludoye et al., 2023). In Ghana, neonicotinoids and pyrethroids are the pesticide classes mostly applied on cocoa farms and account for over 70% of all insecticides utilised in the country (Boateng et al., 2023). Even though pesticide applications are essential for cocoa cultivation, attention must be extended beyond just economic gains to the wider impacts of pesticides imputed for cocoa cultivation to the general vicinity of cocoa plantations.



Figure 1.7 Cocoa spraying activities within Bia West District. A) A Mass Spraying contingent mixing pesticides with water before spraying cocoa farms. B) Cocoa trees being sprayed to protect them against pest and disease infestation (Photos: RGB).

1.5.3 Environmental consequences of pesticide use

Even though pesticides have enhanced crop yields, they have also sparked worries regarding their multifaceted environmental implications and human well-being (Mahob et al., 2014, Pathak et al., 2022, Aminu and Edun, 2019) which are contingent upon the particular types of pesticides utilised, geographical contexts, and specific usage patterns (Goulson, 2013). Whilst insecticides are designed to eradicate insect pests they are renowned for their non-selective nature and often tend to inflict harm on other beneficial insects such as pollinators (Getanjaly et al., 2015, Hatfield et al., 2021). Additionally, they also disrupt populations of natural predators and biological services (Abd Allah et al., 2018). Herbicides which are employed to control the growth of weeds, within cocoa plantations, may potentially impact ecosystems by disturbing the natural composition of plant species, thereby leading to declines in biodiversity (Tuck et al., 2014). For instance, it has been shown that herbicide utilisation may cause imbalances in indigenous flora and fauna and potentially pollute surface water and groundwater and by extension cause adverse effects on aquatic ecosystems and the quality of drinking water (Goulson, 2013). The environmental consequences of pesticide use can vary considerably based on geographical contexts. In tropical regions, characterised by high levels of biodiversity, the risks associated with pesticide use are amplified, as the vast array of species includes numerous endemic and vulnerable organisms that can be disproportionately affected by exposure to pesticides (Simon-Delso et al., 2015). Moreover, the warm and humid conditions prevalent in these regions can enhance the persistence and mobility of pesticides in the environment (Aktar et al., 2009). In contrast, regions dominated by intensive agriculture experience more severe environmental repercussions, as continuous applications of pesticides often lead to the emergence of pest populations that are resistant to pesticides, necessitating the use of even more potent substances (Tabashnik et al., 2013).

The environmental impacts of pesticide use include the loss of biodiversity (Geiger et al., 2010), long-term ecological imbalances and deterioration of soil health (Mubeen et al., 2023, Bisht and Chauhan, 2020, Ansah, 2019), the development of pesticide resistance (Bass et al., 2015), and unintended consequences on non-target species (Rundlöf et al., 2015). The runoff from these landscapes can result in the contamination of nearby bodies of water, thereby posing risks to aquatic life (Schreiner

et al., 2021, Stehle and Schulz, 2015, Gunstone et al., 2021). These factors collectively highlight the urgency of adopting sustainable practices for pest management, such as integrated pest management (IPM) and organic farming, which provide viable alternatives for mitigating these environmental repercussions and safeguarding ecosystems and biodiversity (Pretty & Bharucha, 2015). Short- and long-term exposure to pesticides raises health concerns, especially in environments with limited resources, where vulnerable communities are burdened by social and financial costs associated with pesticide-related health problems (Atreya et al., 2011, Atreya, 2008). Owing to these challenges, it is imperative that for the long-term sustainability of agriculture, there should be a shift towards a balanced approach between food production and the preservation of the environment is of paramount importance (Nuyttens et al., 2013). This is crucial as estimates of the use of pesticides in the USA also point to US\$10 billion worth of environmental and societal harm (Pimentel, 2005). According to Pimentel (2005), the biggest losses are estimated to be in the areas of public health (US\$ 1.1 billion), pests that are resistant to pesticides (US\$ 1.5 billion), crop damage from pesticides (US\$ 1.4 billion), bird losses from pesticides (US\$ 2.2 billion), and groundwater contamination (US\$ 2.0 billion). Accordingly, while pesticides have significantly increased cocoa production, their usage requires a careful balance between agricultural prosperity and environmental and human well-being. By and large, the environmental implications of pesticide use are widespread and may include adverse effects on the general ecosystems, and non-target species, with the added financial challenge associated with managing these effects, not least the direct effects on human health. An informed and balanced approach, which revolves around consideration for both food production and environmental preservation, is indispensable for the long-term well-being of our planet.

1.5.4 Effects of pesticides on pollinators

The interplay between pesticide exposure and pollinator health is a subject of growing concern, with studies indicating that pesticide residues, notably systemic neonicotinoids, pose risks to insect pollinators. According to Goulson et al. (2015), pesticide exposure can weaken the pollinators' immune systems, making them more susceptible to diseases, parasites, and other stressors. Because of the possible harm they could cause to pollinators, pesticide residues, particularly systemic

neonicotinoids, have attracted a lot of attention (Desneux et al., 2007). These residues have sublethal effects that endanger bees' life and pollination effectiveness. They influence pollinators both directly and indirectly. The sublethal effects of pesticides include the impairment of communication, feeding behaviours, and navigational abilities, ultimately undermining their overall fitness and ability to perform effective pollination (Stanley et al., 2016, Giunti et al., 2022, Tosi et al., 2022, Stuligross et al., 2023). The negative impacts on pollinators can also be amplified by complex interactions between stressors, such as pesticides and nutritional shortages (Goulson et al., 2015). A combination of nutritional shortage and exposure to pesticides was found to weaken the immune systems of pollinators and cause them to even become more susceptible to diseases (Di Prisco et al., 2013). There needs to be a careful balance between the application of pesticides for crop cultivation and the preservation of pollinators. Maintaining the delicate balance between crop production and pollinator health requires sustainable practices that take into consideration pollinator health.

1.5.5 Impacts of pesticides on bee products

Through various means, bees encounter contaminants like pesticide residues capable of impacting hive materials and products, raising concerns about the quality and safety of hive products. Bees are exposed to contaminants in a variety of ways, such as through water consumption, contact with pesticide-treated plants and soil, collection of contaminated pollen and nectar, exposure to airborne pesticides in treated areas, and inhalation of pesticide-tainted air (Colin et al., 2004, Manzoor and Pervez, 2021, Rajak et al., 2023, Sanchez-Bayo and Goka, 2014). The hairy bodies of bees then carry these toxins back to beehives (Lambert et al., 2013). Pesticide residues are found in bee colonies as a result of bees being exposed to a wide range of compounds through pollen, nectar, and water contaminated by agricultural pesticides (Wen et al., 2021, Kaila et al., 2022). The quality and safety of bee products are therefore called into question by this contamination. Pollen, a vital source of protein for bees, can absorb pesticides from treated plants, entering bee colonies and contaminating bee colonies and their products (Pervez and Manzoor, 2023, Zhang et al., 2023, Lambert et al., 2013). Beeswax, a necessary component of bee colonies, can accumulate pesticides and potentially harm entire colonies (McAfee et al., 2021, El Agrebi et al., 2020). Even royal jelly, a food source for growing bee larvae, has the potential to

include pesticide residues, raising questions regarding the development and well-being of the next bee generations if exposed to such residues (Wen et al., 2021). According to Lambert et al. (2013), pesticide contamination in bee products can weaken colonies, alter feeding patterns, or even cause colony collapse. These effects could have a cascading ecological impact on ecosystem biodiversity and pollination. There are growing environmental and health issues because of pesticide use, habitat loss, and rising agricultural output on bees, beekeeping, and bee products. Bee colonies and the quality of items made with bees are threatened by chemical residues and environmental degradation.

1.6 Land demand for agriculture

Population expansion and the demand for food to meet nutritional requirements have ushered in increased demand for more arable lands for farming purposes, (Rudel et al., 2009, Zhuang et al., 2022, Tilman et al., 2001). Agricultural products are in high demand around the globe owing to several interrelated factors. The world population, which is projected to hit 10 billion by 2050, comes with the pressure of corresponding increased food production to guarantee food security (Dorling, 2021). Beyond this, dietary preferences are likely to shift in the direction of increased consumption of protein-rich foods and vegetables in response to improved living standards in many parts of the world (Godfray et al., 2010). The concomitant effects include the likelihood of the need for both increased and diversified food varieties which are likely to require more arable lands to achieve these two-pronged demands. Looking beyond this, Lapola et al. (2011) indicated that the growth in the biofuel industry and the demand for bio-energy have also led to increased production of crops like corn and sugar cane for ethanol further putting pressure on demand for arable lands. A similar case may be made for increased land demand to produce raw materials to feed industries which also place demand for more arable lands for agriculture. As nations specialize in the production of particular crops or livestock, they rely on international trade to meet their diverse food preferences and this leads to increased demand for agricultural products for export (Searchinger et al., 2019). The demand for arable lands often leads to transformation in landscapes. This transformation entails the conversion of natural habitats, forests, or grasslands to agricultural areas (Mustard et al., 2012). Over half of the new agricultural land created in the tropics between 1980 and 2000 replaced

intact forests, and another 28% came from disturbed forests (Gibbs et al., 2010). The need for more arable lands, which has been necessitated by population increases and other factors such as dietary changes, and rising industrial development and businesses, has far-reaching effects, such as changing the environment through deforestation and habitat conversion.

1.6.1 Forested areas as pollinator reservoirs

The significance of natural forests for insect pollinators is well documented. As vital habitats to insect pollinators, forested areas play an essential role in their life cycles thereby contributing to the overall survival of pollinators (Ricketts et al., 2008). Many insects depend on natural forests for their dietary resources, breeding grounds, and escape from predators (Didham et al., 1998). They provide a complex web of plant species, each with a distinct set of qualities that give herbivorous insects a variety of food sources (Nichols et al., 2008). Added to this is the fact that the varied canopy layers, which serve as a conduit of structural complexities, provide varying niches and microclimates compatible with different insect communities (Kennedy et al., 2010). The function of the forest as a pollinator reservoir is highlighted in a study by Blanche et al. (2006) that shows a higher rate of single bee trips to longan flowers close to rainforests than to orchards farther away. According to Matheson et al. (1996), several natural species that are absent from coffee farms build their nests in wooded regions. De Marco Júnior and Coelho (2004) indicated that pollinators can find resources, such as materials for nest construction, in native woodlands next to coffee fields. Bailey et al. (2014) noted that male *Andrenidae* bees frequently visited the borders of forests for mating or nesting. The case for land development and the preservation of wooded areas can conflict in many emerging countries in sub-Saharan Africa where agriculture is highly valued (Laurance et al., 2014, Jellason et al., 2021). This could affect the preservation of pollinators. The complex interconnections among insects and forest ecosystems play a crucial role in the overall well-being and equilibrium of this habitat (Tschardt et al., 2012b). Effective monitoring of plant species that are present in the landscape or along forest borders and that offer pollinators food and places to nest is essential (Viana et al., 2012).

1.6.2 Impact of land use change on pollinators

The transformation of natural landscapes into other landforms including urban, agricultural, and industrial areas, which began some 8,000 to 10,000 years ago (Stephens et al., 2019), has implications for pollinators due to alterations caused to the habitats available for insect pollinators (Montero-Castaño and Vila, 2012). The fragmentation, destruction, or degradation of natural habitats which act as refuges for pollinators are results of the conversion of natural and semi-natural vegetation for agricultural purposes (Potts et al., 2010). These modifications cause variations in the biotic and abiotic components of the habitats inhabited by bee and wasp communities, which have an impact on their nesting resources and feeding environments Klein et al. (2007). Changes in land use lower the variety of plants in natural habitats (Goulson et al., 2015, Ollerton et al., 2014), affect pollinators' home ranges (CBD, 2018), and restrict their availability to floral resources including pollen and nectar (Winfree et al., 2011, Scheper et al., 2014). As a result of land-use change, agricultural intensification has been found to impair the pollination services offered by native bee groups (Cane and Tepedino, 2001, Ollerton et al., 2014, Potts et al., 2010). Global biodiversity and agricultural output are significantly impacted by the effects of land use change on pollinators (Winfree et al., 2011). Although the effects of landscape modifications on pollinators are well acknowledged, research findings differ with regard to their impact. According to some studies, insects are less susceptible to land-use change since they only need a modest amount of space to forage (Tscharntke et al., 2002, Winfree et al., 2011). Others contend, however, that agricultural landscapes can adversely impact the diversity of bees (Senapathi et al., 2016, Weiner et al., 2014, Millard et al., 2021). Further investigation is required to fully comprehend their interaction and the ensuing influence on crop yields in light of these divergent viewpoints on the consequences of land-use change on pollinators (Winfree et al., 2011). Research investigating the interactions between various factors that contribute to pollinator reductions should also be a top focus, particularly in light of climate change (Senapathi et al., 2016, Jamieson et al., 2017). Researching how these variables interact through a combination of experimental and observational investigations can offer a more comprehensive viewpoint and help in the creation of successful conservation policies (Figure 1.8). There has been advocacy for future research to concentrate on analysing the relative abundance and composition of pollinator species to ascertain whether species are

gained or lost as a result of changes in land use (Winfree et al., 2011). To address pollinator loss, it is crucial to comprehend how land-use change affects pollinators and their ecosystems.



Figure 1.8 Sampling of forest and cocoa landscapes in Bia West District. A) An aerial pan trap set in a cocoa farm to collect insect foragers in tree canopies; B) A ground pan trap used in combination with aerial pan trap for sampling; C) The carpenter bee (*Xylocopa* spp) collected during field sampling (Photos: RB).

1.6.3 Ecological Harmony

The symbiotic interaction between beekeeping, pollinators, and trees is a story of ecological relevance. A wide variety of plant species are found in forests, which range

from temperate woods to tropical rainforests, giving pollinators access to a variety of nectar and pollen sources (Ulyshen et al., 2023, Mola et al., 2021). Among others, bees, butterflies, birds, and bats are essential to the health and regeneration of the forest (Božek et al., 2023, Rader et al., 2016). Pollinators are crucial to the health of forest ecosystems because they maintain genetic variety and the ability of plant species to reproduce (Hajjar et al., 2008). These ecosystems' very structure is shaped by the complex interactions between pollinators and forest vegetation (Kotze et al., 2022). Beyond ecological intricacies, trees have enormous value for beekeeping as well as pollinators. According to Heller et al. (2019), forests provide a plethora of floral resources that support bee colonies and give bee products unique flavours and attributes. The reciprocal advantages of beekeeping and forests are highlighted by their entangled relationship. Pollination, carbon sequestration, and water management are only a few of the crucial ecological functions that trees offer (Bennett et al., 2009, Brockerhoff et al., 2017, Vargas-Hernández et al., 2023). Additionally, beekeeping's rich cultural heritage harmonises with forest customs, connecting human groups to the diversity of the natural world. However, forest degradation and deforestation pose immediate dangers to the delicate balance between forests, pollinators, and beekeepers. Pollination services necessary for forest regeneration and food production are put at risk when forest ecosystems are lost (Klein et al., 2007). As ecosystems disappear, the connection between beekeeping and forests faces difficulties (Gratzer et al., 2021, Jahan et al., 2021). Maintaining beekeeping practises and ecological balance depends on protecting trees and helping pollinator management techniques (Rader et al., 2016, Katumo et al., 2022, Ulyshen et al., 2023). Conservation activities protect forest ecosystems, enabling pollinators to find sanctuary beneath thriving canopies. For sound judgement and sustainable practises, it is essential to foster a knowledge of these complex interconnections. We can encourage the coexistence of forests, pollinators, and beekeeping through interdisciplinary collaboration, agricultural policies, and community involvement—a tribute to the harmonious choreography of nature.

1.7 Significance of this study

In light of the projected doubling of global demand for cocoa beans by 2050, how can we ensure environmental sustainability while striving to meet production demands?

The inherent validity of such a question is premised on the fact that deforestation and environmental contamination through pesticide use have been linked to cocoa cultivation. This thesis is therefore relevant on many fronts. It sets out to explore the interactions between pollinator health, sustainable agriculture, and societal aspirations. By meticulously evaluating the interactions between cocoa cultivation, pollinators, pesticides, bee products and land use dynamics, this research sought to provide practical insights with the potential to guide effective policies, practices, and interventions (Figure 1.9). Additionally, this study is of relevance to Ghana, where cocoa is an economic cornerstone, and on a global scale occupies a central position in the chocolate industry. Therefore, this study not only serves as a conservation endeavour for a pivotal habitat but also makes a substantial contribution to the worldwide discourse on sustainable agriculture, biodiversity preservation and human health. In summary, this thesis endeavours to show how human development should be in harmony with ecological preservation, making it of paramount importance in the broader context of environmental science and agriculture.

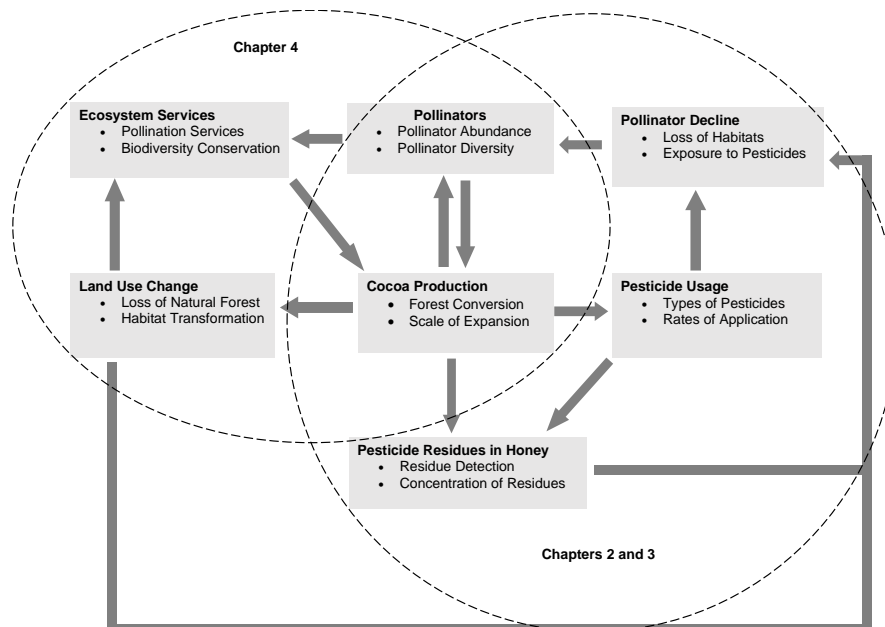


Figure 1.9 A conceptual framework illustrating the key concepts within the thesis and the linkages between them. Cocoa expansion drives extensive land use change, while pesticide usage safeguards yield but has direct effects on insect pollinators and leaves traces of pesticide residues in honey. Pollinators and their vulnerability (pollinator decline) play a pivotal role in the success of both cocoa farming and the delivery of broader ecosystem services.

1.7.1 Research objectives

This thesis aimed to investigate the impacts of cocoa expansion on insect pollinators, and the detection and concentration of pesticide residues in honey. The inquiry consists of three essential studies, each of which illuminates a distinct facet of this research as outlined below:

- i. Chapter 2: Honey contamination from plant protection products approved for cocoa (*Theobroma cacao*) cultivation: A systematic review of existing research and methods. Pesticides play a critical role in cocoa cultivation, but unintended environmental contamination is also likely (Tudi et al., 2021, Nehra et al., 2021, Rosenheim et al., 2020, Aminu and Edun, 2019). Chapter 2 therefore aimed to utilize the relationship between pesticide contamination of honey and pesticide use in the broader environment to evaluate the current knowledge of residues of pesticides used for cocoa cultivation in honey.
- ii. Chapter 3: Exploring pesticide residues in the environment using honey as a proxy: A comparative analysis of cocoa and forest landscapes in Ghana. Cocoa is highly susceptible to insect and disease infestation and therefore its cultivation thrives on pesticide cultivation (Ntiamoah and Afrane, 2008, N'Guessan et al., 2013). However, there is a dearth of information regarding the concentrations and rate of detections of pesticides imputed for cocoa cultivation on bee products such as honey produced in cocoa-producing landscapes. Chapter 3 therefore investigated the presence and concentrations of pesticide contaminants in honey produced in cocoa and forest landscapes in Ghana, the second-ranked cocoa-producing country in the world.
- iii. Chapter 4: Relationships between flower-visiting insects and forest cover in cocoa-growing landscapes in Ghana. Land use change has been found to be one of the leading causes of pollinator decline around the world (Potts et al., 2010). Cocoa cultivation has been one of the leading causes of deforestation with reported disappearance of between 7.5-10 million hectares of natural forest through cocoa cultivation (Wessel and Quist-Wessel, 2015, Adjaloo et al., 2012, Claus et al., 2018). Though land use change through cocoa expansion may affect insect pollinators and by extension, pollination services, this has rarely been assessed. Chapter 4 therefore explored how changes in

landscapes, particularly the loss of natural forests due to cocoa expansion influence flower-visiting insect communities in Ghana.

1.8 Conclusion

This chapter has provided the overarching theme and rationale for the thesis by emphasising the importance of insect pollinators in cocoa and forest landscapes and the need to protect their well-being. Key research gaps identified included the limited understanding of honey contamination by pesticides in cocoa-producing countries, the lack of comprehensive data on pesticide residue levels across diverse landscapes in cocoa landscapes, and the insufficient knowledge of how forest loss and changes in natural vegetation as a result of cocoa expansion impact pollinator diversity. Chapters 2 to 4 address these gaps by examining honey contamination by pesticides, investigating pesticide residue levels in different landscapes, and exploring the effects of forest loss on flower-visiting insects. Overall, this thesis set out to deepen our understanding of the delicate balance necessary for the coexistence of agriculture, environment, and biodiversity by addressing these key gaps.

Chapter 2 Honey contamination from plant protection products approved for cocoa (*Theobroma cacao*) cultivation: A systematic review of existing research and methods

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Chapter 2 Honey contamination from plant protection products approved for cocoa (*Theobroma cacao*) cultivation: A systematic review of existing research and methods

2.1 Abstract

The main component of chocolate, cocoa (*Theobroma cacao*), is a significant commercial agricultural plant that directly sustains the livelihoods of an estimated forty to fifty million people. The economies of many cocoa-producing nations, particularly those in the developing world, are supported by cocoa export revenue. To ensure satisfactory yields, however, the plant is usually intensely treated with pesticides because it is vulnerable to disease and pest attacks. Even though pesticides help protect the cocoa plant, unintended environmental contamination is also likely. Honey, produced from nectar obtained by honeybees from flowers while foraging, can serve as a good indicator for the level of pesticide residues and environmental pesticide build-up in landscapes. Here, we use a systematic literature review to quantify the extent of research on residues of pesticides used in cocoa cultivation in honey. In 81% of the 104 studies examined for this analysis, 169 distinct compounds were detected. Imidacloprid was the most frequently detected pesticide, making neonicotinoids the most frequently found class of pesticides overall. However, in cocoa-producing countries, organophosphates, organochlorines, and pyrethroids were the most frequently detected pesticides. Interestingly, only 19% of studies were carried out in cocoa-producing countries. We recommend prioritizing more research in the countries that produce cocoa to help understand the potential impact of pesticide residues linked with cocoa cultivation in honey and the environment more generally to inform better pesticide usage, human health, and environmental policies.

2.2 Introduction

Cocoa (*Theobroma cacao*) is an essential economic crop with widespread global demand and uses owing to its rich protein, carbohydrate, fat and vitamin content (Lima et al., 2011). Between forty and fifty million people are thought to rely on cocoa farming for a living (Fosu-Mensah et al., 2022, Menkeh, 2021). The annual global production

of 4.2 million metric tons of cocoa beans is estimated to have an economic value of over US\$ 11.8 billion (Beg et al., 2017). The cocoa confectionary market generates about US\$ 80 billion worldwide (Kongor et al., 2018), with West Africa as its primary production engine. However, the cocoa plant is susceptible (Ntiamoah and Afrane, 2008, N'Guessan et al., 2013) to attacks from the cocoa swollen shoot virus, beetles and capsids (miridae) and phytophthora pod rot (commonly called black pod) (Bateman, 2008, Afrane and Ntiamoah, 2011). This results in the loss of 20% to 30% of the cocoa produced worldwide (Adeniyi, 2019), valued at US\$ 3.16 billion (Jung et al., 2020).

To mitigate the impact of disease and pest pressure, (Zainudin et al., 2022) pesticides are widely used in cocoa cultivation (Aminu et al., 2019, Okoffo et al., 2016). For example, an estimated 125,000–130,000 metric tons of insecticides are applied for cocoa cultivation in Nigeria alone (Asogwa and Dongo, 2009). Concerns have been expressed regarding the potential environmental damage caused by pesticides, as well as implications for residues in food (Okoffo et al., 2017), considering that only 0.01% of applied pesticides are determined to reach their targets while the rest filters into the broader environmental ecosystem (Tudi et al., 2021, Llorent-Martínez et al., 2011, Nehra et al., 2021, Rosenheim et al., 2020).

Honey, created when stingless bees and honeybees collect nectar and/or other resources from flowers and plants (Saba et al., 2013, Khan et al., 2018), is used by bees as a nutritious food source (Saba et al., 2013, Bogdanov et al., 2008). While bees are foraging for nectar to make honey, they can also collect potential environmental contaminants, including pesticides (López et al., 2014). Because honey may reflect the chemical conditions of the environment, it can be used as a proxy to assess general ecosystem health (Chiesa et al., 2016, Hungerford et al., 2021). For example, honey and other bee products have been used to assess environmental contaminants, including heavy metals (Kalbande et al., 2008, Perugini et al., 2011), polycyclic aromatic hydrocarbons (Perugini et al., 2009) and pesticides (Mukiibi et al., 2021, Lekduhur et al., 2021).

Pesticide residues in honey can be related to their potential impact on human health using maximum residue limits (MRLs). An MRL (expressed as the milligram of residue

per kilogram of feed commodity) is the highest permissible pesticide residue recommended by the Codex Alimentarius Commission as legally accepted in food commodities and animal feeds (CA, 2022). When determining MRLs, the European Union, one of the world's largest agricultural product markets, considers Codex Alimentarius requirements and good agricultural practices (Son and Vang-Phu, 2021). The European Food Safety Authority (EFSA) calculates MRLs, assuring compliance with globally recognised assessment techniques (FAO, 2002). As a natural food produced by *Apis mellifera*, honey is deemed a food substance of animal origin under Directive 2001/110/EC and therefore needs to meet specified MRL requirements. Unfortunately, different national or regional bodies may set different upper pesticide residue concentration limits, which may lead to confusion in international markets (Malhat et al., 2015). Therefore, MRL harmonisation and standardization is essential.

This review utilizes the relationship between pesticide contamination of honey and pesticide use in the broader environment to evaluate the current knowledge of residues of pesticides used for cocoa cultivation in honey. Specifically, the study aimed to:

- Analyse the time-frame and geographic location of previous studies of honey contamination by pesticides permitted for cocoa cultivation.
- Investigate the extent to which various pesticide classes and varieties have been reported in cocoa-producing countries.
- Evaluate the potential impact of the pesticides reported for human health utilising the European Union maximum residue limits (MRL).

2.3 Materials and Methods

2.3.1 Pesticides utilised for cocoa cultivation

Seven peer-reviewed publications and one international report were identified that contain data on the approved pesticides for cocoa cultivation in four major cocoa-producing nations: Ghana, Nigeria, Cameroon and Ivory Coast, which account for 70% of the world's cocoa production (Wessel and Quist-Wessel, 2015). Using these publications, a pesticide list was compiled of the most important pesticides for cocoa, which included twenty-three insecticides, seventeen fungicides, and two herbicides approved for cocoa growing (Table 2.1).

Table 2.1 Summary of approved active ingredients for cocoa production. These pesticides are recommended for cocoa cultivation in major cocoa-producing countries in West Africa, which account for 70% of the World's cocoa.

Insecticides (active ingredients)	Fungicides	Herbicides	Sources
Acetaprimid Bifenthrin Capsaicin Chlorantraniliprole Chlorpyrifos Lambda-Cyhalothrin Alpha-Cypermethrin Cypermethrin Deltamethrin Dimethoate Etofenprox Fipronil Imidacloprid Indoxacarb Pirimiphosmethyl Promecarb Pyrethrum Sulfoxaflor Teflubenzuron Thiamethoxam	Benalaxyl Benomyl Copper (II) hydroxide Copper (I) hydroxide Copper (I) oxide Dicopper chloride trihydroxide Dimethomorph Fluazinam Maned Mancozeb Mefenoxam Metalaxyl Metalaxyl-M	Glyphosate Paraquat	(Mahob et al., 2014);(Bateman, 2010); (Boamah, 2020);(Ninsin and Adu-Acheampong, 2017); (Antwi-Agyakwa et al., 2015); (Afrane and Ntiamoah, 2011) (Oyekunle et al., 2017), (Mokwunye et al., 2014)

2.3.2 Formulation of search strings

A collection of set-specific search strings was created to systematically search the literature for studies evaluating Table 2.1 pesticides in honey. Each string included a term for honey, plus a list of some of the pesticides of interest and was divided into three: one for insecticides, one for fungicides and one for herbicides (See Appendix B1). To make the search string for the insecticides shorter for the search engine, it was further split into two parts.

2.3.3 Literature search

The search strings created were utilised to conduct searches through the Web of Science Core Collection, PubMed, and Scopus. An initial search was conducted on 12th October 2020, which resulted in 1,360 peer-reviewed studies from Web of Science and PubMed, while a further search was conducted in Scopus on 26th October 2020, which resulted in the retrieval of 524 studies. Books or book portions, theses, and grey literature were excluded (Heymans et al., 2019), as well as any study in languages other than English. After removing duplicates, 1,282 studies were screened based on titles and abstracts for the presence of pesticide active ingredient residues in honey, which produced 91 studies in total. One paper was inaccessible, so 90 studies progressed to quality review. The flow chart (PRISMA table) in Figure 2.1 based on (Moher et al., 2009), shows the procedure taken to arrive at the included studies at the start of this review. A supplementary data search was also carried out on 7th November 2022 for literature released between November 2020 and November 2022 to ensure more recent literature was also captured. The updated flow chart is shown in Appendix B2. After the initial list of 2,610 studies was processed as described above, an additional 23 studies satisfied the eligibility criteria for inclusion. Before text screening and quality assessment, the publication by (Sorokin and Ovcharenko, 2022) was omitted because it was inaccessible. This resulted in an additional 22 papers of the updated search progressing to the quality review stage.

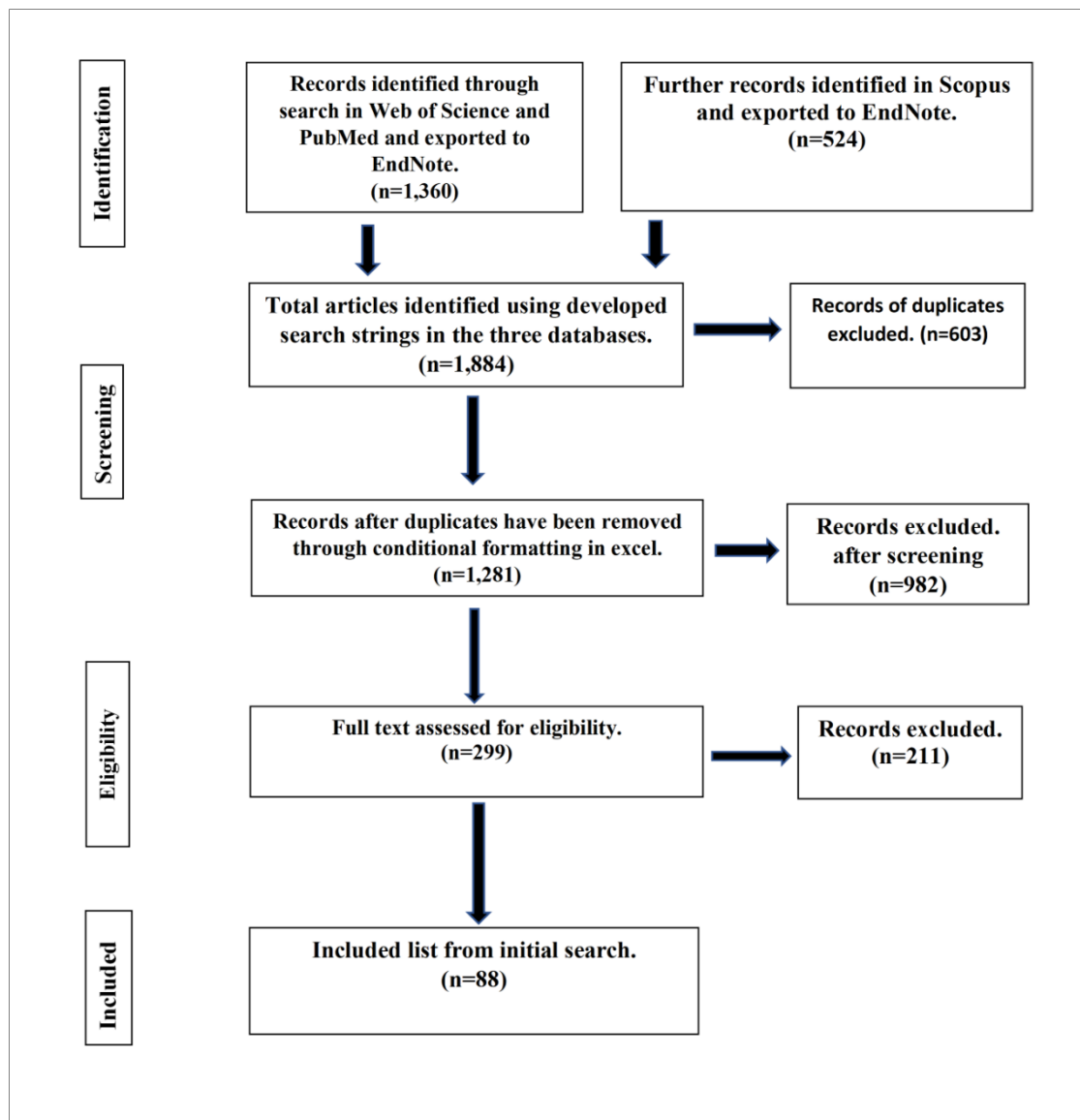


Figure 2.1 Procedure followed to select studies for inclusion in this systematic literature review. Numbers presented are from the first literature search in November 2020 only, while results from the supplementary search in November 2022 are given in Appendix B2. The "eligibility" box covers both the eligibility screen and quality assessment. Based on PRISMA flow chart (Moher et al., 2009).

2.3.4 Quality assessment

Overall, 112 studies from the previous and updated searches were proceeded to quality assessment, and applicability before data extraction (See Appendix B3). We applied a checklist of eleven customised questions (See Appendix B4) based on the

proposed checklists for evaluating quantitative studies by (Kmet et al., 2004) to each study. Two reviewers assessed each paper using a scoring methodology (Kmet et al., 2004). According to this grading scale, particular studies were given a score based on how much they met the criteria “yes”=2, “partial”=1, “no”=0, “NA”=not applicable). The reviewers' consensus on the total scores ranged from 45% to 100%. Eight studies were excluded based on quality assessment results. Three of these eight papers could not be attributed explicitly to one nation for examination. The other five did not measure the levels of pesticide residues in samples, instead using blank honey as a sample matrix to show the analytical method's reliability. One hundred and four studies were determined to have passed the review's quality assessment (See Appendix B2). Table 2.2 summarizes the categories of data extracted from each paper, including information on the publication year, study location, types of pesticides examined and found, and data extraction and analytical methods. The final dataset is presented in Appendix B5. A brief descriptive summary of the main findings of each study included in our systematic review was determined from information provided within each paper and collated for qualitative purposes (See Appendix B6).

Table 2.2 Type of data extracted from included articles. Based on this, a customised data extraction form was developed and used for data capture and processing.

Category	Sub-category
General Reference Information	Author lists and correspondent's contact details
	Title of paper
	Journal name/source
	Volume
	Issue
	Pages of journal
	Year of publication
	DOI
Method	Aim of study
	Study design
	Start date
	End date
	No of samples
	Weight of sample
	Volume analysed
	Extraction technique
	Analytical technique
Statistical analysis	

Geographical locations of the study	Country name
	Continent
	Country status
Pesticide studied	What pesticides were studied
	Were insecticides studied?
	Were fungicides studied?
	Were herbicides detected?
	Concentrations of pesticide residues detected
	Banned pesticides detected
Analytical parameters	Level of detection
	Level of quantification
	MRL of detected pesticides
The type of honey analysed	Unifloral
	Multi-floral
The source of honey analysed	Commercial honey only
	Commercial honey and honey directly harvested
	Honey harvested directly from the production base
Sample treatment	Heated
	Pasteurised
Season when the sample was taken	
Number of times/Seasons samples taken for analysis	Full season (multiple harvest)
	Part season (single harvest)
Matrices analysed	Honey only
	Honey and other matrices
	For multiple harvests: Did the concentration fluctuate between studies?
Honey treatment	Pasteurised
	Blended
Honey characteristics	Electrical conductivity
	% Water
	Colour
Highlights	Key results from the study
	Summary of abstract

Pesticides were recorded in three categories: insecticides, fungicides, and herbicides. Pesticides targeted in each study were recorded, and those detected were subsequently identified and their concentrations recorded. Their concentrations were then compared with the maximum residue limits (MRL) set by the European Union to determine those which exceeded the MRL. All units of concentrations of detected pesticides were converted to mg/kg before comparison. To evaluate the sensitivity of the analytical technique used in each investigation, LOQs applied were compared to compound MRLs.

2.4 Results

2.4.1 Geographical spread and period of study

The first study was published in 1997, but it was in 2015 that study publications began to increase, with 73% of studies taking place between 2015 and 2022 (Figure. 2.2). The geographical locations and the period within which the studies were conducted are documented in Appendix B5.

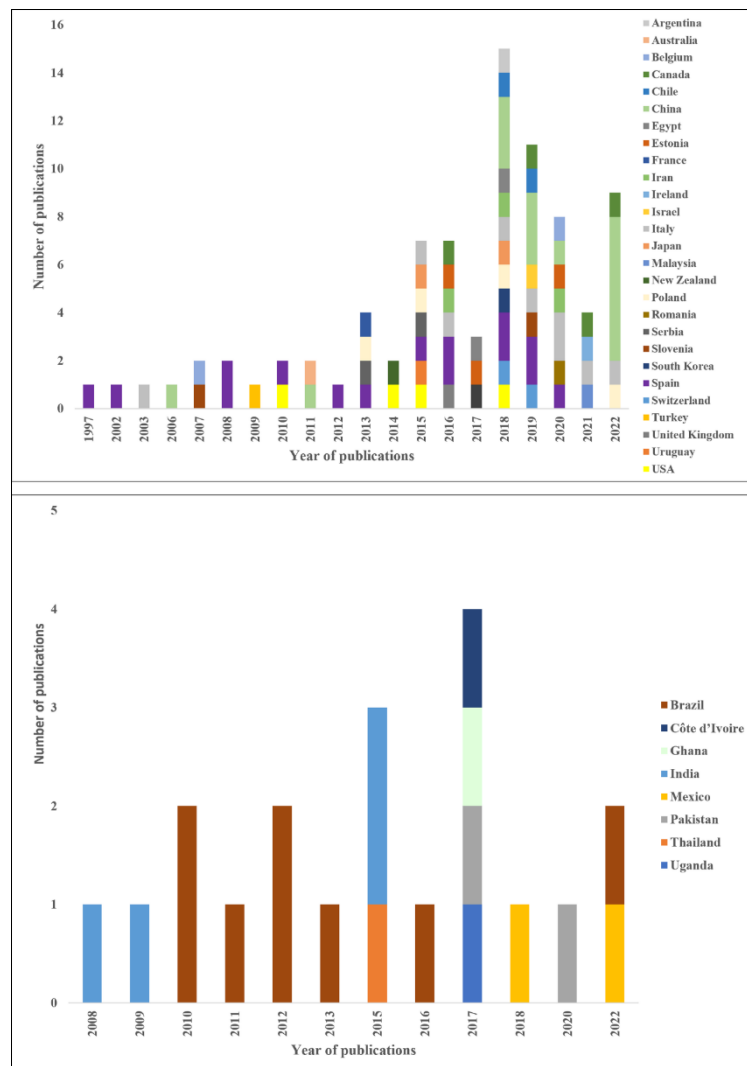


Figure 2.2 The year and country where existing studies were conducted. The studies were published over 25 years across 35 countries. Above) Non-cocoa growing countries where studies were conducted. Below) Cocoa-producing countries where studies took place. Overall, 18 studies were conducted in eight cocoa-growing countries.

Most studies took place in Asia (30%) and Europe (43%). One-third of the 47 studies, that were carried out in Europe, took place in Spain. Similarly, of the 31 studies conducted in Asia, 48% were conducted in China. Overall, 81% of the included studies were conducted in 27 countries where cocoa is not grown (Figure 2.3).

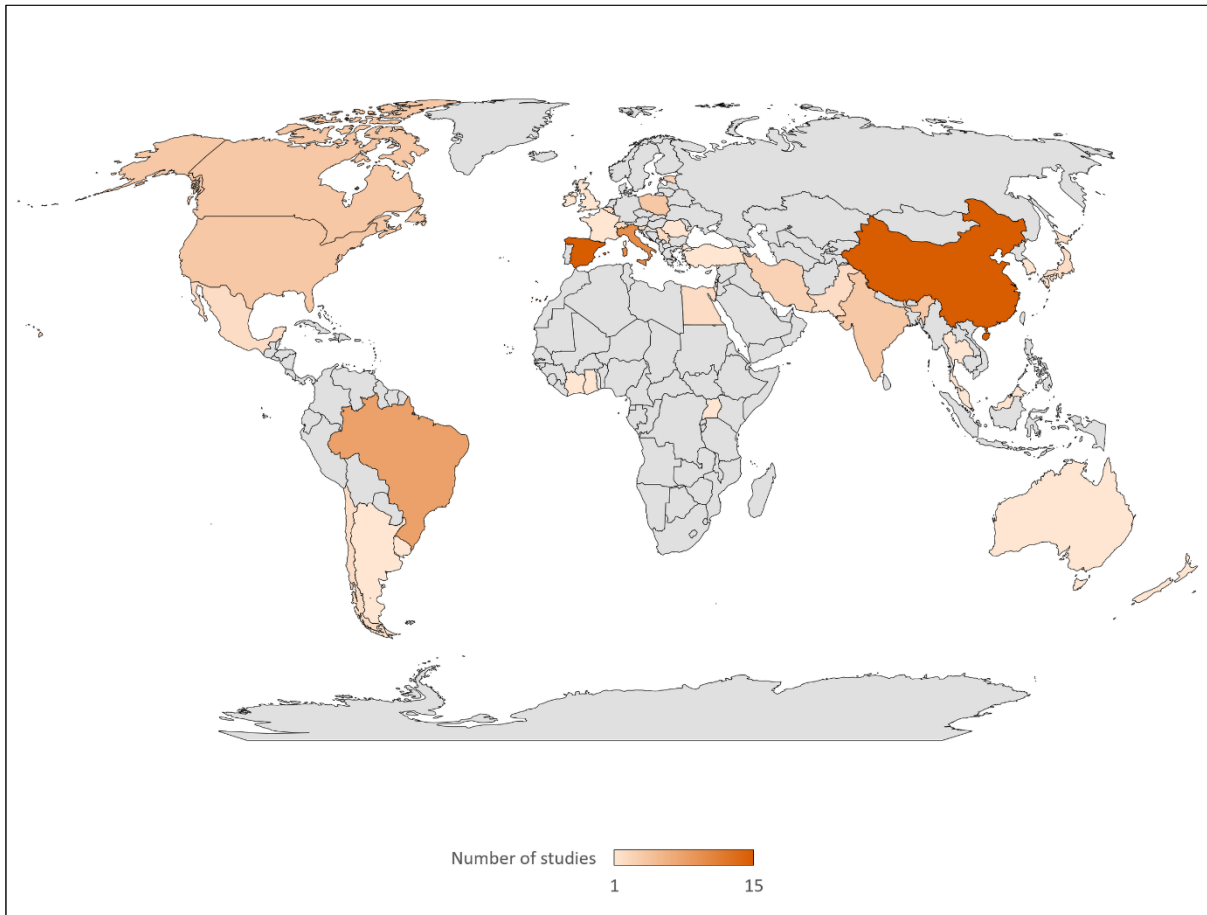


Figure 2.3 Geographical spread of the studies that were undertaken (grey areas=no studies). Spain (15 studies), China (15 studies) and Italy (10 studies) were the top three countries with most studies. One study each was conducted in Ivory Coast and Ghana, which are the first and second-ranked cocoa-producing countries in the world, respectively [Credit: (Geonames)].

There are 57 cocoa-producing countries globally (Figure 2.4), but studies were only conducted in eight. There is an uneven distribution of studies across these eight countries. Of the 20 studies conducted in cocoa-producing countries, 8 studies were carried out in Brazil, the sixth-highest cocoa producer in the world, accounting for 5% of the world's cocoa bean production (See Appendix A). Only one publication was

carried out in each of the Ivory Coast and Ghana, rated first and second with 39% and 17% of the yearly global cocoa production. In contrast, four studies were conducted in India, which accounts for less than 1% of global annual production. The other cocoa-producing countries where studies took place included Mexico (2 studies), Pakistan (2 studies), Thailand (1 research), and Uganda (1 study).

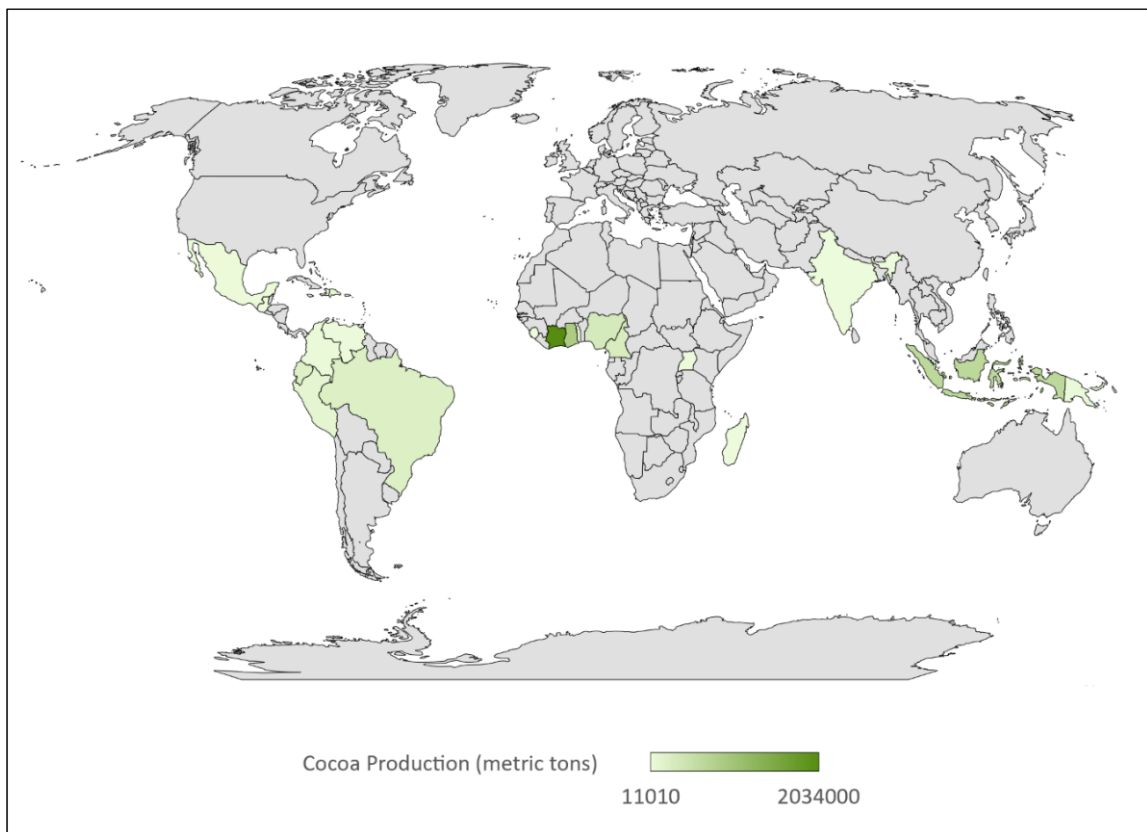


Figure 2.4 The 57 cocoa-producing countries in the world based on the metric tons of cocoa produced annually. Ivory Coast is ranked first with 2,034,000 metric tons of annual production. Nineteen per cent of the included studies evaluated in this review took place in eight cocoa-producing countries. Grey areas = non-cocoa producing areas. Map developed using (Geonames) and data sourced from (WPR, 2022).

2.4.2 The classes and types of pesticides evaluated

Among the classes of pesticides investigated, insecticides received the most research attention, having been examined in 91% of studies. Only four studies examined insecticides, fungicides, and herbicides simultaneously. Pesticide traces were found in 80% of publications included in this review, with a total of 169 different compounds

(comprising some of those recommended as well as those not approved for cocoa cultivation) detected in 86 studies, which took place in 30 of the 35 countries where studies were conducted (Figure 2.5).

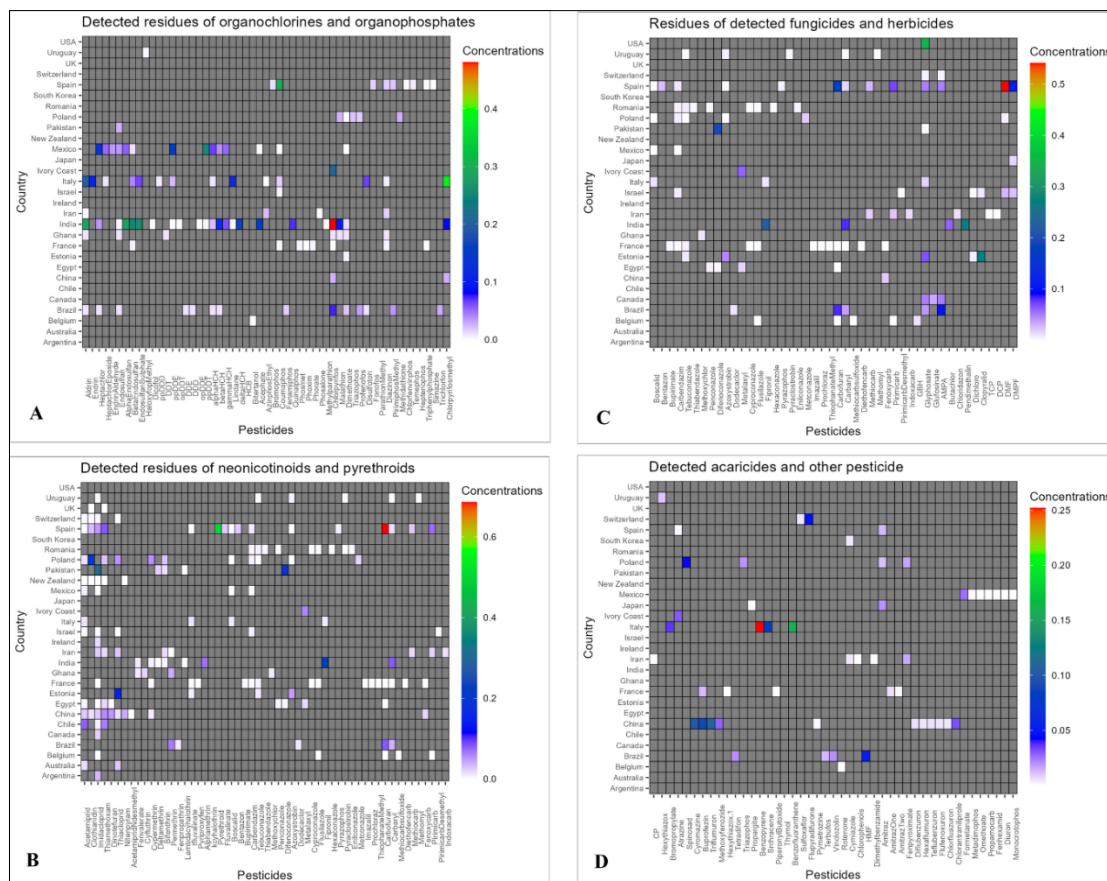


Figure 2.5 Heatmaps with colour scale on the right of each graph showing the detected: A) organochlorines and organophosphates B) neonicotinoids and pyrethroids; C) fungicides and herbicides, and D) acaricides other pesticides which were studied (x-axis) over the period and the respective countries where detections took place (y-axis). Concentrations of each detected pesticide were averaged per the number of detections per country to get one value for each pesticide detected. Units for all detected pesticides were standardised by converting to mg/kg. The individual graphs can be referred to in Appendix B7-B10.

Neonicotinoids were both the pesticide classes most investigated and with the greatest detections overall, with imidacloprid (detected in 20 studies), thiamethoxam (detected in 14 studies), acetamiprid (detected in 13 studies) and clothianidin (detected in 9 studies) being the more commonly detected neonicotinoids. Of the eight cocoa-producing countries, pesticides were only detected in 6 of these countries, with no

detections in Thailand or Uganda, and interestingly, the three most detected pesticide classes in the six cocoa-growing countries were organophosphates, organochlorines and pyrethroids in that order (Figure 2.6 & Appendix A). Eleven approved insecticides for cocoa cultivation, namely capsaicin, chlorantraniliprole, thiamethoxam, acetaprimid, etofenprox, indoxacarb, pirimiphosmethyl, promecarb, pyrethrum, sulfoxaflor, and teflubenzuron and one herbicide (i.e., paraquat) were not detected in any of the studies conducted in the cocoa growing countries. Additionally, our findings showed that only two of the 13 recommended fungicides for cocoa production, namely metalaxyl-M and its isomer metalaxyl (See Appendix B11) were detected in studies conducted in cocoa growing countries. Forty-nine pesticides were detected in studies undertaken in Brazil, Mexico, and India that are not suggested for use in the production of cocoa (Bateman, 2015). However, it should be remembered that whilst cocoa production occurs in these countries, it was not possible to uniformly ascertain whether the honey samples analysed in the studies were collected from cocoa-producing regions within these countries.

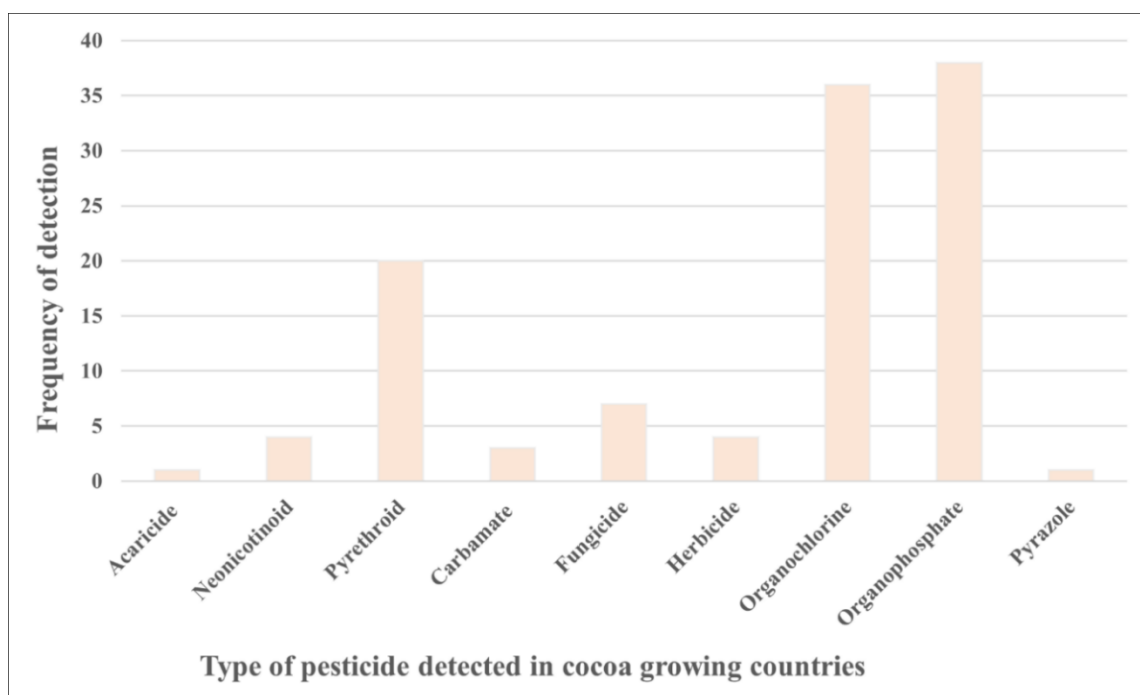


Figure 2.6 The frequency of detection of the different classes of pesticide residues in the 20 studies conducted in six cocoa growing countries: Ghana, Ivory Coast, Brazil, Mexico, India, and Pakistan. No pesticide residues were detected in studies conducted in Thailand and Uganda, the other two cocoa growing countries where studies took place.

Multiple studies were conducted in just over half (54%) of the thirty-five countries where studies took place. Fifteen studies were carried out in Spain and China. There was a far lower incidence of multiple studies in cocoa-producing countries, with only Brazil, India, and Mexico having more than one study conducted. Among the cocoa growing countries, Brazil was the only country where a pesticide (i.e., chlorpyrifos) was detected in different studies. Overall, only seven different pesticides were detected on multiple occasions in all the multiple studies conducted across all countries (See Appendix B12).

2.4.3 Banned pesticides detected

Various jurisdictions have made the use of certain pesticides illegal. Using the Stockholm Convention as the foundation for evaluating "banned" pesticides, 96% of the studies considered in this review did not detect any banned chemicals. Banned pesticides were detected only in three cocoa-producing countries (Ghana, India, and Mexico) and were predominantly organochlorines. One study from a non-cocoa-producing country, Spain, also confirmed the detection of banned pesticides. In the study conducted in Ghana, Dichlorodiphenyltrichloroethane (DDT), an organochlorine insecticide which is the first of the modern synthetic insecticide manufactured primarily to fight malaria, typhus and for agricultural uses (Turusov et al., 2002), was confirmed at 0.01 mg/kg concentration. In Mexico, (Ruiz-Toledo et al., 2018) confirmed the presence of 10 organochlorines, including heptachlor (0.13173 mg/kg); hexachlorocyclohexane (HCF, 0.654 mg/kg), endrin aldehyde (0.03564 mg/kg), and dichlorodiphenyldichloroethylene (DDE, 0.154358 mg/kg) in honey from the Chiapas vicinity where official approval for their usage was withdrawn in the 2000s, prior to the study being carried out. In India, hexachlorocyclohexane (HCH), which is used as an insecticide on fruits, vegetables, and forest crops, and its isomers, endosulfan and aldrin, were detected at concentrations of 0.0028 mg/kg, 0.00253 mg/kg, and 0.00201 mg/kg, respectively. However, it must be noted that DDT is still permitted in India for fumigation against mosquitoes as a malaria control tool, unlike the other three countries where it was detected (Coleman et al., 2015). One of the sixteen studies in Spain found DDE (0.09-0.6598 mg/kg), a metabolite of DDT, to be the only banned pesticide detected.

2.4.3 Exceedance of Maximum Residue Limits

In all, 12% of studies reported pesticide residue quantities in honey that exceeded the MRL established by the European Union (Table 2.3). These studies were conducted in ten different countries. EU MRLs are occasionally revised in light of additional scientific data becoming available to the European Food Safety Authority. During the period of this systematic review, these revisions resulted in an increase in MRL for certain specific pesticides and the 2022 MRLs are the primary focus here while the 2005 ones are also reported in Table 2.3. Among the cocoa-producing countries, MRLs exceedances occurred in Brazil, Ivory Coast, and India. However, in India, malathion levels only exceeded the MRL set by India; the concentration observed was lower than the EU's previous and current MRL. No exceedances of MRLs were detected in North America (4 studies) or Australia (1 study).

Table 2.3 Concentrations of pesticide residues that exceeded the maximum residue limits specified by the European Union by 2022. Those in bold denote pesticide residues that exceed the previous and revised MRLs. Asterisks denote concentrations that were thought to have exceeded the previous MRL established by the EU in 2005. These concentrations, however, are below the updated MRL that the EU has set as of 2022.

Pesticide exceeding MRL	Class of pesticide	Concentration mg/kg	Current MRL (mg/kg)	Previous MRL mg/kg)	Country	Author
Bifenthrin	Pyrethroids	0.0145 *	0.05	0.01	Poland	(Barganska et al., 2013) (Barganska et al., 2013)
Fenpyroximate	acaricides	0.0163*	0.05	0.01		
Methidathion	Organophosphate	0.0257 *	0.05	0.02		
Spinosad	Spinosyns	0.0206*	0.05	0.01		
Thiamethoxam	Neonicotinoid	0.0202 *	0.05	0.01		
Triazophos	Organophosphate	0.0203 *	0.05	0.01		
Azoxystrobin	Herbicides	0.031 *	0.05	0.01	Estonia	(Karise et al., 2017)
Imidacloprid	Neonicotinoid	0.55	0.05	0.05	Pakistan	(Farooqi et al., 2017)
Endosulfan	Organochlorine	0.26	0.01	0.01		
Imidacloprid	Neonicotinoid	0.736	0.05	0.05	Pakistan	(Yaqub et al., 2020)
Difenoconazole	Fungicide	0.386	0.05	0.05		
Bifenthrin	Pyrethroids	15.76	0.05	0.01		

Glyphosate	Herbicides	2.04	0.05	0.05		
Trichlorfon	Organophosphate	0.029	0.01	-	Brazil	(Tette et al., 2016)
Bifenthrin	Pyrethroids	0.0172*	0.05	0.01	Iran	(Mousavi et al., 2020)
Fenpyroximate	Acaricides	0.0154*	0.05	0.01		
Thiamethoxam	Neonicotinoids	0.0183*	0.05	0.01		
Tau-flavulinat	Pyrethroids	0.014*	0.05	0.01	Italy	(Notardonato et al., 2016)
Bromopropylate	Acaricide	0.036	0.01	0.01		
Coumaphos	Organochlorine	0.036	0.01	0.1	Spain	(Lozano et al., 2019)
Dimethylformamide (DMF)	Amitraz	0.541	0.2	0.05		
DMPF	Amitraz	0.107*	0.2	0.05		
Metalaxyl	Fungicide	0.06	0.05	0.05	Ivory Coast	(Ohoueu et al., 2017)
Chlorpyrifos	Metalaxyl	0.208	0.01	0.05		
Atrazine	Herbicide	0.03	0.5	-		
Glyphosate	Herbicides	0.22	0.05	0.05	Brazil	(de Souza et al., 2021)

2.4.4 The types of honey investigated in existing studies

Honey can be obtained in two forms; raw straight from the hive (Aumeeruddy et al., 2019) or processed commercially, including heating and cooling to lower moisture content (Anupama et al., 2003). Except for one study done in Spain, where the source of the honey analysed was not indicated, both commercial kinds of honey (35 studies) and raw honey (61 studies) were analysed in the included studies, with both being analysed simultaneously in 7%. Although it has been established that heating tends to decrease honey quality with the potential to degrade pesticide residues (Tosi et al., 2004), it was not possible to assess how this may have affected the levels of pesticides because information on honey's prior heating or pasteurization was not frequently recorded for in-depth analysis. None of the studies included in our study indicated whether they used blended honey.

2.4.5 Honey sampling rate within studies

Most of the research analysed pesticide residues in honey sampled only once. Only 11 studies repeatedly collected and examined honey samples for pesticide contamination, all using raw honey, except in one study conducted in Uganda, where

commercial honey was used. In eight of these studies, honey samples were gathered and examined over two years or several months within a single year. A unique study analysed honey samples continuously for nine years in Estonia (Laaniste et al., 2016). No trends emerged in studies where honey samples were collected and examined multiple times. No pesticide residues were detected in repeated studies conducted in Uganda (Amulen et al., 2017) and Spain Garcia-Chao et al. (2010). In contrast, in two independent studies conducted in Chile, while no pesticide residues were detected in one study (Mejias et al., 2019), acetamiprid, thiamethoxam, thiacloprid and imidacloprid were confirmed in three honey samples in the other (Bridi et al., 2018). In a study conducted in France, where samples were taken from apiaries in the spring, autumn, and early and late summer, contamination was higher in samples taken in the early spring (Lambert et al., 2013). In a study conducted in Egypt, acetamiprid and imidacloprid were found in honey samples tested in the spring (during the clover season) and summer (during the cotton season) (Codling et al., 2018). One study in Estonia in 2013 found that the amounts of clopyralid and glyphosate were greater than their designated MRL. However, MRL was not exceeded in different studies conducted in Estonia in 2013 and 2014, where honey samples were taken and analysed for two years (Karise et al., 2017). Frequent pesticide residue detections were found over a nine-year investigation in Estonia that began in 2004 (Laaniste et al., 2016). In a different study, glyphosate was examined in honey samples collected in 2015 and 2016 from two distinct locations in the USA and at both locations, its concentration increased in the 2016 samples (Berg et al., 2018).

2.4.6 Limit of detection and limit of quantification applied in studies

The limit of quantification (LOQ) is the smallest chemical concentration that can be successfully quantified (EFSA, 2022, Boneva et al., 2021). The limit of detection (LOD) is the smallest concentration that can be successfully detected (Saadati et al., 2013, EFSA, 2022). The LOD and LOQ used for pesticide analysis in each study were assessed (Table 2.1 & Appendix B6). The analysis methods employed in 77% of the included studies resulted in LOQs that fell below the designated EU MRLs for the investigated substances. In these studies, therefore, it was possible to evaluate if the pesticide concentrations detected exceeded the MRL. There was insufficient

information in seventeen other cases to determine the LOQ employed. Three of the LOQs that were explicitly mentioned were found to be higher than the MRL. For these 20 studies, therefore, it was not possible to conclude that the absence of a pesticide being detected correlated with these pesticide concentrations not exceeding the relevant MRL.

2.5 Discussion

Honey is beneficial to humankind for its nutritive values and as a medium for monitoring environmental quality by assessing its contents for environmental contaminants. In this present study, we undertook a systematic literature review to evaluate honey contamination from plant protection products recommended for cultivating cocoa (*Theobroma cacao L.*), a crop highly dependent on pesticides for cultivation because of its vulnerability to insect and disease attacks.

Our findings demonstrate a steady but low level of analysis of pesticide residues in honey from 1997, with peak reporting periods beginning in 2015. Similar findings were reported by Zioga et al. (2020), who examined the presence of plant protection product residues in plant pollen and nectar, two sources of raw honey. Most of the research they examined was published in 2015, corresponding with the time we observed a sharp increase in studies investigating pesticide residues in honey. The increased growth in studies after 2014 coincided with when the EU placed a moratorium on using some neonicotinoids, namely clothianidin, imidacloprid and thiamethoxam (Goulson, 2013b, EC, 2022). Our findings also indicated that most studies conducted in countries where cocoa is grown occurred around this time. It is possible that the sharp growth in studies could be in response to the reported bee deaths due to the pervasive use of pesticides (Bonmatin et al., 2005) and the reported worldwide decline of pollinators (Potts et al., 2010). Notably, the outdoor use of three neonicotinoids - clothianidin, thiamethoxam, and imidacloprid - was made illegal in 2018 in Europe (Goulson and signatories, 2018, Butler, 2018). Studies increased dramatically again in 2022, with the majority in China (Figure 2.2).

The study's most striking finding is that 19% of studies were conducted in cocoa-producing nations, mostly developing countries. This result correlated with the findings

that most studies on the impacts of herbicides and fungicides on bees were conducted in North America, Europe and Russia (Cullen et al., 2019). A similar trend was observed in a study conducted by Zioga et al. (2020) which evaluated plant protection products in pollen and nectar. Cocoa thrives in hot and humid climatic conditions and tends to flourish in areas around West Africa, East Asia and South America (Fowler and Coutel, 2017). Accordingly, most cocoa-producing countries are located outside North America and Europe. Our finding highlights a dearth of knowledge of the environmental impact of pesticides imputed for cocoa cultivation. Considering honey as a proxy for such assessments, the paucity of knowledge may restrict a better or more detailed environmental impact assessment. Presently, cocoa production levels do not meet demand in several parts of the world, such as China and India (Squicciarini and Swinnen, 2016), and there is currently an increased 2.5% yearly demand for cocoa beans around the world (ICCO, 2008). This is likely to translate into increased cocoa production with a corresponding increased pesticide use to control disease and insect pests. Prioritising evaluation or studies of honey contamination from pesticide application in cocoa growing areas may help reveal the extent to which honey is impacted by pesticides applied for cocoa cultivation and, by extension, the extent to which these compounds are detectable in these regions.

Neonicotinoids were the most detected pesticide substances evaluated. These results support claims elsewhere by Silici et al. (2013), Bonmatin et al. (2015) and (Simon-Delso et al., 2015) that neonicotinoids are the most widely used class of pesticides globally. Neonicotinoids can persist in woody plants for over 365 days with reported half-lives of over 1000 days (Bonmatin et al., 2015). Therefore, their detection is possible even many months or years after application. Moreover, since neonicotinoids were developed in the 1980s to replace the more persistent organochlorines in the environment (Laurino et al., 2011, Simon-Delso et al., 2015), they have been in great demand (Bonmatin et al., 2003). Therefore, it was not surprising that imidacloprid, which along with clothianidin is observed to be highly persistent under certain conditions, was the chemical frequently found in this study. This correlates with the findings of Mitchell et al. (2017) and Kavanagh et al. (2021). Additionally, as of 2009, imidacloprid had sales of US\$ 1091 million, making it the insecticide with the biggest global market share (Jeschke et al., 2011). It is approved for 140 crops, including several crop types such as vegetables, citrus, corn, and oilseed rape pome, among

several others, in about 120 countries (Jeschke et al., 2011, Kleinschmit and Lilliston, 2015). It is therefore not surprising that imidacloprid was detected most frequently in our included studies (Poletti et al., 2007, Chang et al., 2018).

Even though neonicotinoids were the most detected class of pesticide residues across all research in this study, our findings show that the top three most frequently detected classes of pesticides in the six cocoa-growing countries were organophosphates, organochlorines, and pyrethroids, in that order (Figure 2.6 & Appendix B11). Among the plausible reasons for this finding are that these pesticides are inexpensive and easily accessible and are, therefore, frequently used in developing countries where most cocoa-producing countries are located (Hernik et al., 2014, Fu et al., 2022, Keswani et al., 2022). From our study, we can confirm that 60 pesticides, which are largely not approved for cocoa cultivation (Bateman, 2015), were detected in studies conducted in cocoa-producing countries, though again, it should be noted that it was not possible to uniformly ascertain if the honey collected came from cocoa-producing areas within these countries. Many of these pesticides were found in Mexico and India, for which cocoa production is not the dominant agricultural crop. Implementing laws and regulations governing the use of pesticides in developing countries continues to be a challenge. The ban on using OCPs in developed countries has witnessed remarkable success (Hernik et al., 2014). Still, the same may not be vouched for developing countries where pesticides are highly valued as a means of breaking into the global market of food production (Ecobichon, 2001). Organophosphorus pesticides (OPPs) continue to be widely applied in developing countries due to their ability to inhibit disease attacks and enhance productivity (Fu et al., 2022).

Three distinct studies conducted in three different nations—Ghana, Mexico, and India—detected pesticides designated as illegal under the Stockholm Convention. However, it is noteworthy that pollutants of organochlorine (OC) derivatives, such as PCBs, DDT, and a number of other pesticides no longer approved for use, have been found to persist in the environment (Dron et al., 2022). It was beyond the scope of this work to determine whether the detected illegal pesticides were administered recently or were present in past applications. Even though research findings by (Bayoumi, 2022) point to the continued use of substantial amounts of banned chemical pesticides in developing countries, it must also be recognised that in some countries

such as India, DDT, which has received a worldwide ban, is still approved for use against mosquitoes in controlling malaria (Coleman et al., 2015). This may explain the frequent detection of DDT and its various derivatives in research conducted in India (See Appendix B7-B10).

In the present study, we found that 12% of included studies detected pesticides whose concentrations exceeded allowable limits required for human consumption (Table 2.3), one-quarter of which occurred in three cocoa-producing countries. One further important observation from our study shows that some detected pesticide residues that previously exceeded specified MRLs set by the EU at the time of the study are presently below the revised MRLs that have since been implemented in the EU. This is significant as it implies that products previously deemed to pose a risk to human health would now be assessed as not posing an unacceptable risk. It should also be considered that while the revision of MRL can impact the assessment of honey as a food product, it does not alter its relevance as an indicator for the assessment of pesticide contamination in the surroundings of the hive location. The finding of pesticides exceeding MRL is significant in at least two major respects. Human exposure to levels of pesticides exceeding MRL can cause many health-related problems. The consumption of unacceptable levels of pesticides via food is known to have many acute and chronic health implications (Zikankuba et al., 2019). Exploration of the causes of exceedances of MRLs is beyond the scope of this work. Nonetheless, it should be noted that as only 0.01% of applied pesticides reach their target, with the rest entering into the general ecosystem, the exceedance of MRLs should serve as a warning for the potential impact of these compounds on the surrounding environment (Tudi et al., 2021, Llorent-Martínez et al., 2011).

The LOD and LOQ employed in the bulk of the studies under review were often lower than the MRLs set for honey by the EU, which range from 0.05 mg/kg to 0.2 mg/kg (Vichapong et al., 2021). This finding suggests that studies included in this review largely applied analytical methods with sufficient sensitivity to allow the potential health implications of pesticide detections to be evaluated. It must, however, be noted that the LODs for the three studies were not suitable for detecting pesticide residues below EU MRLs, compromising the extent to which their results could be considered within this study. In particular, even though no pesticide residues were found in one Brazilian

study by Bezerra et al. (2010), their reported LOQs mean that the study's reports of no pesticides detected cannot expressly be interpreted to mean that no pesticides were present at concentrations that could cause harm. In the studies by Sampaio et al. (2012) and Cesnik et al. (2019), the LOQs attained for the method were at concentrations so high that their results cannot be construed to suggest that the pesticides detected were the only ones that were of concern.

Even though the scope of this study did not extend to assessing the effects of pesticides on bees, the high frequency of detection of neonicotinoids in honeys as observed in our study suggests there is a potential risk that bees could be impacted by neonicotinoids through exposure during foraging. In our study, concentrations of 0.736 mg/kg of imidacloprid, (Farooqi et al., 2017); 0.0274 mg/kg of thiacloprid (Tanner and Czerwenka, 2011) and 0.0202 mg/kg of thiamethoxam (Bargańska et al., 2013) were confirmed in Pakistan and Poland respectively. Although these concentrations are below the known LD₅₀ for these compounds for bees (Kumar et al., 2020), they are within the range of concentrations shown to induce sub-lethal effects. For instance, Straub et al. (2021) confirmed that the survival of honeybees was reduced by 51% when exposed to 0.0043 mg/kg and 0.0011 mg/kg concentrations of thiamethoxam and clothianidin respectively. Brood development was stunted when honey bees were exposed to field-realistic concentrations of thiamethoxam (0.2 mg/kg) and clothianidin (0.001 and 0.01 mg/kg) (Williams et al., 2015). Bumble bees were found to experience reduced learning capability, and have changes in foraging and homing success when exposed to field-realistic levels of up to 0.0024 mg/kg of thiamethoxam (Stanley et al., 2015a), which is a 10-fold lower concentration than what was detected by Bargańska et al. in Poland. Therefore, the possibility of sub-lethal effects of the detected pesticide residues on honeybees should not be ruled out.

It was observed that pesticide residues were detected in 80% of commercial and raw honey analysed in the included studies. This discovery is consistent with the findings of Mitchell et al. (2017), who, in a global study of neonicotinoids in honey, verified the presence of neonicotinoids in 75% of 198 honey obtained directly from producers. However, the most striking observation made in our study was that 90% of studies that analysed raw honey confirmed the presence of pesticide residues. This was higher than previous findings by Mitchell et al. (2017) evaluating raw honey. However, it

should be noted that our findings were not confined to neonicotinoids. Our finding highlights the frequent occurrences of pesticides in the general environment. Raw honey from a broad spectrum of natural and agricultural landscapes were assessed in the studies of interest in this review. These included raw honey from agricultural farmlands within forest belts in Ghana (Darko et al., 2017), apiaries located within 2 miles of an oilseed (Jones and Turnbull, 2016), various agroclimatic zones (Khan et al., 2004), agricultural landscapes with mostly intensively managed fields, forested areas and human settlements (Karise et al., 2017), unifloral and multifloral sources (Mejias et al., 2019) among several others. In the present study, a very small number of studies evaluated the floral background of the honey. Therefore, it was not possible to correlate pesticide contamination to any specific floral resources.

2.6 Conclusion

The current knowledge of studies of honey contamination from pesticides approved for cocoa cultivation has been evaluated through a systematic literature review. The studies conducted to date have been disproportionately focused on non-cocoa growing countries, leaving a huge gap in knowledge of what residues of pesticides approved for cocoa cultivation are found in honey and, by proxy, how prevalent these pesticides are in the environment in cocoa growing areas. Future research should therefore prioritize cocoa-producing nations, particularly the top producers, Ghana, and Ivory Coast, who together produce 70% of the world's cocoa. Continuous monitoring and rigorous adherence to pesticide application regulations are crucial in cocoa production to ensure pesticide residues are kept below harmful levels. Using analytical techniques with appropriate sensitivity, stakeholders can ensure that residue levels can be evaluated using MRLs to minimise potential negative impacts. Outcomes from these studies could contribute to policy formulation of pesticide usage, human health, and sustainable beekeeping, especially in cocoa production landscapes.

2.7 Acknowledgements

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Chapter 3 Exploring pesticide residues in the environment using honey as a proxy: A comparative analysis of cocoa and forest landscapes in Ghana

3.1 Abstract

Cocoa (*Theobroma cacao*) cultivation contributes immensely to the economic growth of several Western African countries. However, the plant's susceptibility to pest and disease infestations tends to lead to extensive pesticide use for protection. Nonetheless, the extent to which these pesticides occur within the environment in these regions has rarely received attention in research. Through this study, we examined for the first time, residues of approved pesticides for cocoa cultivation present in honey sourced from natural (forest), and agricultural (both agroforest cocoa and fullsun cocoa) landscapes in Ghana. By analysing the presence and concentration of pesticide residues in honey, the study intended to shed light on the extent to which these compounds permeate the environment at the landscape scale. Pesticide residues were found in honey from all the landscape categories studied. Even though eight pesticides were investigated, only six pesticides namely acetamiprid, indoxacarb, sulfoxaflor, thiamethoxam, difenoconazole, and imidacloprid were detected. Overall, the agroforest cocoa honey had the highest number of pesticide types detected, while forested landscapes had the lowest, but differences among landscapes were not significant. Intriguingly, imidacloprid was universally present, and pesticide residues were even detected in honey samples from forested landscapes 2 km away from cocoa plantations, implying long-range pesticide transport. With the exception of difenoconazole, the remaining discovered pesticide residues were successfully quantified, and all concentrations were below the EU Maximum Residue Limits (MRLs), indicating they were safe for human consumption. However, the measured residual concentrations, which ranged from 0.003 mg/kg to 0.051 mg/kg, are within the range that has been shown to have sub-lethal consequences for honeybees. Our study provides insights into the extent to which pesticides applied for cocoa production are detectable in the surrounding landscapes thus contributing to regulatory measures, sustainable agriculture, and informed beekeeping strategies.

3.2 Introduction

Human-induced activities can generate contaminants in the environment which may pose risks to human health and other forms of life. Agricultural activities have become one of the leading causes of pollution through the use of pesticides, especially in many developing countries (Vasco et al., 2021). Natural forests present in many of these countries have been determined to be a hub of food resources for biodiversity, as well as providing sanctuary from the impact of plant protection products (PPPs), given their inherent potential to buffer the adverse effects of pesticides (Park et al., 2015). Indeed, recent research confirms that pesticides were less detected in pollen from forests compared to pasture, agriculture or urban landscapes (Fulton et al., 2019).

Often, the quantity of contaminants emanating from anthropogenic activities may exceed the homeostatic capability of the environment to cleanse itself (Bargańska et al., 2016, Bashir et al., 2020). The continuous and timely monitoring of environmental quality ensures food safety, the protection of human health and the health of the ecosystem more generally (Cunningham et al., 2022, Panseri et al., 2020). Honey is deemed a suitable sentinel biomonitor for the environment owing to its ability to reveal contaminants (Al-Alam et al., 2017, Cunningham et al., 2022). As a product generally produced alongside other agricultural activities (Tette et al., 2016), an evaluation of pesticide residues in honey can give an idea about the extent and types of pesticides applied in environments surrounding hives (Kumar et al., 2018). This is because, during foraging to collect pollen and nectar from crops, honeybees encounter many PPPs (Migdał et al., 2018, Xiao et al., 2022, Bogdanov, 2006) as they usually cannot discriminate between treated and untreated forage sources (Karise et al., 2007, Zawislak et al., 2019, Cunningham et al., 2022). Additionally, potential exposure of bees to pesticides may also occur from co-flowering wild non-target plants (Russo et al., 2020). As such, bees can end up transporting applied pesticides in pollen and nectar into their hives which may contaminate the honey and other hive matrices (Al-Waili et al., 2012, Sadowska et al., 2019, El-Nahhal, 2020). The exposure of honeybees to applied pesticides and resulting contamination of hive products could have sub-lethal implications for bees, such as on behaviour or reproduction (Stanley et al., 2015, Straub et al., 2016). It should, however, also be acknowledged that some pesticides may be more likely to find their way into nectar and pollen than others due

to various factors, contributing to their presence in hive products. Even so, the analysis of hive products remains a meaningful method to assess pesticide contamination within the vicinity of the hive and has been used as such in previous work (Gierer et al., 2019, Murcia-Morales et al., 2022, Xiao et al., 2022). As honeybees can forage beyond 2 km from the location of their hives (Garbuzov et al., 2015, Visscher et al., 1996, Beekman and Ratnieks, 2000), what they collect, and therefore the profile of pesticide residues in honey, may be indicative of pesticide residues found within the wider landscape.

One of the most important crops in many developing countries is cocoa (*Theobroma cacao L.*) (Boakye et al., 2023). Cocoa is rated among the 13 globally essential commercial commodities (Claus et al., 2018) and contributes immensely towards the economic growth of several Western African countries (Duguma et al., 2001, Enu, 2014, Coulibaly and Erbao, 2019), but is highly susceptible to insect and disease attacks (Ntiamoah and Afrane, 2008, N'Guessan et al., 2013). Globally, an estimated 20% to 30% of cocoa produced (Adeniyi, 2019) valued at 3.16 billion dollars (Jung et al., 2020), is lost to pests and disease. PPPs are therefore routinely applied for cocoa cultivation to prevent losses (Okoffo et al., 2016, Aminu et al., 2019). For instance in Ghana, the second-highest cocoa-producing country in the world (Bangmarigu and Qineti, 2018), the government initiated a nationwide spraying program in 2001 which was aimed at protecting cocoa plants from black pod disease and cocoa pests (Abankwah et al., 2010). The program, formally named the National Cocoa Disease and Pests Control (CODAPEC) and popularly called Cocoa Mass Spraying Program (Gyimah, 2019), was started as a remediation measure because cocoa farmers found it difficult to bear the high cost of pesticides and maintenance of cocoa farms (Abankwah et al., 2010). Even though this has resulted in increased yields (Oduro and Omane-Adjepong, 2012), potential unintended effects in the general ecosystem (Fenner et al., 2013, Bernardes et al., 2015) are also a possibility.

The European Union has set maximum residue limits (MRLs) for many pesticides applied for crop production in foodstuffs (including honey) through Regulation (EC) No 396/2005 to guarantee safe human consumption. In Ghana, efforts have been made to evaluate pesticide residues in some food matrices such as maize and cowpea (Akoto et al., 2013), fermented dried cocoa beans (Okoffo et al., 2017), fruit and

vegetables (Bempah et al., 2011), cocoa beans (Frimpong et al., 2012b), cabbage (Amoako et al., 2010), fish (Darko et al., 2008) and tomatoes (Essumang et al., 2008). However, even though beekeeping is being promoted in Ghana as an additional source of income for many poor farmers in many forest belts, cocoa-growing areas and around some protected areas, limited study has been conducted to establish the status of pesticide residues in honey produced in Ghana (Darko et al., 2017). Currently, there exists a dearth of information regarding the presence of pesticides imputed for cocoa cultivation on bee products such as honey produced in cocoa-producing landscapes in Ghana.

The aim of this study was therefore to:

- i) evaluate the presence and concentrations of eight approved and widely used pesticides (acetamiprid, bifenthrin, difenoconazole, fipronil, imidacloprid, indoxacarb, sulfoxaflor and thiamethoxam) for cocoa cultivation in honey in Ghana and
- ii) determine if the presence and number of detected and quantified pesticides (pesticidal loads) could be related to the landscape type where honey samples were collected.

3.3 Materials and Methods

3.3.1 Study area

This study took place in Bia Conservation Area (BCA), Bia North Forest Reserves (BNFR), and cocoa landscapes within the Bia West District (BWD) in the Western North Region of Ghana (Figure 3.1). BWD (1,287 km², 6°6' N, -3°1'W) is one of the leading cocoa-producing districts in Ghana (West, 2022). Agriculture is the mainstay of the economy of the district with cocoa as the foremost cultivated crop. An estimated 75% of the 88,204 populace are engaged in cocoa enterprise either as land/farm owners, tenants or farm labourers (West, 2022, GSS, 2014). Beekeeping has been promoted by the Ghana Forestry Commission and other organizations to enhance livelihoods and alleviate poverty for residents near the Bia Conservation Area.

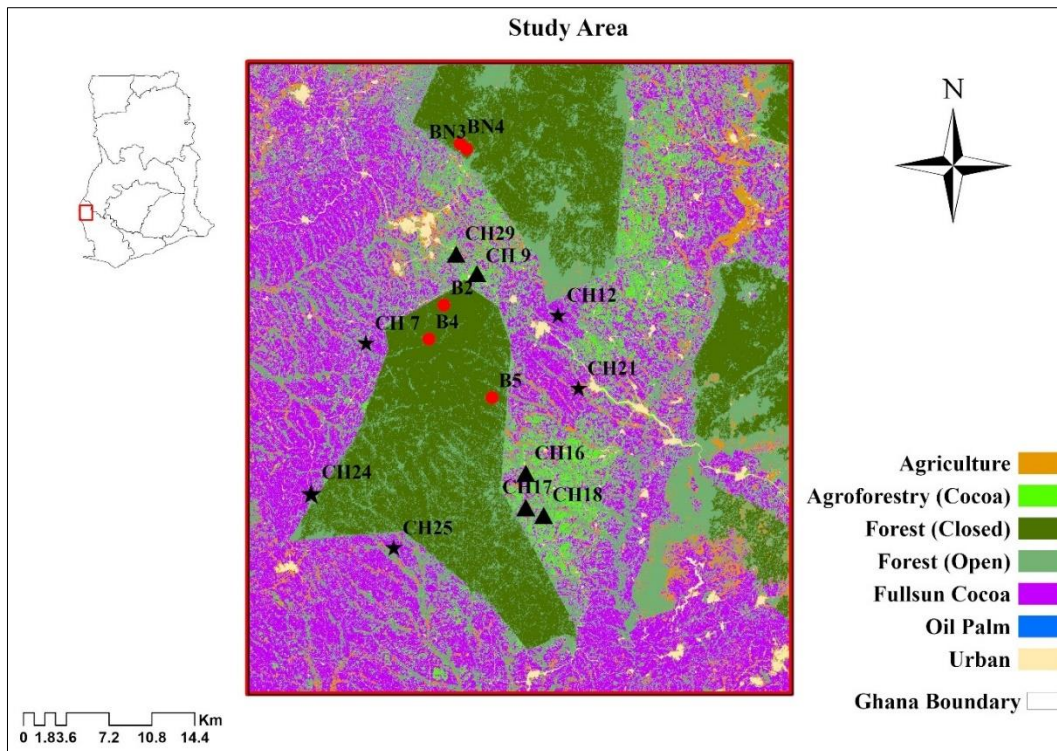


Figure 3.1 Locations of sampled beehives (five per landscape type). ● = beehives located in protected areas representing natural vegetation (NV) landscapes; ★ = beehives located in fullsun cocoa (FC) landscapes representing intensive agricultural use and ▲ = beehives located in agroforest cocoa (AC) landscapes representing extensive agricultural use. See Appendix C1 for detailed geographic information.

3.3.2 Study design and site selection

The sampled beehives were located in landscapes that represented three different landscape intensities: (1) natural forest landscapes where pesticide application is prohibited, (2) landscapes with dominant fullsun cocoa monoculture (intensive agriculture) and (3) landscapes dominated by agroforest cocoa where cocoa is integrated with shade trees (extensive agriculture) (Figure 3.1 & Appendix C2). Five independent landscapes were selected to represent each landscape type with a beehive in the centre and were selected to be as intermixed geographically as possible to avoid spatial autocorrelation. All the beehive locations (landscape centres) were kept at least 4 km apart to ensure the independence of beehives based on the known foraging ranges of honeybees (Garbuzov et al., 2015, Visscher et al., 1996, Beekman and Ratnieks, 2000, Steffan-Dewenter and Kuhn, 2003). In addition, beehives mounted in natural forests were also kept at least 2 km away from agricultural/cocoa

landscapes based on the foraging range of honeybees. The landscapes were selected based on the land use/land-cover map developed by Satelligence (2019) and field visits and categorised by the most dominant land use category within the 2km radius around each sampled beehive. A summary of landscape analysis which was conducted to establish the percentages of dominant habitat types is presented in Appendix C1. Beehives located in fullsun cocoa and agroforestry cocoa plantations were provided by beekeepers. The beehives in natural forests were placed in two protected areas for the purpose of this study, namely Bia Conservation Area (three hives) and Bia North Forest Reserves (two hives). The authorities of the Ghana Forestry Commission granted approval to construct and mount 10 beehives inside these protected areas overall. The beehives were mounted during the swarming season from August to December 2020 during which beekeepers baited for bee colonies. However, at the end of the swarming season, only five of the ten beehives mounted in the natural forests were colonised and these were used for the study. All the beehives used in our study were the top bar hive designs and had equal dimensions (See Appendix C3).

3.3.3 Sample collection

Honey samples were collected within one week from the end of March (31/03/2021) to the start of April (07/04/2021) to minimize variation in climatic factors, flowering, and pesticide treatments during the period of sample collection (Lambert et al., 2013). For this study, only freshly built honeycombs constructed by honeybees were utilised to avoid any potential contamination. There was no reuse of beeswax from other colonies or previous years. Two honey samples from two different honeycombs from each of the 15 sampled hives were collected for the study. The honey samples were scooped directly from the honeycombs to pre-labelled sample tubes (Appendix C3) and placed in ice and subsequently transported from the field to the park headquarters and kept at 4–8°C (Gajger et al., 2019) until being shipped to the School of Chemical Science, Dublin City University, Ireland in dark and airtight containers for pesticide residue analysis. Upon arrival, the honey samples were kept at 4–8°C until the start of the analysis.

3.3.4 Pesticide selection

Eight active ingredients were selected for study as the most applied pesticides for cocoa cultivation in BWD from 2018 to 2021 under the National Cocoa Disease and Pest Control (CODAPEC) program, in terms of the number of cartons received for distribution (See Appendix C4). The selected pesticides consisted of 3 neonicotinoids (acetamiprid, imidacloprid and thiamethoxam), 1 oxadiazine pesticide (indoxacarb), 1 sulfoximine (sulfoxaflor), 1 triazole fungicides (difenoconazole), 1 insecticide belonging to the fipronil phenylpyrazole chemical family (fipronil) and 1 pyrethroid (bifenthrin; Appendix C5). Etofenprox was excluded from this study due to suspected contamination from beehive construction (Appendix C6).

3.3.5 Materials

Certified analytical standards for imidacloprid, acetamiprid, thiamethoxam, bifenthrin, fipronil, difenoconazole, indoxacarb and etofenprox, of >97% purity, extraction salts [magnesium sulphate (MgSO₄) anhydrous, sodium chloride, sodium citrate tribasic dihydrate and sodium citrate dibasic sesquihydrate], Reagent Plus(R) ≥99%, purifying salt PSA silica, corning (R) centrifuge tubes, Millipore Millex syringe filters with the hydrophilic membrane (pore size 0.22 μm and 20 μm diameters), 1.5 mL autosampler vials and conical inserts were procured from Merck Life Science Ltd (Ireland). The sulfoxaflor standard was purchased from LGC (UK). The deionised ultrapure water used during the analysis was generated using the ELGA Purelab Ultra SC MK2 (ELGA, UK). The stock solutions, from which calibrations were prepared, were strictly preserved at -25 °C in dark conditions.

3.3.6 Sample preparation

Pesticide extractions were conducted using QuEChERS (Paradis et al., 2014, Mitchell et al., 2017, Kavanagh et al., 2021). The QuEChERS method (an acronym for Quick, Easy, Cheap, Effective, Rugged and Safe), was developed by Anastassiades et al. (2003) to determine different classes of pesticide residues and is presently recognised as the most efficient extraction technique for multi-residues pesticides (Lambropoulou and Albanis, 2007, Lesueur et al., 2008). Samples were prepared by weighing 2.5g of honey into a 15 mL polypropylene tube to which 9 mL of H₂O: ACN (50:50, v/v) and

20 μL of a 500 ng/mL Internal Solution (IS) containing deuterated IS thiamethoxam-D3 and clothianidin-D3, purchased from Merck Life Science Ltd (Ireland), were added to the extracts to compensate for any possible matrix effects or recovery losses and response drift during chromatographic analytical and detection procedures (EC, 2022, Melo et al., 2019, Commission, 2019). The honey was dissolved by manual agitation and ultrasonication for 10 min and the resulting solution was transferred into a 15 mL tube containing the extraction salts [2 g magnesium sulphate (MgSO_4), 0.5 g sodium chloride, 0.5 g sodium citrate tribasic dihydrate and 0.25 g sodium citrate dibasic sesquihydrate]. One millilitre of H_2O : CAN (50:50, v/v) was then added to the first tube, and after a brief agitation period the remaining solution was transferred to the extraction salt tube. The mixture was then vigorously shaken by hand for approximately 2 min and then centrifuged at 4000 g for 10 min. The upper phase (approx. 4.5 mL) was collected in a second 15 mL tube containing the purification salts (0.15 g MgSO_4 and 0.1 g PSA). After vigorous shaking for 1 min, the tube was centrifuged at 4000 rpm for 10 min. The recovered supernatant (4.5 mL) was dispensed into three 1.5 mL Eppendorf tubes (Lennox Laboratory, Ireland). The solution was concentrated to dryness under a gentle stream of nitrogen in a CentriVap centrifugal evaporator (Labconco, Thermo Scientific, Ireland) thermostatic at 40°C and dried residue remaining re-suspended in 0.25 mL of methanol (MeOH) 25%. The tubes were finally vortexed and filtered through 20 mm PTFE hydrophilic syringe filters into LC-MS vials containing 250 μL conical inserts.

3.3.7 HPLC-MS/MS analysis

The targeted pesticide residues were analysed using an Agilent 1290 Infinity II LC multi-sampler, binary pump, and multiple-column thermostatted compartment coupled to a 6470A triple quadrupole mass spectrometer (MS) (Agilent Technologies, Cheshire, UK). The studied compounds were successfully separated on Waters XBridge C18 4.6 x 100 mm, 3.5 μm (p/n: 186003033) using a column oven at 30 °C and a flow rate of 0.5 mL min^{-1} . Mobile phase A consisted of 5 mM ammonium formate with 0.1% formic acid in ultrapure water with mobile phase B comprising 0.1% formic acid in acetonitrile. The gradient program used increased linearly from 30% to 80% B in 8 min, 94-98% B in 2 min, holding at 100% B for 4 min, and finally returning to the initial conditions at 30% B in 2.0 min. The injection volume was 10 μL .

Mass spectrometry was performed in ESI+ through Multiple Reaction Monitoring (MRM) mode using the following parameters: capillary voltages of + 3.5 kV and -3 kV; desolvation temperature 340°C; curtain gas (N₂) flow 8 L min⁻¹, nebuliser gas pressure 35 psi; sheath gas flow 11 L min⁻¹; and sheath gas temperature 375 °C. Data acquisition, analysis and control of the LC-MS/MS system were achieved using the Agilent Mass Hunter Workstation Data Acquisition Version 10.0 software. The chromatographic parameters for studied compounds were retention time (R_t), quantifier ion, and qualifier ion. The elution of all eight targeted analytes was completed in a time range from 3.378 to 14.075 min (Appendix C7- C8).

3.3.8 Quantification and validation

Quantification and validation experiments were based on methods previously applied (Mitchell et al., 2017, Zioga et al., 2022, Kavanagh et al., 2021). The method was validated by utilising SANTE/2019/12682 guidelines for sample pre-treatment procedures usually undertaken prior to pesticide residue analysis in food and feed (Commission, 2019). The studied pesticides were quantified by internal calibration using calibration solutions in MeOH 25% at 0.1, 0.25, 0.5, 1, 10 and 50 µg L⁻¹ with each containing internal standards at a concentration of 20 µg L⁻¹. A validation assay was conducted using honey samples (n=6) free from pesticide residues. Blank samples (containing no honey) were analysed to assess the method's efficiency along with contamination checks to ensure that no external contamination occurred during the sample collection, handling, or analysis process. The blank samples were spiked with IS solution prepared and 20 µg L⁻¹ of a mixed solution of targeted pesticides as previously applied (Thevenet et al., 2017, Mitchell et al., 2017). The ranges of linearity of each analyte, limit of detection (LOD), limit of quantification (LOQ) and recoveries for three concentrations were established using six-point calibrations (Sudeep et al., 2015, Mitchell et al., 2017). The method was validated to ensure the reliability of its performance (Chandran and Singh, 2007, Booth and Simon, 2004). The validation parameters namely linear range, linearity, LOD and LOQ were assessed for all targeted pesticides using standard calibration (Appendix C9-11).

3.3.9 Statistical Analysis

Firstly, we assessed the relationship between the pesticides detected and the landscape categories. We used binomial Generalised Linear Models (GLMs) to analyse the detection of pesticides as a binary outcome (present/absent), with a separate model for each pesticide detected (Zuur et al., 2009). In these models, the explanatory variable was the landscape which had three categories (natural vegetation, full sun cocoa, and agroforest cocoa). To ensure the reliability of our results, we performed several diagnostic tests designed specifically for binomial Generalised Linear Models (GLMs). The Hosmer-Lemeshow test ('pROC') was used to assess goodness of fit by comparing predicted and observed probabilities (useful for scenarios involving grouped data). ROC analysis, facilitated by the 'Proc' package was used to the model's ability to discriminate using the Area Under the Curve (AUC). Additionally, the 'carat' package was employed to construct a confusion matrix to visualise classification performance (including accuracy).

Secondly, we also compared the pesticide load across the landscape categories. The total number of pesticides found at each site was used as the response variable, while the landscape categories served as the explanatory variables in GLMs. Data visualisations were first performed for an overview of the distribution and mean values of pesticide counts across the different landscape categories using the 'ggplot2' package. Residual analysis was then performed to evaluate model performance and indicate a good fit. A cross-validation (Browne, 2000, Berrar, 2019) was performed and also provided insightful information about the model's predictive power. All statistical analysis was conducted in R version 4.2.2 (Team, 2022).

Furthermore, we compared the concentrations of the residues of detected pesticides found in this study to the European Union Maximum Residue Limits (EU MRLs; Appendix C9), to determine whether the concentrations detected of these pesticides were within acceptable ranges for human consumption. Additionally, we compared pesticide concentrations identified in our study to those in existing studies that have established the sublethal effects of pesticides on honeybees. We focused only on sub-lethal effects because of their immediate relevance to real-world scenarios where the

majority of honeybee colonies come in contact with pesticide residues usually at sublethal levels (Francisco and Koichi, 2016). Furthermore, since sublethal impairments can gradually weaken bee colonies and make them more vulnerable to different stresses, focusing on sublethal effects feeds into the evolving regulatory criteria for pesticides and highlights the potential risks which pesticides pose to pollinators (Tosi et al., 2022, Gill et al., 2012). With the help of Boolean operators ('sub-lethal' AND 'pesticide' AND 'honeybees'), we searched three important scientific databases, namely PubMed (30 publications), Scopus (58 publications) and Web of Science (44 publications). Initially, 132 publications were found using this search approach following which duplicates were removed using EndNote X9. We then evaluated each publication by screening the titles and abstracts to find those that had assessed the sub-lethal impact of at least one of the pesticides we investigated on honeybees. This was undertaken to help identify papers directly relevant to our objective. As a result of this screening process, we identified twelve studies that had successfully established sub-lethal pesticide effects on honeybees (See Appendix C12). Next, we conducted a comparative analysis, cross-referencing the pesticide concentrations found in our dataset, with those that detected sub-lethal effects in these studies.

3.4 Results

3.4.1 Pesticide residues detected

Overall, six pesticide residues were detected (acetamiprid, imidacloprid, thiamethoxam, indoxacarb, sulfoxaflor and difenoconazole), with at least two pesticides and up to six being detected in a single honey sample (Table 3.1 & Appendix C1). Only imidacloprid was persistently detected in all honey samples. The other five pesticides (namely sulfoxaflor, thiamethoxam, indoxacarb, acetamiprid and difenoconazole) were detected in 93%, 73%, 67%, 33% and 7% of the 15 honey samples respectively (Figure 3.2 & Table 3.1). Bifenthrin and fipronil were not detected in any of the honey samples analysed.

Table 3.4 Summaries of the detected and quantified pesticides and the distribution of detection per landscape type. The minimum and maximum concentrations are given in mg kg⁻¹ with the standard errors for all honey samples. The number quantified is the number of honey samples with concentrations quantified to be above LOQ for each analyte. “Number” columns represent the number of landscapes (e.g., acetamiprid was detected in five of the 15 landscapes, but in only 1 instance was it detected above LOQ).

Analytes	Number of samples with detections	Number of samples with measured concentrations	Concentrations (mg kg ⁻¹)				Number detected in NV	Number detected in FC	Number detected in AC
			Minimum	SE	Maximum	SE			
Acetamiprid	5	1	NA		0.051	0.0740	ND	2	3
Imidacloprid	15	14	0.004	0.003	0.046	0.065	5	5	5
Thiamethoxam	11	4	0.013	0.004	0.017	0.009	2	5	4
Bifenthrin	ND	ND	ND				ND	ND	ND
Indoxacarb	10	8	0.003	0.002	0.042	0.059	4	4	2
Difenoconazole	1	0	<LOQ				ND	ND	1
Fipronil	ND	ND	ND				ND	ND	ND
Sulfoxaflor	14	14	0.004	0.002	0.026	0.013	4	5	5

ND: not detected; SE=Standard error; NA: not applicable; pesticide detected only in one sample.

<LOQ denotes pesticide concentration quantified to but below individual analyte's Limit of Quantification (LOQ)

3.4.2 Quantification of pesticide residues

Even though six pesticide residues were detected, only five compounds were detected at sufficiently high concentrations to be quantified. These were acetamiprid with a concentration of 0.051 mg kg⁻¹ in one sample and imidacloprid whose concentrations ranged from 0.004 to 0.046 mg kg⁻¹ in 14 honey samples. Other pesticides detected were thiamethoxam (0.013 to 0.017 mg kg⁻¹ in 4 honey samples); indoxacarb (0.003 to 0.042 mg kg⁻¹ in 8 honey samples) and sulfoxaflor (0.004 to 0.026 mg kg⁻¹ in 14 honey samples). None of the concentrations of the pesticide residues quantified exceeded their specified EU Maximum Residue Limits (MRL) (Appendix C 9).

3.4.3 Relationships between pesticide detection and landscape categories

Imidacloprid was found in all samples across all landscapes including natural forest areas, highlighting its persistence. However, there were no significant relationships between presence and absence of the other pesticides detected and landscape categories (acetamiprid: $\chi^2 = 5.64$, df = 2, p = 0.06; thiamethoxam $\chi^2 = 5.66$, df = 2, p = 0.059, indoxacarb $\chi^2 = 2.36$, df = 2, p = 0.31, difenoconazole $\chi^2 = 2.34$, df = 2, p = 0.31 and sulfoxaflor ($\chi^2 = 2.34$, df = 2, p = 0.31). Even though none of the relationships attained any statistical significance, some trends emerged. For example, acetamiprid was endemic to fullsun and agroforest cocoa landscapes while difenoconazole was only detected in honey samples from agroforest landscapes. The other pesticides detected in our study were found in all the landscapes (Figure 3.2). Further to this, honey samples where acetamiprid was successfully quantified were completely localised to the agroforest cocoa landscape. Conversely, honey samples with measured concentrations of thiamethoxam, exceeding the LOQ, were limited to fullsun and agroforest cocoa landscapes. Only imidacloprid, indoxacarb, and sulfoxaflor were observed with quantifiable concentrations in all the landscape categories.

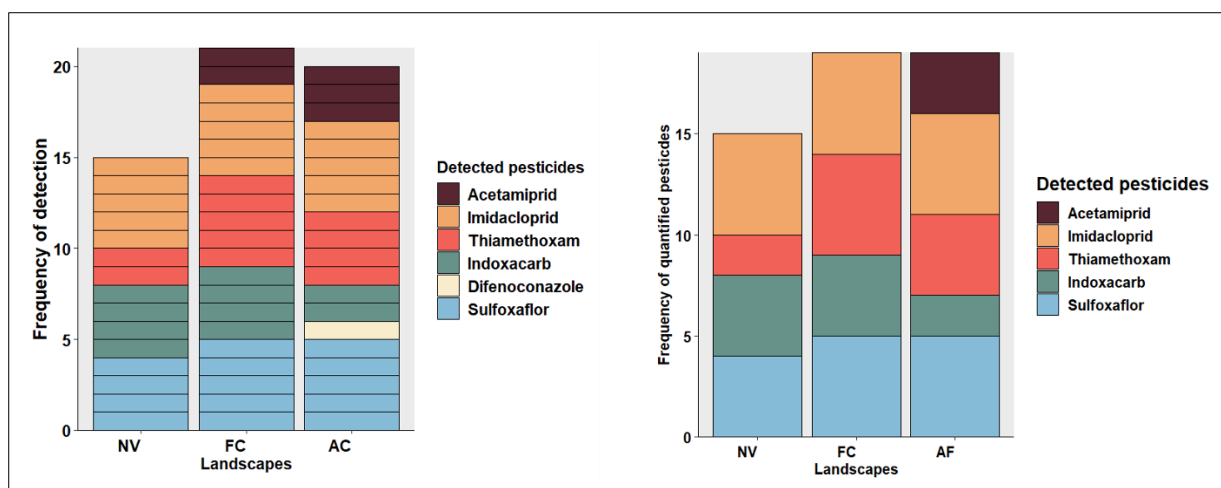


Figure 3.2 Pesticide detection and quantification across landscape categories. Left: Presence of detected pesticide residues. The agroforest cocoa landscapes had the highest number of pesticide types detected. Right: Frequency of pesticide with measured concentrations i.e., those whose concentrations were higher than the limit of quantification (LOQ). Difenoconazole was detected but could not be quantified because its concentration was below the LOQ.

3.4.4 Pesticide load

Among the studied landscapes, agroforest cocoa areas displayed the highest number of pesticide compounds with six distinct residue types, while the lowest pesticide load was found in natural vegetation (4 in total) but on average five per landscape (Figure 3.3). However, despite these trends, there was no significant difference in pesticide loads among landscape categories ($\chi^2 = 1.15$, $df = 2$, $p = 0.56$).

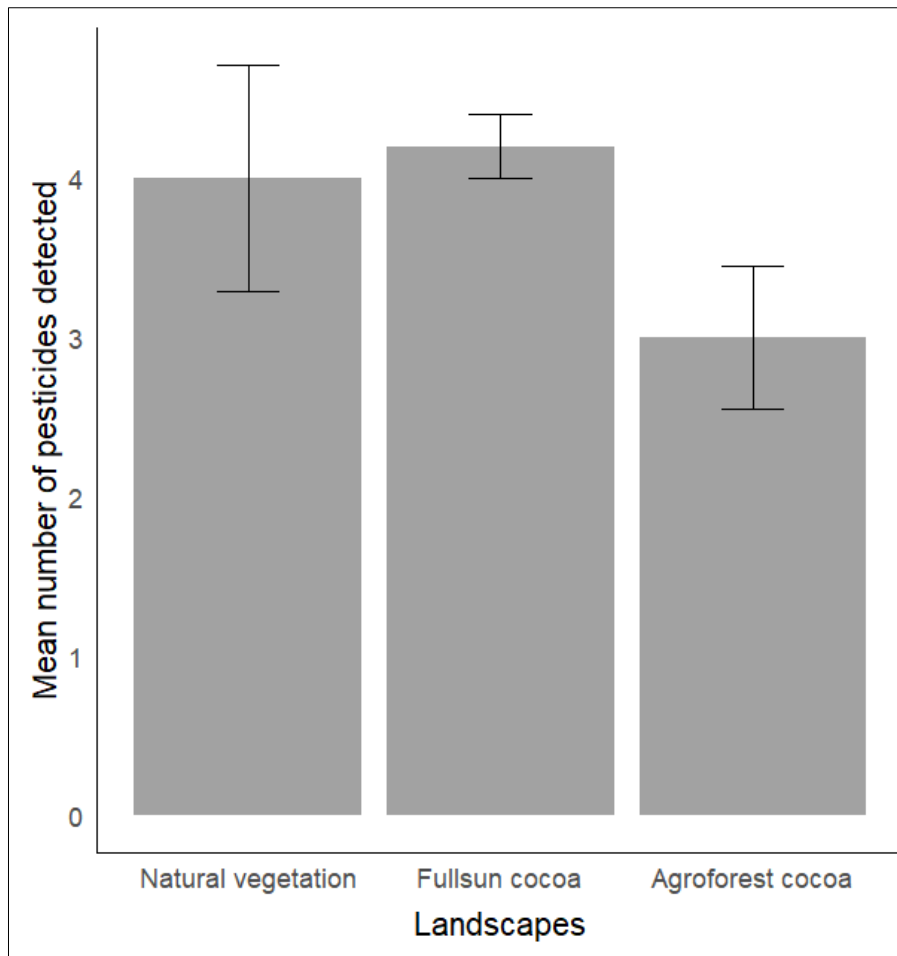


Figure 3.3 The mean number of pesticides found per landscape in each landscape category (natural vegetation (NV), fullsun cocoa (FC) and agroforest cocoa (AC)). The error bars indicate the standard error of the mean. There was no significant difference in the number of pesticides found among landscape categories.

3.4.5 Pesticide concentration and sub-lethal implications for honeybees

From the comparative analysis conducted, we observed that the residues of two of the detected pesticides with measured concentrations in our study, namely thiamethoxam (0.017 mg/kg) and imidacloprid (0.046 mg/kg), (Table 3.1), exhibited quantities surpassing those observed in previous studies that have successfully identified sub-lethal effects on *Apis mellifera* (as detailed in Appendix C11). In queens, immunology, sperm viability and gene expression were all found to be affected by imidacloprid at concentrations of 0.02 mg/kg (Chaimanee et al., 2016). Honeybee larval tissues experienced apoptosis in response to thiamethoxam at a concentration of 0.0004 mg/kg (Gregorc and Ellis, 2011), whereas bee colonies had significant harm at higher

concentrations (0.1 mg/kg) (Thompson et al., 2019). Further emphasizing the complexity of these effects, Forfert et al. (2017), found that queens mated less frequently, leading to reduced worker bee genetic variation when exposed to 0.004 mg/kg of thiamethoxam. This gives an indication that the concentrations of imidacloprid and thiamethoxam found in our research may cause concern about potential sub-lethal effects on honeybees. Unlike thiamethoxam and imidacloprid, our observed concentrations of acetamiprid (0.051 mg/kg) and sulfoxaflor (0.026 mg/kg) were lower than the concentrations applied in a previous study by (Capela et al., 2022) which showed the concentration of acetamiprid and sulfoxaflor at 0.26 mg/kg impaired the homing ability of honeybees. Overall, twelve of the existing studies that demonstrate an array of sub-lethal effects of imidacloprid and thiamethoxam, ranging from alterations in honeybee queens' gene expression to sperm viability, immunological responses, foraging behaviour, cognitive abilities, colony expansion, and reproduction, used concentrations far lower than those found in our work.

3.5. Discussion

Honey's production, occurring largely in agricultural landscapes, makes it potentially susceptible to chemical contamination, as bees may feed on nectar and pollen possibly contaminated with pesticide residues. Evaluating pesticide contaminants in honey can therefore provide insights into the prevalence of pesticides in the environment more broadly. This study assessed honey samples from cocoa and forest ecosystems in Ghana for pesticide presence and concentration.

The results from this study indicate that honey samples from fullsun cocoa, agroforest cocoa and natural vegetation landscapes all contained pesticides with at least three pesticide residues detected in each landscape type (Table 3.1 & Figure 3.2). This finding is in line with those of Han et al. (2022) who found four metabolites in 94% of the honey samples they examined and validated their presence at various doses and detection rates. This highlights the pervasiveness of pesticide residues in the landscape and provides evidence that even honey in hives located up to at least 2 km inside natural vegetation is not exempted from the impact of pesticides applied in nearby cocoa landscapes. The observation made by Zioga et al. (2023) who confirmed

the presence of pesticides from fields without any recorded application of the chemicals in that corresponding year is similar to our finding of pesticide residues in honey from natural vegetation where pesticides are not applied. While their study highlights the presence of pesticide residues in crop and wild plant pollen and nectar, our research expands on this knowledge by demonstrating the existence of pesticide residues in naturally occurring landscapes that are not directly susceptible to pesticide treatments. Our study emphasizes the possibility of widespread pesticide dispersion outside the bounds of typical agricultural practices and raises intriguing issues regarding the mechanisms by which these residues can reach non-agricultural areas. Again, the detection of pesticides in honey from natural vegetation in our study aligns with a previous study by Darko et al. (2017) which was also conducted in Ghana. Their study showed that honey from forested areas in Ghana contained pesticides. However, it is noteworthy to mention that in our study, it was observed that honey samples from the natural vegetation were found to contain the lowest number of pesticide residues (4 pesticides) compared to fullsun cocoa (5 pesticides) and agroforest cocoa (6 pesticides), even though no significant differences were detected in the number of detected residues across the landscape categories. The higher detection rates of pesticide residues in landscapes with more extensive agricultural activities observed in our study are in line with an earlier observation made by Ponce-Vejar et al. (2022) who found that insecticide detections were generally higher in locations with high-intensity agricultural practices and highly technical agricultural practice. The highest number of residues in agroforest cocoa compared to the other landscapes may be explained by a variety of factors. For example, agroforest cocoa landscapes typically have a higher diversity of plants and tree species compared to fullsun cocoa areas. This increased diversity might attract a wider range of pests (Altieri et al., 1984, Lamond et al., 2019) which may require a higher rate of pesticide application to manage various pests effectively. However, this explanation may not fully explain our findings if pesticide application is standard across the landscape due to the Mass Spraying programme, although over-application has been cited as an issue in some cocoa-growing regions (Fianko et al., 2011, Kwakye et al., 2019). Also, the timing of pesticide application during nectar-gathering by honeybees and co-flowering wild plants could influence the level of pesticide residues in honey samples as agroforest landscapes may have more wild plants which are attractive to bees which become contaminated with pesticide drift or runoff. To gain a comprehensive

understanding, further investigations and data collection are necessary. Analysing pesticide use records, studying bee behaviour and forage choice, and conducting more detailed studies on the specific pesticide products applied in both landscape categories could help to unravel the underlying reasons for the observed differences in pesticide residues.

In contrast to the findings of Ohoueu et al. (2017), who examined the levels of pesticide residues of cypermethrin, profenofos, metalaxyl, chlorpyrifos, and atrazine, in honey coming from the Ivory Coast - a country similarly engaged in cocoa production - our study found a noticeably higher rate of pesticide residue detection. Their study also confirmed widespread contamination with pesticides, with their results including the presence of cypermethrin and profenofos in 70-80% of the 40 honey samples analysed, which is comparable to our findings of at least two pesticide residues in every sample. The differences in findings may be attributable to both the different compounds analysed, as well as methodological differences, as our analytical method had higher sensitivity and selectivity. Due to the improved capabilities of our analytical approach, the higher rate of detection in our study compared to theirs is plausible. Again in assessing global exposure of pollinators to neonicotinoids namely acetamiprid, clothianidin, imidacloprid, thiacloprid and thiamethoxam through the analysis of 198 honey samples from around the world, Mitchell et al. (2017) found that 75% of samples contained at least one analyte with 45% of the samples two or more of the analytes. Furthermore, Lu et al. (2016) detected the presence of at least one neonicotinoid in 72% of the honey samples which is below the rate of detection of residues as observed in our samples. The reason for the higher rate of detection of pesticide residues in our study compared to some previous studies may be related to the intense application of pesticides for cocoa cultivation in the study area. The cocoa plant is very susceptible to disease and pest attacks such as cocoa swollen shoot virus, beetles and capsids (miridae) and phytophthora pod rot (commonly called black pod) (Bateman, 2008, Afrane and Ntiamoah, 2011) and therefore to ensure sustainable production, pesticides are intensely applied to prevent losses (Ntiamoah and Afrane, 2008, Okoffo et al., 2017, Aminu et al., 2019). Even though pesticides are needed to sustain cocoa cultivation, some previous studies suggest possible misapplications or abuses in certain localities in Ghana. Kwakye et al. (2019) found pervasive overdose use of synthetic pesticides in many parts of Ghana. In an earlier

review, Fianko et al. (2011), indicated that there is substantial evidence of an over-use of agrochemicals especially pesticides in Ghana. Antwi-Agyakwa et al. (2015) confirmed that some farmers in Ashanti, Eastern, Western and Volta regions of Ghana, undertook as many as eleven applications per year which was seven more applications than the approved four applications per year. Together, this indicates that pesticide usage in Ghana may be even higher than reported; which may also explain the high rate of detection of pesticides across all samples.

Although no significant difference was detected in the number of detected pesticide residues across the landscapes, our study provides valuable insights into pesticide residue distribution. It supports a previous study conducted in India, another cocoa-growing country, by Choudhary and Sharma (2008) who found that honey from natural vegetation had a lesser number of pesticide residues. The Bia Conservation Area and Bia North Forest Reserves, where honey from natural vegetation was collected, are protected areas and as such pesticide applications are completely prohibited. The detection of pesticide residues in the honey samples is therefore likely attributable to the nearby cocoa and agricultural landscapes. In our study, sampled hives were placed at least 4 km apart to ensure spatial independence. However, some beehives within protected areas were situated approximately 2 km from cocoa-producing landscapes. Generally, when floral resources are abundant, both social and solitary bees tend to localise their foraging activities within distances of 2 km around the hive location (Garbuzov et al., 2015, Visscher et al., 1996, Steffan-Dewenter and Kuhn, 2003). However, foraging to a distance of 5.5 km by honeybees has also been confirmed (Beekman and Ratnieks, 2000). The probability of honeybees foraging beyond the 2 km into the cocoa-producing landscapes for floral resources and in the process transporting pesticides into the hive is possible. Alternatively, pesticide residues may move into forest areas via drift or runoff. However, the remit of our study did not extend to evaluating the availability of floral resources or the foraging activities of honeybees around mounted hives, and the reasons for this deserve further investigation.

One of the most remarkable results to emerge from our study is the presence of imidacloprid in all honey samples, along with sulfoxaflor (93%) and thiamethoxam (73%) in the majority. This finding aligns with our findings from Chapter 2, where in a

systematic review that synthesised honey contamination from plant protection products approved for cocoa (*Theobroma cacao*) cultivation, we confirmed imidacloprid as the most frequently detected pesticide. Imidacloprid belongs to a broad group of pesticides classed as neonicotinoids which are the most applied classes of pesticides around the world (Silici et al., 2013, Bonmatin et al., 2015b, Simon-Delso et al., 2015). The high rate of detection of imidacloprid observed in our study also reflects the conclusion reached by Kavanagh et al. (2021) who found imidacloprid as one of the highest detected neonicotinoids in Irish honey. Similar observations were also made by Mitchell et al. (2017) who found imidacloprid as one of the neonicotinoids present in 79% of European honey samples and Lu et al. (2016) who also identified imidacloprid as the most detected neonicotinoid (72%) in the honey samples analysed in the USA. Presently, imidacloprid is registered for the cultivation of over 140 different crop types in about 120 countries (Jeschke et al., 2011, Kleinschmit and Lilliston, 2015). Data on pesticide use for cocoa production in the study area (2018-2022) revealed imidacloprid as the second most applied pesticide. It was therefore not surprising that imidacloprid was consistently detected in honey samples studied. The steady detection of imidacloprid provides evidence of its wide coverage of use and persistence in the environment. Neonicotinoids, like imidacloprid, are highly persistent and can bio-accumulate in soil (Goulson, 2013) with their half-lives exceeding 1000 days and can also persist in woody plants for more than 365 days (Bonmatin et al., 2015). Their detections are thus possible even over a lengthy period long after application.

Our findings revealed that the measured pesticide concentrations varied between 0.005 mg kg⁻¹ to 0.051 mg kg⁻¹ in the agroforest cocoa and between 0.003 mg kg⁻¹ to 0.02mg kg⁻¹ in both the fullsun cocoa and in wild vegetation (Table 3.1). It is significant to highlight that the measured concentrations of the targeted pesticides did not exceed the permissible limits, i.e., the EU MRLs, which could have caused safety issues for human honey consumption (Regulation, (EC) No. 396/2005). This finding is consistent with a past baseline study of honey samples from forest and agricultural landscapes by Darko et al. (2017) in Ghana who found eleven pesticide residues but whose concentrations did not exceed specified EU MRLs. Therefore, the integration of beekeeping with cocoa cultivation could be a viable and practical approach to support rural livelihoods. The coexistence of these two practices can be facilitated with proper

management strategies to minimize potential risks and ensure the sustainability of both cocoa production and honeybee populations. Although all quantified residue concentrations in the honey samples were found to be below acceptable limits for human consumption, it was observed that some concentrations were higher than those reported in previous studies. For instance, in New Zealand, Switzerland, and Canada, acetamiprid was quantified at 0.0002 mg/kg, 0.029312 mg/kg, and 0.0045 mg/kg, respectively, as reported by Chen et al. (2014), Kammoun et al. (2019) and Codling et al. (2016). On the other hand, our observed concentration of thiamethoxam, ranging from 0.014 mg/kg to 0.017 mg/kg, was lower than the concentration of 0.0252 mg/kg detected in Poland by Bargańska et al. (2018).

Even though our results showed that the pesticide residues detected in the honey examined do not compromise the quality of the honey for human consumption as required under (Regulation, (EC) No. 396/2005), the measured concentrations for acetamiprid, imidacloprid, thiamethoxam, indoxacarb, and sulfoxaflor highlight possible concerns for honeybee populations (Table 3.1). This worry is premised on the fact that study has established that bees may have reduced pollination efficiency when exposed to pesticides (Stanley et al., 2015c) and more particularly exposure to imidacloprid, acetamiprid, and thiamethoxam has been established to negatively impact the cognitive capabilities of bees (Bass and Field, 2018, Sánchez-Bayo et al., 2016). The resultant effects may include paralysis and death (EC, 2022). Interestingly, we observed that the concentrations of pesticides quantified were relatively higher than the concentrations of some of the existing studies found to have negative effects on honeybees. At a concentration of 0.0045 mg/kg of thiamethoxam, much lower than that observed in our results, the longevity, viability and quantity of live sperm in drones became limited thus adversely impacting the reproductive capability of male honeybees (Straub et al., 2016). A dose of 0.005 mg/kg, a concentration much lower than our observed concentration, was found to disrupt the age-based divisions of labour ultimately impacting bee behaviour (Zhang et al., 2020). Similarly, a 51% reduction in flight activity was observed when honey was subjected to 0.0043 mg/kg of thiamethoxam which is lower than our observed concentration (Straub et al., 2021). Additionally, the foraging activities of honeybees were reduced and vitellogenin gene expression increased when exposed to 0.001 mg/kg of thiamethoxam, according to Christen et al. (2016). Although many of our concentrations were higher than those at

which sublethal impacts have been established, more work is needed to elucidate how they compare to LC50s. Nevertheless, implications of pesticide residues in honey for bees and other pollinators are raised by these findings. Although our results imply that the honey is safe for human consumption, the detected pesticide concentrations give rise to potential concerns regarding their sub-lethal effects on honeybees and the crucial ecosystem services they offer.

The detection of pesticide residues in all landscape categories evaluated in our study gives credence to the assertion by Simon-Delso et al. (2017) that any attempt to assess the effects of pesticides on pollinators should be limited to their toxicity but should extend to how they get exposed to it in the field. Our results established that bees were exposed to pesticides in natural forests in landscapes devoid of pesticide applications. Bees' exposure to pesticides in non-crops is likely through unintentional contamination of non-crop areas with pesticides (Knapp et al., 2022). In a study, Simon-Delso et al. (2017), found that pesticides applied to crops unattractive to bees, such as cereals or sugar, can potentially be a source of contamination through weeds or drift to surrounding or succeeding crops. Consistent with the observation by Simon-Delso et al. (2017), we found that residues of pesticides applied for cocoa cultivation were detected in honey from natural vegetation (i.e. no crop landscapes). Bees are not known cocoa pollinators and therefore may not forage cocoa trees for nectar. However, they ended up picking pesticide residues in cocoa-growing landscapes. Analysing the plant origin of honey samples in our study could undoubtedly have provided valuable insights into understanding the routes of pesticide exposure for honeybees across different landscape types in our study. However, the remit of this study did not extend to the plant origin of detected pesticide residues even though that could have better elucidated the pathways through which pesticides enter hive matrices. Nonetheless, our findings show that pesticides applied for cocoa cultivation find their way to non-cocoa growing areas.

The detection of multiple pesticides in honey samples in our study highlights the significance of considering how pesticide combinations may affect both human health and pollinators. While individual pesticides can pose risks on their own as evaluated in our study, the combined impact of chemicals could result in higher toxicity levels or unexpected reactions (Sanchez-Bayo and Goka, 2014). In a study which evaluated

the toxicological interaction effects of imidacloprid, thiamethoxam and chlorpyrifos on *Bombus terrestris*, Zhang et al. (2022) found that all dual or triple combinations showed either synergistic or additive effects. Similarly, in a review, Simon-Delso et al. (2015) found that exposure to mixtures of pesticides can cause synergistic toxicity effects on bee populations leading to adverse effects on foraging behaviour, weakened immune systems and increased vulnerability to diseases. The multiple pesticide residues detected in honey samples in our study underscore the need for further investigations of the possible interactive effects on honeybees and also calls for extensive monitoring practices to reduce the risks associated with pesticide combinations in agriculture and their impacts, on ecosystems and human well-being.

Although bifenthrin was the most frequently used pesticide in the Bia West District of Ghana from 2018 to 2021 according to data available on pesticide application for cocoa cultivation under the National Cocoa Disease and Pest Control (CODAPEC) program, it is interesting to note that our results did not confirm any detectable or quantifiable concentrations of bifenthrin in any of the honey samples across all the different landscapes. This result is at variance with the observation made by Yang et al. (2018) that bifenthrin is easily detected in environmental media, residential areas and biota; an observation further given credence to the work by Allinson et al. (2015) which found bifenthrin as the most prevalently applied pesticide in Melbourne, Australia. Jeppe et al. (2017) also established quantifiable levels of bifenthrin at 78% of their sampling sites lending support to the observation by Yang et al. (2018) and Allinson et al. (2015). Where LOD and LOQ are not sufficiently low, detections and quantifications of pesticides may be missed during analysis. For instance, Bezerra et al. (2010) reported no detections of pesticide residues in a study conducted in Brazil. However, their reported LODs and LOQs ranged from 0.07 mg kg⁻¹ to 0.25 mg kg⁻¹ and 0.02 mg kg⁻¹ to 0.08 mg kg⁻¹, respectively. These concentrations were higher than the EU MRLs and therefore cannot be deemed to be sufficiently low to be assured of no risk due to pesticide contamination. In our study however, the LOD achieved for bifenthrin was 0.022 mg kg⁻¹ which was lower than its MRL, providing reassurance that bifenthrin was not present in any honey samples at levels which pose a risk to human health. The LODs for all targeted pesticides applied in our study ranged from 0.001 to 0.022 mg kg⁻¹ with the LOD of each targeted being below its specified MRL (See Appendix C9). Despite the sensitivity of our method, we interpret the non-

detection of bifenthrin with caution owing to the fact the recoveries for bifenthrin were only 41.2% and 84.1% respectively, which were the lowest compared to the other pesticides studied.

3.6 Conclusion

This study examined the presence and concentrations of pesticides used in cocoa cultivation in the Bia West District, a significant cocoa-producing region in Ghana, by specifically analysing honey samples from cocoa and forest landscapes. Our findings indicated that honey samples from fullsun cocoa, agroforest cocoa, and natural vegetation landscapes all contained pesticides, with at least four pesticide residues detected in each landscape type. This observation highlighted the pervasive nature of pesticide residues in the environment, emphasizing that even honey collected from hives situated up to 2 km within natural vegetation was not exempt from pesticide impact. Although the tested honey samples complied with legal requirements for human consumption, concentrations of quantified pesticides in these samples raised questions about their possible sub-lethal effects on honeybees and, consequently, the ecosystem services they provide and this deserves further study. These results stress the need for effective use of pesticides for cocoa cultivation to reduce risks to non-target organisms and ensure ecosystem sustainability. In line with this, our study emphasizes the need for continued law enforcement regulations governing pesticide use in cocoa cultivation and discourages any relaxation of monitoring and law enforcement practices. Future studies should encompass multiple cocoa-growing districts and other non-cocoa farming landscapes where pesticides are employed to gain a comprehensive understanding of pesticide impacts on the broader environment. One of the most important issues worth investigating in future studies is the processes that allow pesticides to spread into non-agricultural areas, including source tracing of pesticide residues, studies on bee foraging behaviour, and accurate records of pesticide use. In conclusion, our study contributes valuable insights into the prevalence of pesticides in bee products in a prominent cocoa-producing area in Ghana, and how this relates to landscape type. In the end, combining beekeeping with cocoa farming can be a successful strategy if the right management techniques are used to ensure the sustainability of both practices and reduce any potential dangers.

3.7 Acknowledgements

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Chapter 4 Relationships between flower-visiting insects and forest cover in cocoa-growing landscapes in Ghana

“The fate of insect pollinators is like a thread woven into the fabric of human influence” RGB

Chapter 4 Relationships between flower-visiting insects and forest cover in cocoa-growing landscapes in Ghana

Abstract

Increased cultivation of Cocoa has led to the conversion of over six million hectares of rainforest to cocoa farmlands in West Africa. This land use change may have implications for pollinating insects and the pollination services they provide to crops and wild plants. This study, conducted in Ghana, examined the effects of the changing proportion of natural forests in the landscape, due to cocoa expansion, on flower-visiting insects. Ground and aerial pan traps were deployed to capture flower-visiting insects across 18 sites selected along a gradient of an increasing proportion of natural forests. A total of 19,927 flower-visiting insects were collected across the rainy and dry seasons with the Order Diptera being most abundant. The ground pan traps were more effective in trapping different taxa of insects than the aerial traps, capturing 61% of the total insects. Furthermore, whereas trap type did not influence the abundance of Hemiptera, Coleoptera, and Hymenoptera, the abundance of Diptera was significantly related to trap type. Overall, it was observed that the Hemiptera, Coleoptera, and Hymenoptera increased in response to an increasing proportion of natural forests, but the Dipterans exhibited an inverse relationship. Further exploration of the bee data showed that natural forest and trap type did not significantly influence bee abundance, species richness, and Shannon diversity. However, bee community composition differed along the forest gradient. Overall, our results show that increasing proportions of natural forests are positively related to some groups of insects. Therefore, preserving patches of natural forest in cocoa plantations will guarantee the coexistence of diverse insect communities in cocoa and forest landscapes, and may help ensure sustained delivery of pollination services.

Keywords: Pan trap, Bee Bowls, Insect Pollinators, Bees, Cocoa, Land use change, Forest, Ghana

4.1 Introduction

Cocoa, which is rated among the 13 globally essential commercial commodities (Claus et al., 2018, Daniels, 2006), has become one of the leading causes of deforestation in the tropics (Yao Sadaïou Sabas et al., 2020, Ruf and Zadi, 1998, Ruf and Schroth, 2004). The increased cultivation of cocoa to meet global demand, which has been increasing at an annual average of 2.5% (ICCO, 2008), has led to the conversion of between 14-15 million hectares of tropical rainforest into cocoa farmland over the last 50 years (Somarriba and Lopez-Sampson, 2018). In West Africa, where 70% of the world's cocoa beans come from, between 7.5-10 million hectares of natural forest have been cleared to pave the way for cocoa cultivation (Wessel and Quist-Wessel, 2015, Adjaloo et al., 2012, Claus et al., 2018). Particularly in Ghana, this expansion has encroached upon numerous biodiversity-rich hotspots protected through designated areas (Hackman, 2014). As cocoa's global significance continues to rise and its cultivation expands leading to deforestation, especially in vital biodiversity-rich regions like Ghana, research into impacts on biodiversity is urgently needed to inform sustainable management and conservation.

Insect pollinators provide essential pollination services to both crops and wild plants, thereby sustaining human and other life in the general ecosystem (Singh and Adhikary, 2021, Potts et al., 2010). Approximately 87.5% of flowering plants benefit from biotic pollination (Ollerton et al., 2011), with the estimated combined global economic value of pollination services to crops estimated to range between US\$ 195 billion and US\$ 387 billion annually (Porto et al., 2020). Overall, insect-pollinated crops account for one-third of all food supply required by humans (Khalifa et al., 2021). Global estimates suggest that the absence of animal pollination services could lead to a substantial loss, ranging between 3% and 8%, of total agricultural production (Aizen et al., 2009). The substantial economic value attributed to pollination services underscores the vital significance of these services in ensuring healthy ecosystems and global food security.

The recently reported decline in insect pollinators around the world has raised a growing concern owing to their crucial role in sustaining biodiversity and global food

production (Stevenson et al., 2022, Potts et al., 2010, Hallmann et al., 2017). Evidence suggests that, over 50 years, some wild bees and other pollinators have become threatened with extinction suggestive of a pollinator crisis. The reported trends of insect pollinator declines emphasize the critical need for sustained and interdisciplinary research to comprehensively understand the complex factors driving these declines.

One of the leading drivers of pollinator decline is land use change, mainly due to its role in causing either habitat loss or fragmentation (Ramos-Jiliberto et al., 2020, Kovács-Hostyánszki et al., 2017, Lynch et al., 2021) and also because land use change for crop production may come along with increased pesticide uses (Tudi et al., 2021, Schreinemachers and Tipraqsa, 2012). The loss or fragmentation of habitats may reduce suitable habitat sizes and alter both biotic and abiotic habitat parameters potentially leading to the isolation or reduction of insect populations (Tscharntke et al., 2012b, Klein et al., 2002, Fahrig, 2003). This may be because of the adverse effects on the insect phenology, behaviour, and distribution patterns (Morante-Filho et al., 2016). Disruptions in the landscape adversely impact plant density, pollinator density, and pollinator behaviour (Hadley and Betts, 2012, Steffan-Dewenter and Westphal, 2008, Gebhardt et al., 2023, Millard et al., 2021). The impact extends to monoculture associated with land-use change, particularly affecting pollinators reliant on specific crops for resources (Brittain et al., 2013). The consequences stemming from land-use change, do not just threaten pollinator populations through habitat loss and fragmentation, but also resonate through altered phenology, behaviour, and distribution patterns, impacting both plant and pollinator communities.

Land use change, characterised by the loss of natural forests, may have implications on pollinators. Natural forests in agricultural landscapes are considered vital for supporting insect pollinators (Raderschall et al., 2021, Ricketts et al., 2008). Many native species build their nests in forested areas (Matheson et al., 1996, Ulyshen et al., 2023, Felderhoff et al., 2023) utilising these habitats as reservoirs for nest-building materials (De Marco Júnior and Coelho, 2004, Power et al., 2022). Nery et al. (2018) have suggested that areas with high proportions of natural forests tend to provide high resources for bees and other flower visitors. Forests serve as critical sources of forage

availability for pollinators, providing diverse floral resources and habitats essential for their sustenance and reproduction (Nery et al., 2018, Jha and Dick, 2010, Mensah et al., 2017, Danner et al., 2016). Understanding how pollinators respond to the loss of natural forests due to land-use change is essential for management purposes and therefore calls for scientific enquiries.

In exploring the dynamics of insect populations, sampling methods play a crucial role. Insect populations may be sampled using diverse methods, each varying in efficiency and effectiveness across different habitats (McCravy, 2018, Hyvärinen et al., 2006). Pan trapping emerges as the preferred technique for sampling bees globally, offering broad coverage, high species collection, reduced biases, and the ability to detect similar species (Westphal et al., 2008). In tropical rainforests, where many pollinating insects are canopy feeders, elevated pan traps enable the assessment of vertical dispersal within the habitat (Bağ-Badowska, 2012, Nuttman et al., 2011, LeBuhn et al., 2016). While pan traps are widely used for insect sampling, they favour colour preferences, limiting their representation of pollinator diversity (Campbell and Hanula, 2007, Vrdoljak and Samways, 2012). The attractiveness of certain colours may therefore disproportionately attract or deter specific insects, potentially leading to skewed abundance and diversity estimations. The selection of the right traps is therefore very pivotal for obtaining representative insect communities and achieving study objectives (Leather, 2008, Doxon et al., 2011). Recognizing the inherent biases of different sampling methods, researchers, as emphasised by Doxon et al. (2011), should carefully select techniques aligning with study objectives. The selection of trapping methods, with a preference for pan trapping in this context, is vital for revealing comprehensive insights into populations of flower visitors.

An understanding of the impact of land use change on insect pollinators is particularly relevant when those changes are driven by the expansion of agricultural cultivation of crops that depend on pollinators. Therefore, this study aimed to assess how changes in landscapes, particularly the loss of natural forests due to cocoa expansion, affect flower-visiting insect communities in Ghana. Additionally, the differences between aerial and ground pan trap sampling were investigated. The study addressed four main research questions: 1) How does the abundance of different insect taxa relate to the changing proportion of natural forests in cocoa-growing landscapes? 2) How are the

species richness, abundance, and diversity of bees influenced by the changing proportion of natural forests in cocoa-growing landscapes? 3) How does bee community composition vary in response to the changing proportion of natural forest in cocoa-growing landscapes? and 4) To what extent does the trap type influence insect abundance, bee species richness, and diversity in cocoa-growing landscapes?

4.2 Materials and Methods

4.2.1 Study area

The study was conducted in and around Bia Conservation Area (BCA), a wildlife-protected area lying in two districts, namely Bia West and Juaboso Districts of the Western North Region of Ghana (Figure 1). BCA also falls under the traditional authority of the Sefwi-Wiawso Traditional Council at Sefwi-Wiawso (Wildlife Division, 2010). Characterised by a moist evergreen forest, BCA experiences bi-modal rainfall, primarily occurring from May to June and September to October. The surrounding area comprises approximately 42 communities with varying population sizes (Wildlife Division, 2010). The main economic activity for the inhabitants in the vicinity of the protected area is agriculture (West, 2022, GSS, 2014). The two forests, which previously shared boundaries with BCA, Sucusuku Forest Reserve and Bia Tawya Forest Reserve, have been cleared to pave the way for cocoa cultivation, leading to a landscape dominated by cocoa plantations outside of BCA (Wildlife Division, 2010). Approximately 53% of the study area is now covered by cocoa plantations, including both fullsun cocoa monoculture and agroforestry cocoa which is integrated with other tree crops. In addition to cocoa, the area also produces other major crops, including palm trees, maize, cassava, cocoyam, and plantains. These crops contribute significantly to the agricultural activities and livelihoods of the communities surrounding BCA.

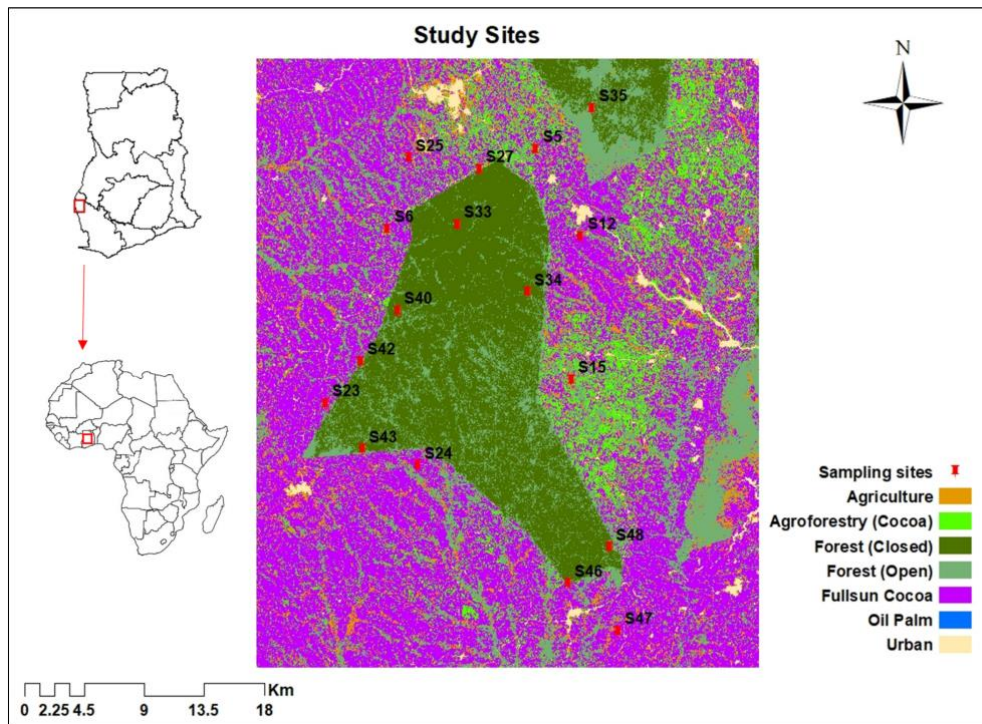


Figure 4.1 Study area. Eighteen study sites with varying proportions of natural forests within a 2 km buffer around each site (range 9-100%) located within and around Bia Conservation Area, at Bia West District in Ghana, were selected for the study.

4.2.2 Sampling sites

Eighteen sites were selected along a gradient of increasing proportion of natural forest cover for this study. The land-cover map developed by Satelligence (2019), alongside field visits, was used to select 48 co-ordinates/sites which were kept a minimum of 4 km apart to ensure spatial independence based on the foraging distances of both social and solitary bees which tend to localise their foraging activities within 2 km around the hive location (Garbuzov et al., 2015, Steffan-Dewenter and Kuhn, 2003, Visscher et al., 1996). Land use types in the landscape included agriculture, agroforestry cocoa, closed forest, open forest, fullsun cocoa, oil palm plantation, and urban settlements (Figure 4.1). An analysis of habitat types was carried out using the ArcMap to establish the percentage of each dominant habitat/vegetation type around each study site within a 2 km radius. Appendix D2 shows the proportion of each habitat type within each 2 km radius around each study site based on the analysis conducted and shows sites dominated by cocoa plantations and those dominated by natural forests. Natural forests included both open and closed forest categories combined. Principal Component Analysis (PCA) was then used to select 18 sites along the

dominant gradient of forest cover within the landscape at a 2 km radius while keeping other land uses as consistent as possible (Appendices D1-2; Figure 4.1). Sites were then ground-truthed to ensure forests also met international definitions as being areas spanning more than 0.5 hectares where native species or trees taller than 5m spontaneously developed, free from human influence, and with canopy cover exceeding ten per cent (Kuuluvainen and Aakala, 2011, GEMET, 2023, FAO, 2023).

4.2.3 Sampling of flying insects

Overall, the eighteen study sites were sampled on four different sampling periods between 2021 to 2022, to capture temporal fluctuations in pollinator communities (Thompson et al., 2021, Graham et al., 2021, Banaszak et al., 2014). The initial two sampling periods ran from September to October 2021, during the rainy season whilst the third and fourth sampling periods took place in the dry season, specifically in January and February 2022. To capture a comprehensive representation of the insect communities, we employed both ground and aerial pan traps (bee bowls) (Westphal et al., 2008, Nuttman et al., 2011, Bąk-Badowska, 2012) because in tropical rainforests, many bees and other insect pollinators are canopy feeders (LeBuhn et al., 2016). The ground pan traps (GPT) were placed in low vegetation at the height of the surrounding vegetation (Figure 4.2). The aerial pan traps (APT) were carefully guided and hung in trees at 20 to 30 m high to enhance the visibility of flower pollinator visitors in tree canopies (Nuttman et al., 2011) (Also see Appendix D3). Each trap consisted of three bowls, which were UV sprayed painted in white, blue and yellow colours which have been established as standard colours for capturing a wide array of species (Leong and Thorp, 1999, Westphal et al., 2008). The bowls used to make the traps were 6" in diameter at the tip, 5" deep and 4" at the base. Even though pan traps are designed to attract flower-visiting insects and were deployed in our study we took notice of the fact that they are not the optimal method when it comes to capturing butterflies and moths (Shrestha et al., 2019, Kral-O'Brien et al., 2021).



Figure 4.2 Mounted pan traps: A) An aerial trap (APT) consisting of a metal frame with three bee bowls (blue, white, and yellow) kept 30cm apart. The order of the bowl colours was randomised among the three aerial traps per site; B) APT hoisted on an emergent tree branch in the tree canopy. Both ends of the fishing line used to raise the aerial pan traps were firmly tied on the ground to provide stability to pan traps against animal or wind disturbances; C) A triangular ground pan trap (GPT) which was fitted to a polyvinyl chloride pipe to prevent ground insects from climbing into the bowls.

At every sampling site, a 100 m transect oriented along an East-West axis was established, with the GPS coordinate of the site precisely aligning to the 50 m mark on the transect (Appendix D4). Along the transect, at the 0 m, 50 m, and 100 m marks, one aerial and one ground trap were, resulting in 3 aerial and 3 ground traps per site (total of 18 bowls) (Droege, 2005). The bowls were half-filled with water, and a small amount of unscented detergent was added to disrupt surface tension. Additionally, we created small drainage or overflow holes at the top edges of the bowls (sized such that bees could not pass through) to allow water to drain out during overnight showers. The pan traps were exclusively deployed during favourable weather conditions in the morning, specifically between 7:00 AM and 10:00 AM, when temperatures ranged from 26°C to 29°C. After placement, the traps were left for 72 hours, following which they were removed in the same order they were initially set. The collected samples were carefully sieved to separate the insects and subsequently preserved in 70% ethanol (Figure 4.3). These samples were then transported to the entomological laboratory at

the University of Cape Coast, Ghana, where the specimens were sorted into different insect orders.



Figure 4.3 Field and lab work: A) Remote places on cocoa farms reached on motorbikes to set pan traps; B) Collected specimens in a bowl; C) Collected specimens being sieved on the field; D) Alcohol being poured on bottled to preserve it during transport; E) Collected bees being separated and dry mounted for identification and F) Sorting of specimen at the Department of Conservation Biology at the University of Cape Coast into the different insect orders.

4.2.4 Specimen curating and identification

During sorting, the abundance of insects in each bowl was recorded at the order level. The main orders found were Diptera, Hemiptera, Coleoptera, Hymenoptera, and Lepidoptera, while any other orders were grouped as “others”. All bees collected were then separated and subsequently dry-mounted for identification (Nuttman et al., 2011). All bees collected were identified to the morphospecies level. The bee identification was undertaken using existing taxonomic keys and descriptions by Eardley and Urban (2010) and Combey and Kwapong (2016). Insects collected were deposited at the insect museum at the Department of Conservation Biology and Entomology, University of Cape Coast, Ghana where the bee identification was conducted.

4.2.5 Data analysis

All statistical analyses in this study were conducted using R version 4.2.2 (R Core Team, 2022). GLMMs were employed to assess the relationship between the abundance of each insect order and the proportion of natural forest within a 2 km radius using the 'glmmTMB' package (Brooks et al., 2017), with each order analysed separately. Insect abundance was used as the response variable and was calculated using the “dplyr” package (Wickham et al., 2019), pooling together data at the trap type level in each season, resulting in 4 data points per site (two APT points and two GPT points). The calculations were based solely on the available data after the missing values, caused by the accidental pouring of the content of one aerial trap at one site, (i.e., 3 bowls in one aerial trap) were removed from the dataset (indicated as NA). The impact of the missing data on our results was assessed by estimating the missing data using Multiple Imputation by Chained Equations (MICE) (Van Buuren and Groothuis-Oudshoorn, 2011, Royston and White, 2011, Zhang, 2016). There was no difference between datasets with or without the estimated missing data; therefore, all analyses were restricted to only the original dataset with the missing data for one trap. The proportion of natural forest within the 2 km around study sites, trap type and their interaction were used as fixed effects whereas the site was specified as a random intercept nested within the season, to account for variability among sites. Model fit was evaluated using diagnostic tools such as QQ plots of residuals, residuals vs. predicted

values, and tests for overdispersion, homogeneity, and zero inflation provided by the “DHARMA” package (Hartig, 2022). Models were initially fitted with Poisson distribution but were updated to negative binomial distribution 2 (estimated using ML and nlminb optimiser) where necessary to improve model fit. Two models, one involving natural forest with an interaction term with the trap and another without an interaction term, were fitted following which the ‘AUCcmodavg’ package (Mazerolle and Mazerolle, 2017, Mazerolle, 2020) was applied for model selection. The Akaike Information Criterion with a correction for small sample sizes (AICc) was used to determine the better parsimonious model (Harrison et al., 2018, Bevans, 2022). Model performance was then assessed after which the 'predict' function was used in conjunction with the “ggplot2” package (Wickham et al., 2021) to visualise the impact of the fixed effects on the predicted insect abundances, at 95% confidence intervals. Capture per trap and seasonal distributions were subjected to statistical analysis to explore potential differences in insect abundance.

We also explored the influence of the proportion of natural forests specifically on bee abundance, species richness and Shannon-Weiner diversity (hereafter referred to as the Shannon diversity). As with the insect abundance, data for each site were pooled at the trap type level within each season resulting in four data points per site. The “vegan” package (Oksanen et al., 2017) was used to calculate the species richness and Shannon diversity. The same model structure and procedure, as described above for insect abundances, was applied to the species richness owing to its inherent nature as count data (Zeileis et al., 2008, Maxwell et al., 2018, Zuur et al., 2009). Model fit was attained using the Poisson distribution for the species richness. Unlike species richness, a linear mixed model (LMM) was chosen for the analysis of the Shannon diversity, which is a continuous variable (Nolan and Callahan, 2006, Ramezani, 2012) using the “lme4” package (Bates et al., 2014). Shannon diversity was square-root transformed to improve model fit.

A dedicated analysis was conducted to assess the adequacy of our bee sampling efforts in capturing the regional diversity. First, we generated a species richness curve using the vegan function "spacaccum." This visualization allowed us to observe the accumulation pattern of bee species throughout our sampling efforts. Additionally, in response to the need for a more comprehensive understanding of total species

richness, an abundance-based coverage estimator, namely AC and Chao, which tends to give satisfactory estimates (Rosenberg, 2005) was used to estimate potentially possible species richness present at a regional level. The total bee species richness per site across all sampling sessions was used for this analysis.

Bee community composition was visualised using Non-Metric Multidimensional Scaling (NMDS) (Zuur et al., 2007). Community matrices were generated to represent the abundance of all bee species pooled at the site level (i.e., across both seasons and trap types). The *decostand* function of the “vegan” package was then used to apply the Hellinger transformation to standardise both the dominant and rare species to ensure that the true ecological patterns were captured. The integrated ‘metaMDS’ function in the *vegan* package was used to fit the NMDS (Oksanen et al., 2013). The output of the NMDS was then utilised to represent the gradient in forest cover around each sampled site through colouration, employing the ‘ggplot2’ package (Wickham et al., 2021). The stress value of a Shepard diagram generated using the ‘shepard’ function in the BinMat package to evaluate the goodness-of-fit was 0.1363, indicative of a satisfactory representation of the bee community composition. The Spearman correlation for the Shepard diagram was computed at 0.8, further affirming the robustness of the ordination in capturing the underlying patterns in species composition. The observed high correlation (close to 1) implies that the NMDS distances accurately represent the dissimilarity structure in the original data.

The Mantel test, using the ‘mantel’ function in the *vegan* package, was then employed to assess whether there was a significant association between the similarity in bee community composition and changes in the proportion of natural forest and whether geographic proximity corresponds to similar bee community composition. To achieve this, the geographic coordinates were extracted from longitude and latitude data and organised into a data frame. The dissimilarities in bee abundance along the 18 sites were quantified across different species from a distance matrix, which was computed using Bray-Curtis dissimilarity with the function ‘vegdist’ from the *vegan* package. The proportion of natural forest was then converted into a distance matrix using Euclidean distance by using the function base R function ‘dist’. The conversion of the proportion of natural forest into a distance matrix using Euclidean distance was performed to represent the dissimilarity between sites based on their forest cover. The geographic

data were then employed to compute a Haversine distance matrix, representing the great-circle distance (in kilometres) between locations on the Earth's surface using the 'distm' function in the geosphere package (Hijmans et al., 2017) for the analysis. A pairwise plot was then generated to visualise the relationship between the proportion of natural forest and Bray-Curtis dissimilarity in species composition represented as a pairwise comparison between each of the 18 sites, with the distance between sites represented by colour.

4.3 Results

4.3.1 Insect abundances

Overall, we collected a total of 19,661 individual insects representing 15,947 Diptera, 2603 Hymenoptera, 723 Coleoptera and 388 Hemiptera (Table 4.1; See Appendix D5 for further details on Lepidoptera). The ground pan traps outperformed the aerial pan trap by collecting 61% of the total insects. The abundance of insects collected in the rainy season (51%) was higher than in the dry season but was not significantly different. Among the bowl colours, the highest number of insects were collected in the yellow bowls (42%) followed by white (30%) and blue (27%; See Appendix D6 for the full dataset containing both the insect abundances and geographic information).

Table 4.5 An overview of the total abundances of each insect order, organised by trap type and season. The table displays the breakdown of the counts for each insect order. The totals are first broken down by trap type used and per season.

Trap/Season	Coleoptera	Hemiptera	Diptera	Hymenoptera
APT	362	184	5823	1324
GPT	361	204	10124	1279
Dry season	392	292	7019	1833
Rainy season	331	96	8928	770

(GPT= Ground pan trap; APT=Aerial pan trap)

The abundances of Hemiptera, Coleoptera, and Hymenoptera were positively related to the proportion of natural forest, but the Dipterans showed a negative relationship

(Table 4.2; Figure 4.4). Among the different insect orders, trap type was found to be a significant predictor of only the Diptera abundance, with more individuals caught in ground traps (Table 4.2 & Figure 4.4). There was no significant interaction between the proportion of natural forest and trap type for any of the insect orders.

Table 4. Summary of test results of the effects of natural forests and trap types (APT and GPT) on the abundances of the different insect taxon. The chi-square statistics (Chisq), and degree of freedom (DF) alongside the significance levels denoted by asterisks (*p<0.05; **p<0.01; ***p<0.001) are displayed.

Response	Predictor	Chisq	Df	Pr >Chisq
Coleoptera	NF	6.51	1	0.01*
	Trap	0.03	1	0.09
Diptera	NF	15.24	1	<0.001***
	Trap	48.44	1	<0.001***
Hemiptera	NF	24.58	1	<0.001***
	Trap	0.12	1	0.73
Hymenoptera	NF	10.37	1	0.001**
	Trap	0.01	1	0.92

(NF=Proportion of natural forest)

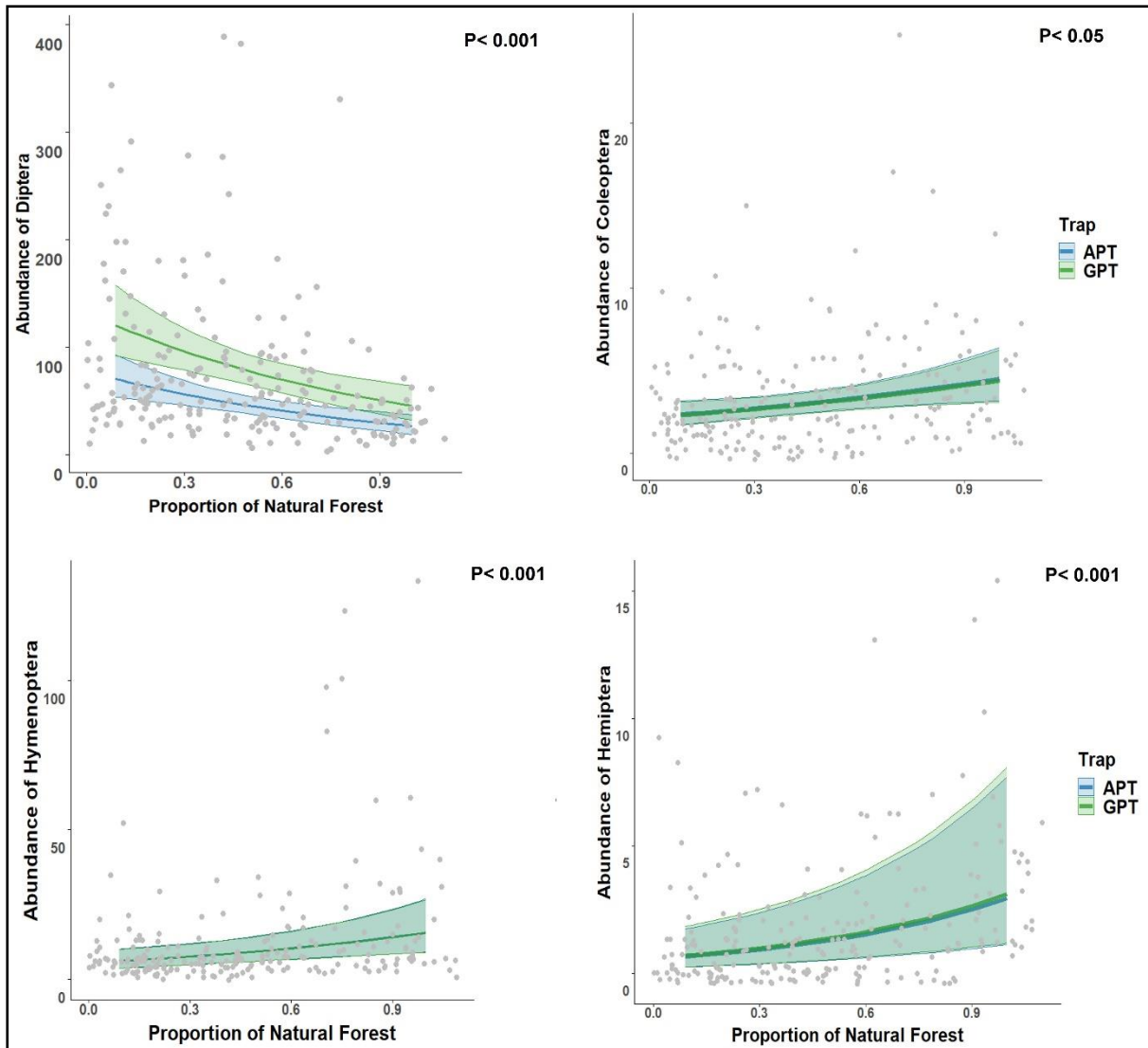


Figure 4.4 Diptera (top left); Coleoptera (top right); Hymenoptera (bottom left) and Hymenoptera (bottom right) as predicted by natural forest. A generalised linear mixed model (GLMM)-derived smoothed trend line with a $\pm 95\%$ confidence interval and aerial pan trap (APT) and ground pan trap (GPT) represented by blue and green colours respectively are shown.

4.3.2 Bee abundance, species richness and diversity

After four rounds of sampling across two seasons, a total of 332 individuals of 23 bee species were collected across all the study sites. Most bees (60%) were collected in the dry season (See Appendix D7) while the ground pan traps collected a greater percentage (59%) of total bee abundance. No significant difference was observed between the observed species richness and the estimated species richness across the whole sampling region (Welch Two Sample t-test, $t = 1.9401$, $df = 4.0416$, $p =$

0.1236) indicating sufficient sampling effort (See Appendix D8). The estimated species richness ranged between 26.06 (boot estimator) and 33.32 in the total bee community (Jack1 estimator).

Overall, three bee families, the Apidae, Halictidae and Megachilidae of the seven known African families, were recorded throughout this study. The most dominant species were the *Compsomelissa nigrinervis* (Smith, 1859) (28%), *Apis mellifera* (23.1%), *Braunsapis facialis* (16.3%) and *Meliponula (Axestotrigona) ferruginea* (11.1%). Among the bee species identified, this was the first time the carpenter bee *Xylocopa flavonifa* was recorded in Ghana's wet evergreen forest ecosystem (Figure 4.5). The proportion of natural forest in the landscape and trap type were not related to bee abundance (forest: $\chi^2=2.03$, $df=1$, $p=0.15$, trap: $\chi^2=0.7854$, $df=1$, $p<0.38$) species richness (forest: $\chi^2=0.4477$, $df=1$, $p=0.50$; trap: $\chi^2=1.28$, $df=1$, $p=0.26$) or Shannon diversity (forest $\chi^2=0.68$, $df=1$, $p=0.41$; trap: $\chi^2=2.32$, $df=1$, $p=0.13$) (Figure 4.6).



Figure 4.5 Samples of bee species identified. A) *Ceratina calcarata*, B) *Xylocopa flavonifa* (De Bees), the first time to have been identified in tropical evergreen forest in the southern part of Ghana; C) *Dactylurina staudingeri*; D) *Lipotriches cirrita* of the Halictidae family; E) *Meliponula ferruginea* of the Apidae family and F) *Xylocopa nigrita* of the Apidae family

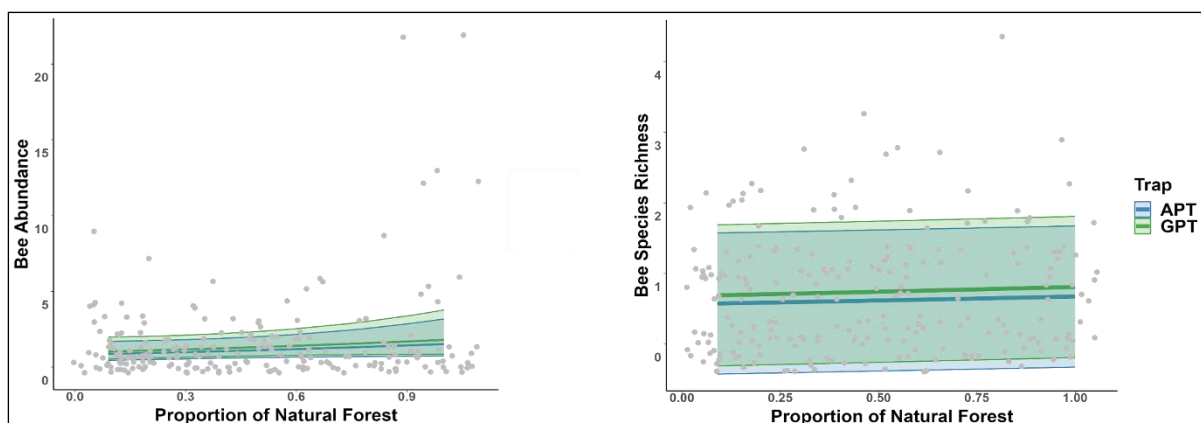


Figure 4.6 The graphs illustrate the predicted effects of the proportion of natural forest on bee abundance (left) and species richness (right), differentiated by trap types. A generalised linear mixed model (GLMM)-derived smoothed trend line with a $\pm 95\%$ confidence interval and aerial pan trap (APT) and ground pan trap (GPT) represented by blue and green colours respectively are shown. Raw data points are also included, providing insight into the observed richness with jittered points for clarity and the colours distinguish between trap types.

4.3.3 Patterns in bee species composition

The NMDS plot (Figure 4.7) illustrates some of the key species that may be driving the similarities in sites characterised by a higher proportion of natural forest; for example, species like *Dactylurina staudingeri* (Dacty), *Meliponula ferruginea* (MeliF), *Halictus jucundus* (Hali) and *Xylocopa varipes* (Varipes) all seem to be particularly influential. On the other hand, *Lasioglossum duponti* (Lasio), *Lipotriches cirrita* (Cribo), *Ceratina calcarata* (Cere) and *Lipotriches orientalis* (Lipsp) are all influential in driving the similarities between sites with lower proportions of natural vegetation. The stress value, denoted as 0.1363 on the plot, signifies a satisfactory representation of the bee community composition. (See Appendix D9 for the Shepard Diagram evaluating the NMDS goodness-of-fit).

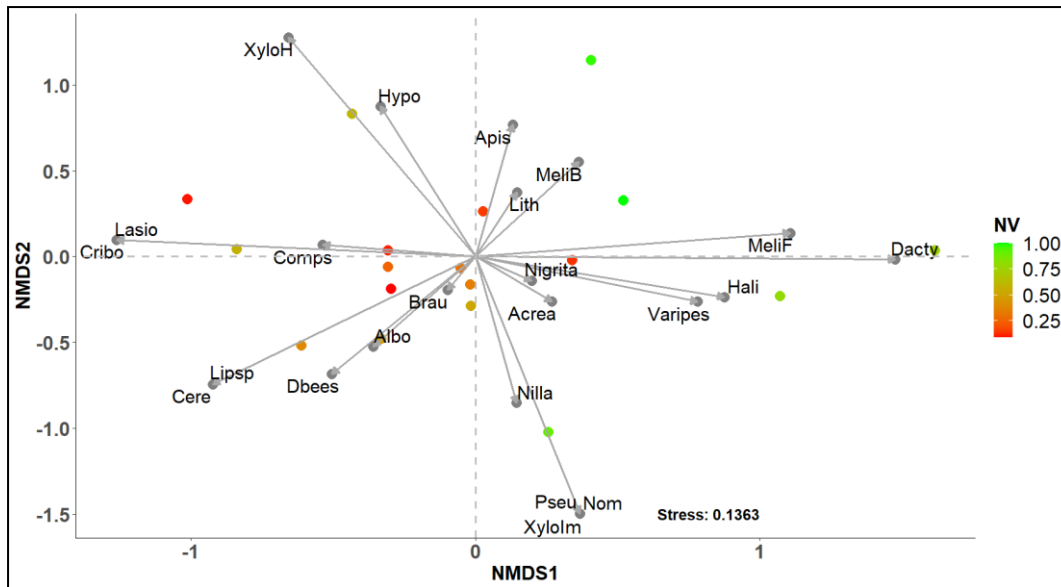


Figure 4.7 An NMDS plot showing a two-dimensional space visualisation of species and sampling sites based on their compositional dissimilarities. The sampling sites are represented as points (coloured by the proportion of natural forest at each site), while arrows indicate the position of individual species about the origin (0,0). The dashed lines intersecting at the origin aid in interpreting the directionality of the species' dissimilarity. (See Appendix D7 for the full names of the abbreviated bee species names).

From our results, we observed that the environmental matrix (natural forest) exhibited a strong correlation with the species Bray-Curtis dissimilarity matrix (Mantel $r=0.41$ $p=3e-04$) indicating that sites with similar bee communities tend to have a similar proportion of natural forest (Figure 4.8). In contrast, the species Bray-Curtis dissimilarity matrix and the geographic matrix were not significantly related (Mantel $r = -0.08$, $p=0.66$). This lack of relationship suggests that distance between sites was not a predictor of bee community composition. Instead, the proportion of natural forest has an overriding impact on the patterns in bee community composition.

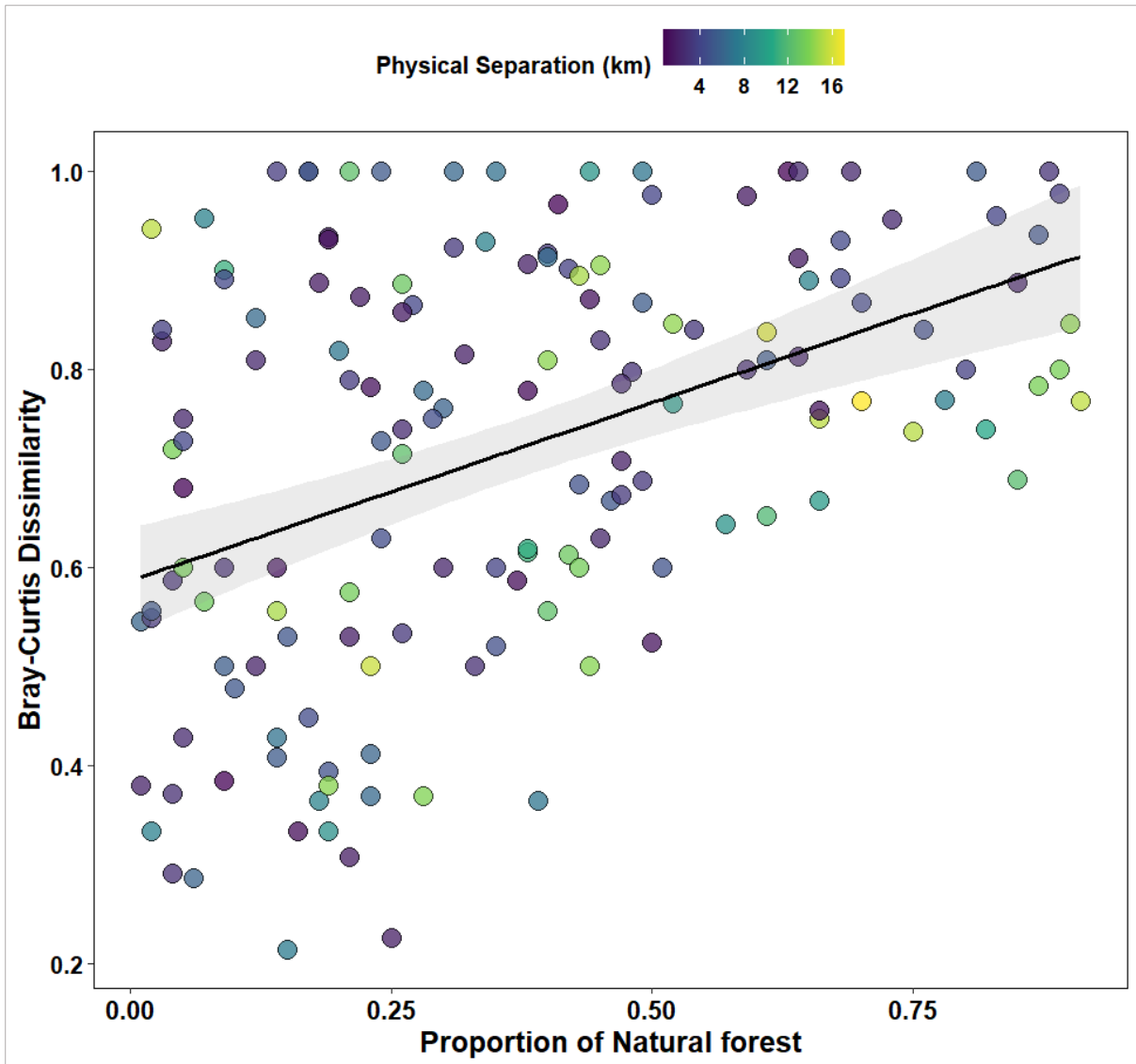


Figure 4.8 A pairwise comparison of community dissimilarity and changing proportions of natural forests with an overlay of the geographic distance. Each point represents a pairwise combination of 18 sampled sites (324 pairwise combinations in all). The linear regression line (black) with a shaded confidence interval, provides insights into the trend. The x-axis and y-axis denote the proportion of natural forest and Bray-Curtis Dissimilarity, respectively. Points colour gradients in the legend depict greater physical separation. The plot highlights potential patterns and trends in the dissimilarity of bee compositions as dictated by the proportion of natural forests.

4.4 Discussion

In this study, we evaluated the influence of changing proportions of natural forests, brought about by cocoa expansion, on insect pollinators. Our findings show a positive association between the abundance of Hymenoptera, Hemiptera, and Coleoptera and the proportion of natural forests. This finding is consistent with other studies where natural forests have been positively associated with the abundance of insect pollinators (Kambach et al., 2012, Montes et al., 2022, Forrest et al., 2015, Vance et al., 2014, Smith et al., 2021), which may be linked to forests providing important nesting resources (De Marco Júnior and Coelho, 2004, Power et al., 2022) or floral resources (Nery et al., 2018). Notably, in tropical rainforests, many canopy-feeding insects exploit the rich resources available in the upper canopy, including diverse plant species and foliage and this specialised feeding strategy allows them to access a unique array of nutrients and plant compounds which may not be on the ground (Lowman, 2009).

However, not all studies show positive relationships between flower-visiting insects and forests. For example, Winfree et al. (2007) and Toledo-Hernández et al. (2016) reported an increased abundance of insect pollinators on farmlands as opposed to areas with increasing forest cover. Evidence suggests that where human disturbances result in increasing levels of food resources, the pollinator per flower visit may not be affected (Samejima et al., 2004). However, the conversion of natural forests to accommodate cocoa cultivation may not necessarily translate into floral resources for insect pollinators in cocoa landscapes. According to Adjaloo and Oduro (2013), the floral structure of cocoa inhibits access to many cocoa flower visitors. This observation is supported by O'Doherty and Zoll (2012) who asserted that the evolutionary structure of the cocoa flower makes it unsuitable for many insects to pollinate it. It thus appears that the increasing abundance of Hymenoptera, Hemiptera, and Coleoptera in correlation with an increasing proportion of natural forests suggests a potential association with the availability or accessibility of greater floral resources in natural forest landscapes compared to monoculture cocoa landscapes. Thus, the specific characteristics of cocoa landscapes may not provide the necessary floral resources, thus impacting insect abundance compared to more natural forest environments.

Unlike the Hymenoptera, Hemiptera and Coleoptera, the abundance of the Diptera had an inverse relationship with the increasing proportion of natural forests. In the landscapes dominated by cocoa cultivation, the increased abundance of Diptera may be linked to cocoa expansion. Cocoa, being an insect-pollinated crop, primarily relies on ceratopogonid midges (*Forcipomyia*) as its primary pollinators (Winder, 1978, Tscharrntke et al., 2012a, Young, 1982, Adjaloo and Oduro, 2013). Ceratopogonid midges, classified within the order Diptera (Borkent and Wirth, 1997) were observed to be more abundant in cocoa farms compared to areas with increased forest cover. However, it is acknowledged the taxonomic resolution in our samples may pose limitations on the precision of these findings, and further investigation with higher taxonomic resolution would be beneficial to enhance the robustness of these observations. This finding contrasts with the conclusions reached by Kaufmann (1975) and Young (1986) that midges are recognised for their widespread distribution in highly diverse tropical forests characterised by multi-strata vegetation which provide favourable conditions, including cool and dark habitats, as well as organic substrates suitable for their development. The tropical forest in our study area may be exhibiting greater heterogeneity through cocoa expansion or variation in microhabitats that could influence midge distribution differently than the more homogeneous forests studied by Kaufmann and Young. Our observation of the abundance of Diptera is supported by Harrison et al. (2018b) that some insects become beneficiaries of some form of human-induced landscape modifications. The differential responses to the changing proportion of natural forest observed in the studied insect orders indicate that different species respond differently to anthropogenic drivers.

The findings of this present study, revealing no significant influence of changing proportions of natural forests through cocoa expansion on bee abundance, species richness, and Shannon diversity, challenge conventional assumptions and present intriguing deviations from previous research. Our finding is a sharp departure from studies which highlight a positive relationship between bee abundance, species richness and diversity indices with natural forests (Carper et al., 2014, Ricketts, 2004, Ockermüller et al., 2023, Knoll and Penatti, 2012) or the reverse through loss of natural forest such as the study by Zattara and Aizen (2021) which confirmed negative impacts on pollinator communities. Again, our results contrast the conclusions reached by Winfree et al. (2007) that bee abundance and species richness decrease with

increasing forest cover in the surrounding landscapes. Several hypotheses are relevant to the observations made in our study. According to Klein et al. (2002), some bee species may not necessarily experience declines in response to habitat modifications. The observed effects of changing proportion of natural forests on the diversity metrics of bees such as abundance, species richness, and the Shannon index suggest some measure of persistence even in areas undergoing significant land use change (Henle et al., 2004). The observed lack of effects of land use change on bee abundance may also be attributed to the unique dynamics of cocoa-growing landscapes, where the interplay between land use changes and bee populations may differ from other crop types. Besides natural forests in the landscapes, agroforestry cocoa cultivation is practised in the landscape and entails the integration of trees or forest patches in cocoa cultivation. The tree or forest patches in cocoa landscapes may serve as a sanctuary for bees (Chacoff and Aizen, 2005, Carvalheiro et al., 2010). The effects of land use changes on bees may therefore be crop-dependent, and contingent on the specific nature of the alterations in the landscape. The complexity of these relationships is further underscored by the shifting responses of bee populations within cocoa-growing landscapes. However, this study did reveal an effect on the overall composition of bee communities. However, it is acknowledged that the traditional diversity metrics alone may not capture the full extent of the ecological implications of decreasing the proportion of natural forests through cocoa expansion (Dormann et al., 2007). Our result therefore highlights how crucial it is to consider not just the traditional diversity metrics but also the larger ecological context when evaluating how habitat changes affect bee populations. Changes in bee community composition over time or in response to land use change may help reveal the extent of general changes in the environment. Since some bee species may be resistant to particular environmental stressors, they are useful markers of an area's general ecological stability (Hopfenmüller et al., 2014).

From our results, we observed that the Bray-Curtis dissimilarity matrix (bee community composition) showed a strong correlation with the environmental matrix (natural forest) (Mantel $r=0.41$ $p=3e-04$) indicating that sites with similar bee communities tend to have a similar proportion of natural forest. The study aligns with and reinforces existing research, showcasing how bee community composition is influenced by the natural forest (Brosi et al., 2007, Ealy et al., 2023, Mayes et al., 2019). Bee species, including

Apis mellifera adansonii, *Meliponula ferruginea*, and *Meliponula bocandei* were found to drive composition in sites with higher proportions of natural forests (Figure 4.7). This may suggest their preference for sites with higher proportions of natural forests. The *Meliponula ferruginea*, a stingless bee, is commonly found in tropical forests making its nests in tree cavities or soil (Heard, 1999). The *Meliponula bocandei*, also a stingless bee, is commonly found in tropical regions and may utilize tree hollows for nesting (Roubik and Roubik, 1992). The honeybee, *Apis mellifera adansonii*, is a generalist species and has been found to thrive in different landscapes including natural habitats and agricultural landscapes (Winston, 1991). Our observation of bee species driving composition in less forested sites also highlights some of their general characteristics. *Xylocopa nigrita*, *Xylocopa varipes*, *Xylocopa hottentotta*, *Xylocopa imitator*, and *Xylocopa flavonifa* found to drive sites with less proportion of forest, are typically found in open habitats, including fields and open forests (Michener, 2000). Similarly, *Amegilla nila*, *Amegilla albocaudata*, and *Amegilla acraensis* are known to forage in different landscapes, including gardens and agricultural fields (Michener, 2000, Buadu, 2016). *Lithurgus pullatus* are found in open habitats and tend to utilize plant stems for nesting (Litman, 2012). *Braunsapis facialis* is a ground-nesting bee generally found in open habitats such as grasslands (Michener, 2000). Overall, our study revealed the effects of natural forest in dictating bee community composition and this highlights how certain bee species, including those that are generalists and habitat-specific ones, may be associated with different land uses.

The ground pan traps (GPTs) demonstrated superior performance, capturing 61% of total insects, highlighting the critical role of trap selection (Su and Woods, 2001, Yi et al., 2012). However, among the different insect orders, only the abundance of Diptera was found to be predicted by the trap type even though no significant interaction between the proportion of natural forest and trap type for any of the insect orders was observed (Table 4.2 & Figure 4.4). Variations in sampling outcomes may be attributed to elevation and possibly to trap designs. The triangular design of the GPT, offering multiple entry points with bowls independently oriented towards the sky, potentially rendered it more attractive and efficient for insect capture. In contrast, the vertical arrangement of the aerial pan trap (APT) restricted access to flying insects, particularly those adapted to higher elevations. Furthermore, the GPT was less susceptible to wind disruption compared to the APT interference (Tuell and Isaacs, 2009). While the

GPTs showed a pronounced increase in Diptera abundance (Table 4.2), no interactions between trap type and forest proportion on insect abundance were observed, emphasizing the independent influences of each predictor. Our study highlights the necessity for methodological consistency and awareness of trap-specific biases when comparing and interpreting insect abundance across diverse ecosystems. The observed preferences for yellow bowl colours align with the importance of visual cues for flower-visiting insects (Saunders and Luck, 2013, Vrdoljak and Samways, 2012, Grootaert et al., 2010). The performance of the ground pan traps (GPTs) and trap influence on the abundance of Diptera highlight the importance of trap selection in insect abundance studies.

Moreover, a combination of pan-trap with other sampling techniques, such as sweep netting, could have been complementary in sampling the insect fauna (Wilson et al., 2008). However, even though sweep netting is a widely-used method for sampling insects (Berglund and Milberg, 2019, Prendergast et al., 2020, Doxon et al., 2011), it was not considered in our study due to its limitations in reaching the tree canopies within our forested and cocoa landscapes. Besides, there is no protocol on how to use a sweep net for sampling in the tropics (Prado et al., 2017). As a sampling technique that is typically restricted to ground-level sampling, sweep netting would have been inadequate for capturing the full spectrum of insect diversity in the vertical plains present in our study sites, particularly those inhabiting higher elevations and canopy environments. Therefore, we opted for trap-based methods specifically designed to attract and capture flower-visiting insects and are capable of being elevated to allow assessment of the vertical distribution of insect taxa in different habitats (Bağ-Badowska, 2012) for a more comprehensive sampling approach aligned with the objectives of this study.

4.5 Conclusion

Our study evaluated the shifting responses of flower-visiting insects to the changing proportion of natural forest within cocoa-growing and forest landscapes in Ghana. Beyond contributing to the advancement of our knowledge and understanding of insect dynamics within cocoa-growing landscapes, this study also provides a foundation for further research aimed at reconciling agricultural productivity with the preservation of

flower-visiting insects. The positive correlations between insects of the order Hymenoptera, Hemiptera and Coleoptera orders and natural forest cover highlight the potential benefits of natural forest to many diverse insects. Nevertheless, the inverse response observed with an abundance of the insect taxon Diptera and the lack of a significant correlation between bee diversity metrics and influence on bee community composition by natural forest showed that land use changes do not affect all insects equally prompting a reconsideration for well-designed and targeted conservation efforts that account for the specificities of cocoa cultivation. With demand for cocoa products rising on an annual basis, which is likely to usher in the accelerated conversion of natural forests to pave the way for cocoa cultivation, conservation efforts should consider the context-specific responses revealed in this study, emphasising the need or importance of maintaining natural forests within cocoa landscapes for sustaining diverse insect communities.

4.5 Acknowledgements

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Chapter 5 General Conclusions

“Man in his misguidance has powerfully interfered with nature. He has devastated the forests, and thereby even changed the atmospheric conditions and the climate. Some species of plants and animals have become entirely extinct through man, although they were essential in the economy of Nature”

Johann Wolfgang von Goethe (1749-1832)

Chapter 5 General Conclusions

5.1 Overview

This thesis aimed to explore the nexus among cocoa expansion, insect pollinators, pesticide use and honey contamination from plant protection products. Three studies, consisting of two field experiments and a review study were conducted as part of this thesis with each study addressing key components of the overall aim of the thesis. In Chapter 2, I used a systematic literature review approach to evaluate the extent to which existing studies have analysed honey contamination from plant protection products approved for cocoa (*Theobroma cacao*) cultivation. In this review study, I examined both the time-frame and geographic scopes of the existing studies, prevalence, types and concentrations of pesticides reported in cocoa and non-cocoa growing countries and the potential implications of the concentrations of identified pesticides on insect pollinators and human health, employing the European Union's maximum residue limits (MRL) as a benchmark. Through this study, I detected disparities in the number of existing studies between cocoa and non-cocoa growing countries with 81% of existing studies taking place in the latter. Chapter 3 extended my investigation into pesticide residues in honey through a field experiment where I collected honey samples from fullsun cocoa, agroforestry cocoa and natural landscapes in Ghana and analysed for the presence and concentrations of the widely applied pesticides for cocoa cultivation. None of the concentrations of the six pesticides detected, namely acetamiprid, indoxacarb, sulfoxaflor, thiamethoxam, difenoconazole, and imidacloprid exceeded the EU MRL. In Chapter 4, the effects of changing proportions of natural forests caused by cocoa expansion on flower-visiting insects were investigated through a field experiment. Through the field experiments conducted in Chapters 3 and 4, I was able to evaluate the effect of deforestation on pollinator diversity and honey contamination from pesticide use. In this final chapter, I provide a synthesis of the key results across the three studies and outline policy and management strategies for addressing emerging findings. In addition, I outline what could have been done to enhance the overall success of this thesis. Following this, I summarise future research in the context of this thesis and end with a general conclusion.

5.2 Summary and synthesis of key findings

5.2.1 Implications for pesticide input for cocoa cultivation

The production levels of cocoa beans do not meet demands in several parts of the world such as China and India (Squicciarini and Swinnen, 2016). In the midst of this, an estimated 20% to 30% of cocoa produced around the world, valued at 3.16 billion dollars, is lost to pests and disease (Jung et al., 2020, Adeniyi, 2019). As cocoa cultivation intensifies, pesticide application helps mitigate losses from pests, diseases, and other threats, contributing to sustainable production levels (Okoffo et al., 2016, Aminu et al., 2019). While acknowledging these benefits, potential implications of pesticides inputted for cocoa cultivation in the surrounding environment are a possibility (Pisa et al., 2015, Bonmatin et al., 2015, Sánchez-Bayo et al., 2017). Through the systematic literature review (Chapter 2), I observed that geographically pesticide residues in honey from cocoa-producing countries, which are largely in Africa and other developing countries, were under-studied. This is consistent with a similar systematic literature review about the impacts of herbicides and fungicides on bees which showed that most studies are concentrated in North America, Europe and Russia (Cullen et al., 2019). This trend was also observed by Zioga et al. (2020) who evaluated plant protection products in pollen and nectar. Findings from Chapter 2 showed that among the cocoa-producing countries where studies took place, MRL exceedances of pesticide residues occurred only in Brazil, Ivory Coast, and India. Out of the eight approved pesticides investigated in honey from cocoa and forest landscapes in Ghana, I detected six pesticides but the concentration of none exceeded their specified MRLs (Chapter 3). This is consistent with my results in Chapter 2 where pesticide residues in honey from the major honey-producing forest belts did not exceed the MRLs. I also observed in Chapter 2 that only one study each was conducted in both Ivory Coast and Ghana, two countries which account for 70% of the cocoa beans placed in the world market (Yao Sadaïou Sabas et al., 2020, Bangmarigu and Qineti, 2018, Wessel and Quist-Wessel, 2015). In Chapter 3, I observed the pervasiveness of pesticides inputted for cocoa cultivation in the landscapes to the extent that pesticide residues were detected in honey from natural forests where pesticides are not allowed. This shows that natural forests which support a broad spectrum of pollinator diversity, as observed in Chapter 4, are not immune to pesticides applied in cocoa landscapes. For instance, honey from natural forests about

2 km from cocoa landscapes was found to contain pesticide residues (Chapter 3). This is suggestive of pollinator exposure to pesticides within natural forest landscapes near cocoa landscapes.

A study conducted by Pasaru et al. (2023) showed that pesticides applied for pest and disease control on cocoa farms reduced pollinator diversity. In both Chapters 2 and 3, a broad spectrum of pesticides across different categories including neonicotinoids (e.g., imidacloprid, acetamiprid, etc.); pyrethroid (e.g. k-cyhalothrin, hydroxymethylfurfural, etc.), organochlorine (e.g. lindane, endosulfan, etc.), organophosphate (e.g. chlorpyrifos, malathion, etc.); pyrazole (e.g. fipronil, etc.); carbamate (e.g. carbaryl, carbofuran, etc.); fungicide (e.g. metalaxyl, difenoconazole, etc.) and herbicide (e.g. fenpropathrin, atrazine, etc.) were detected in honey from cocoa growing countries. Beneficial insects may come into contact with several of these pesticides when applied to pest insects (Desneux et al., 2007) because only 0.01% of applied pesticides are determined to reach their targets while the rest filter into the surrounding environments (Tudi et al., 2021, Llorent-Martínez et al., 2011, Nehra et al., 2021, Rosenheim et al., 2020). I observed that the concentrations of several pesticides exceeded concentrations found to cause sub-lethal effects on bees (Chapters 2-3). The measured residual concentrations of detected pesticides, which ranged from 0.003 mg/kg to 0.051 mg/kg, are within the range that has been shown to have sub-lethal consequences for honeybees (Chapter 3). For instance, a concentration of 0.0045 mg/kg of thiamethoxam, much lower than that observed in our results was shown to affect the longevity, viability and quantity of live sperm in drones thereby impacting the reproductive capability of male honeybees (Straub et al., 2016). Decreased proportion of natural forest was found to impact pollinator diversity (Chapter 4) and this may be exacerbated by pesticide usage in the cocoa landscapes particularly looking at the concentrations of pesticide residues detected (Chapter 2-3).

From Chapters 2 and 3, I observed that imidacloprid (a neonicotinoid pesticide) emerged as the most detected pesticide. Imidacloprid is widely used in several parts of the world because it offers effective protection to crops (Simon-Delso et al., 2015, Jeschke et al., 2011). It is approved for approximately 140 crops in about 120 countries (Jeschke et al., 2011, Kleinschmit and Lilliston, 2015). Once translocated after being taken up by plants, it provides long-lasting protection against insect pests (Bonmatin

et al., 2015). The problem however is that it can persist in woody plants for over 365 days with reported half-lives of over 1000 days (Bonmatin et al., 2015, Krupke et al., 2012). This may account for its prevalent detection in honey samples particularly in areas of extensive use. The prevalence of imidacloprid as the most detected pesticide (Chapters 2 & 3) brings into focus the effects of neonicotinoids on pollinator health especially as it was detected even 2 km into natural forests (Chapters 3) which harbour higher abundances of insects (Chapter 4). The effects of neonicotinoids on bees are documented and range from adverse effects on pollinator behaviour, reproductive capability and foraging activities, among others (Straub et al., 2021, Stanley et al., 2015b, Stanley et al., 2015a, Zhang and Nieh, 2020). Because cocoa cultivation relies on both pollinators and pesticides, an integrative approach to cocoa cultivation which guarantees the responsible use of pesticides that causes less harm to pollinators is essential. Co-management of crop pests, natural enemies and beneficial insects need to be handled holistically (Merle et al., 2022). This feeds into the ideals of integrated pest and pollinator management (IPPM) (Biddinger and Rajotte, 2015, Egan et al., 2020) which is aimed at managing pests while protecting pollinators and other beneficial insects in a balanced way. The preservation of insect pollinators within IPPM is essential for agroecosystem resilience and this helps mitigate the threats to pollinator populations and also contributes to crop yields (Gurr et al., 2017).

Research has established that one hectare of cocoa plantation could produce 2000 kg of cocoa beans per hectare per year (Goenaga et al., 2015), but in 2016 the average worldwide production per hectare per year was 438kg of cocoa (FAOSTAT, 2020). Cocoa yields benefit from insect pollination (Toledo-Hernández et al., 2017, Kofi et al., 2014) and pollination services can therefore be a limiting factor in cocoa production. Low pollinator visitation and inefficient transportation of pollen grains have been cited as causes for low cocoa pollination (Toledo-Hernández et al., 2017). The potential effects of pesticides inputted for cocoa cultivation (Chapters 2 & 3) and the impact of reduced natural forests (Chapter 4) contributing to low pollination services on cocoa farms may be likely. However, as pointed out by Claus et al. (2018), the effects of insecticides on cocoa pollinators' load and efficiency of pollination of cocoa flower visitors besides *Forcipomyia* spp require further investigation. Beyond the potential effects of pesticides, how this is exacerbated by rising temperatures increasing droughts, and climate change on cocoa pollinators needs further

exploration as suggested by Toledo-Hernández et al. (2017). This is essential owing to the small number of cocoa pollinators recorded in other cocoa landscapes. For instance, Schawe et al. (2016) recorded only 13 cerapogonid individuals belonging to seven species in cocoa plantations during a study. Groeneveld et al. (2010) also recorded only 28 cerapogonids in cocoa plantations in Indonesia. The abundance of cocoa pollinators in our study landscapes (Chapter 4) is not suggestive of sufficient insect pollination as hand pollination of cocoa is extensively practised in Ghana (Asante et al., 2023, Nyamekye, 2021). This brings into focus the role of natural forests in supporting a diverse group of insect pollinators (Chapter 4). For instance, even though Frimpong-Anin et al. (2011), indicated that nearness to natural forest did not offer any pollination advantages, they did admit that secondary forest patches surrounding the cocoa farms could offer the pollinating resources.

5.2.2 Need for natural forests in cocoa landscapes

Cocoa cultivation has resulted in the disappearance of between 14 and 15 million of tropical forests globally over 50 years (Nasser et al., 2020, Kalischek et al., 2023, ICCO, 2008, Somarriba and Lopez-Sampson, 2018). I observed in Chapter 4 that Hemiptera, Coleoptera and Hymenoptera shift to natural forests with cocoa expansion, and this is indicative of push factors in cocoa landscapes. As vital habitats to insect pollinators, forested areas play an essential role in their life cycles thereby contributing to the overall survival of pollinators (Ricketts et al., 2008). Many insects depend on natural forests for their dietary resources, and breeding grounds, and escape from predators (Didham et al., 1998). However, the conversion of natural and semi-natural vegetation for farming purposes comes with its attendant problems of the fragmentation, destruction or degradation of natural habitats which serve as sanctuary to pollinators (Potts et al., 2010). Alterations caused by land use change in natural habitats lead to loss of plant diversity (Goulson et al., 2015, Ollerton et al., 2014) and change the home range of pollinators (CBD, 2018). This restricts pollinators' accessibility to floral resources in the form of pollen and nectar (Cox-Foster et al., 2007).

Given the positive relationship observed between natural forests and Hymenoptera, Hemiptera and Coleoptera (Chapter 4), the need to maintain natural forests in cocoa landscapes becomes a necessity. But this can be more tenable if the drift of pesticides

from cocoa landscapes to natural forests (Chapter 3) is properly assessed and addressed. Therefore, conservation strategies, which consider cocoa expansion and the adaptive strategies for different insect species become essential (Klein et al., 2002, Ricketts, 2004, Harrison et al., 2018b). This is because the pollination of other flowering plants and the maintenance of biodiversity including the pollination of other crop types would require pollination services from bees and other insect pollinators in the landscapes (Ollerton et al., 2011, Eeraerts et al., 2019). The broader implications discussed in Chapter 4 highlight the necessity to balance cocoa expansion with biodiversity conservation (Brosi et al., 2007, Mayes et al., 2019). Additionally, dissimilarity patterns in bee species composition emphasize the crucial role of natural forest cover in sustaining diverse bee communities and this highlights conservation strategies in response to evolving land use patterns (Forrest et al., 2015, Smith et al., 2021). Overall, this study provides the foundation for further research which can assess how the benefit of pesticide application for cocoa protection does not negate the benefit of pollination services by cocoa pollinators through adverse effects of pesticides.

5.2.3 Study outcomes in relation to Sustainable Development Goal 15

The outcomes emerging from this thesis (Chapters 2-4) have significance for advancing Sustainable Development Goal 15 (SDG 15) which is aimed to “protect, restore, and promote sustainable use of terrestrial ecosystems, sustainably manage forests, combat desertification, and halt and reverse land degradation and halt biodiversity loss” (Cooper et al., 2023, UN, 2020, Mohieldin and Caballero, 2015). The observed pesticide residues in honey from both cocoa and natural landscapes and their implications on insect pollinators (Chapters 2-3) as well as the observed influence of changing proportion of natural forest on pollinator diversity (chapter 4), which are integral components of terrestrial ecosystems, emphasize the dynamic relationships between agricultural practices, pesticide exposure, and biodiversity loss (Goulson et al., 2015, Powney et al., 2019). The exceedance of MRL for some pesticides in honey (Chapter 2) can have direct effects on humans and other forms of life. Broadly, pesticide concentrations and deforestation pose potential threats to affect life on Earth. As SDG 15 seeks to sustainably manage forests or terrestrial life, my research emphasizes the need for sustainable land management practices that can ensure a

balance between cocoa cultivation with biodiversity conservation (Gibbs et al., 2010). Overall, the findings contribute to the global understanding of the challenges posed by changing landscapes on pollinator communities, providing valuable insights for policymaking and conservation efforts aimed at achieving SDG 15's overarching goals.

5.2.6 Integration of beekeeping in cocoa plantations

Fluctuating cocoa prices tend to affect cocoa farmers whose livelihoods depend entirely on cocoa cultivation thereby necessitating the need for additional incomes to support their livelihoods (Franzen and Borgerhoff Mulder, 2007, Gilbert, 2016, Muojama, 2016, Salifou et al., 2019). Beekeeping emerges as a viable solution, offering diversified revenue streams which may generate additional income for cocoa farmers (Ahikiriza, 2016, Adamte, 2023, Vittaz, 2021). Integrating beekeeping with cocoa cultivation aligns with sustainable agricultural practices, providing farmers with honey-related income while enhancing pollination for other crop types. Outcomes which emerged from Chapter 3 showed that, even though pesticides were detected in honey produced in cocoa landscapes, the concentrations of detected pesticides did not exceed allowable limits to raise human health concerns. This result highlights the potential of beekeeping to augment livelihoods in cocoa-producing regions (Darko et al., 2017). This is supported by the fact that cocoa expansion did not necessarily influence the abundance of bees in the landscapes (Chapter 4). This may be suggestive of available floral resources or support beekeeping. Natural forests provide an array of floral resources for bees and other insect pollinators, and this contributes to foraging habitats (Matheson et al., 1996, Felderhoff et al., 2023, Ulyshen et al., 2023). Beekeeping may be promoted as an alternate income by empowering cocoa farmers through beekeeping. This may not only foster economic resilience but also support conservation goals through the provision of pollination services by managed honeybees. However, competition for floral resources, between honeybees and other native bee species or insects could emerge with intensively managed honeybees for honey production and crop pollination in agricultural landscapes (Rader et al., 2016). The problem is that honeybees may likely outcompete native bees for floral resources due to their larger colony sizes and foraging populations (Goulson, 1999). According to Mallinger et al. (2017), existing studies show considerable variability about the

effects of managed honeybees on other insect pollinators, with 53% reporting adverse effects on wild bees, 28% indicating no effects, and 19% reporting mixed effects with the added risks to wild bees through pathogen transmission. Increased competition may result in reduced resource availability for native solitary bees and other pollinators, impacting their ability to meet nutritional needs and reproduce successfully potentially leading to negative consequences for biodiversity and ecosystem functioning (Potts et al., 2010, Goulson, 1999). Further to this, evidence exists to suggest the possibility of the co-existence of honeybees and other floral visitors with no variation in the rate of visitations in forested African landscapes (Coppinger and Stanley, 2023). Maintaining or restoring diverse natural habitats can help provide floral resources or suitable conditions for both honeybees and native bees (Tonietto and Larkin, 2018, Vaughan and Black, 2008). Additionally, supplemental feeding for honeybees, if needed, can reduce resource competition (Standifer, 2003, Topal et al., 2022, Page and Williams, 2023). Furthermore, apiary sites may be selected away from known habitats of native bee bees to help reduce the competition between honeybees and native bees. This should be complemented with the education of beekeepers for adherence to regulations to ensure that beekeepers act to support the beneficial relationship between managed and native bees.

5.2.7 Adaptability of bees to changing proportions of natural forests

In Chapter 4, I observed that bee abundance, species richness and Shannon diversity were unaffected by the changing proportion of natural forests in the cocoa landscape, but it influenced bee community composition. This is divergent from other studies where the abundance of bees has either been found to be either positively or negatively related to land use changes (Hoehn et al., 2008, Kearns et al., 1998, Winfree et al., 2007, Goulson et al., 2008). This finding is significant with respect to the contribution of bees to pollination services. Approximately 87.5% of all flowering plants depend on insect pollinators for pollination (Ollerton et al., 2011) out of which honeybees and other bees are estimated to account for 62% of insect pollination (Rader et al., 2016).

As revealed by Klein et al. (2002) some bee species do not necessarily experience declines in response to habitat modifications. However, the effect of natural forests in

altering bee community composition (Chapter 4) shows that pollination services by bees to other insect-dependent plants in the landscapes may be threatened by cocoa expansion but this will have to be tested through a study. On the other hand, bee community composition as illustrated in the non-metric multi-dimensional scaling plot in Chapter 4, shows that the proportion of natural forest had an overriding influence on the patterns in bee community composition, emphasizing the fundamental role of natural habitats in sustaining diverse bee communities. Patterns of plant diversity, nesting resource availability, and habitat shading, present at the scale of a few hundred meters have been found to determine bee community patterns in the mosaic open–savanna–forest landscape (Grundel et al., 2010). Generally, the relationship between bees and plants is grounded in the principle of mutualism, where bees contribute to forest health through efficient pollination, while forests reciprocate by sustaining diverse and abundant floral resources, supporting robust bee communities (Gikungu, 2006, Miller-Struttman et al., 2015). Different bee species exhibit varying foraging preferences, leading to more effective and widespread pollination across plant species (Hoehn et al., 2008). The provision of ecosystem services, such as pollination, nutrient cycling, and pest control may be provided by different species within a bee community to contribute to the general health of the ecosystem (Mori et al., 2013). Therefore a well-balanced community composition ensures that these services are consistently available, benefiting both the ecosystem and human well-being (Cardinale et al., 2012).

5.2.8 Methodical approach

In this thesis, two distinct methodological approaches were employed in field experiments to quantify landscape composition. Three landscape categories were selected in Chapter 3 and evaluated for pesticide residues while in Chapter 4, I employed a gradient approach to examine landscape composition (Winfree et al., 2007). The categorical approach in Chapter 3 allowed for a systematic comparison between cocoa-dominated landscapes and natural forested areas and this allowed me to assess the variations in pesticide residues among these distinct environments. On the other hand, the gradient approach adopted in Chapter 4 was designed to capture the subtleties of ecological variations along a natural forest proportion gradient within cocoa landscapes. This method acknowledged the inherent heterogeneity present in

transitional zones between cocoa and forested areas. Sampling along a gradient provided a better understanding of how insect pollinator communities responded to varying proportions of natural forest cover.

A broad spectrum of sampling techniques, such as suction traps, malaise netting, etc are available for sampling insect populations (Leather, 2008). In Chapter 4, pan traps comprising blue, white and yellow colours were used for sampling. Pan trapping is reputed as the best technique for sampling bees in all geographical areas and tends to have the largest sample coverage, highest collection of species, reduced collector biases alongside being able to detect similar species (Westphal et al., 2008). In tropical rainforests, many bees and other pollinating insects are canopy feeders (LeBuhn et al., 2016) and elevating pan traps allows for the assessment of the vertical dispersal of insect communities within the habitat (Bąk-Badowska, 2012). While pan traps are widely used for insect sampling, they favour colour preferences, limiting their representation of pollinator diversity (Campbell and Hanula, 2007, Vrdoljak and Samways, 2012). The attractiveness of certain colours may therefore disproportionately attract or deter specific insects, potentially leading to skewed abundance and diversity estimations. Given this, the research question for Chapter 4 was designed to focus on flower-visiting insects highly attracted to colours. The traps were designed to sample flying flower-visiting insects and consisted of both ground and aerial pan traps to sample eighteen sites on four occasions to capture flower-visiting insects. The use of aerial pan traps in this study ensured that insects in the vertical plane were not missed during sampling.

5.3 Policy and Management

5.3.1 Overview

As the global demand for cocoa intensifies (ICCO, 2008), cocoa expansion, alongside pesticide application, may pose challenges to biodiversity conservation. This thesis has established the extent to which cocoa expansion influences pollinator diversity, how honey is contaminated by plant protection products and the potential effects of concentrations of pesticides on both humans and insect pollinators. In light of the comprehensive investigation into the influence of cocoa cultivation on flower-visiting insects and honey quality, this section outlines key policy and management

recommendations derived from empirical evidence. However, in light of the multiple findings and associated implications on pollinators and honey quality, an integrated and multidimensional policy strategy capable of addressing the different facets of challenges posed by cocoa cultivation is needed. By bridging the gap between research findings and actionable strategies, I aim to contribute to sustainable practices that balance cocoa production with biodiversity conservation. The policy framework proposed extends to align with international agreements, fostering a holistic approach towards sustainable cocoa cultivation

5.3.2 Cocoa expansion and biodiversity conservation

Land use change, notably the expansion of cocoa cultivation and the consequential reduction in natural forest coverage highlights the critical need for a harmonious blend of agricultural development and biodiversity conservation. My research findings, as detailed in Chapter 4 demonstrate that the integration of trees and patches of natural forest within cocoa plantations may provide vital support and habitats for the diverse pollinator taxa (Tscharntke et al., 2012b, Harrison et al., 2018b). Considering, cocoa landscapes should be designed to have patches of natural forests in the landscapes. A monitoring mechanism, ensuring compliance, must accompany these initiatives. In tandem, collaborative efforts between government agencies are imperative to incorporate policies that safeguard forest patches which directly feeds SDG 15 which aims to protect, restore, and promote sustainable use of terrestrial ecosystems. Furthermore, leaving patches of natural forest feeds directly into the Bonn Challenge, a global effort which was launched in 2011 and aimed to restore 350 million hectares of degraded forest landscapes by 2030 (Verdone and Seidl, 2017).

5.3.3 Sustainable pesticide use

The extensive presence and concentrations of pesticide residues uncovered in cocoa honey, as elucidated in Chapters 2 and 3, underscores the necessity for informed policy decisions regarding responsible pesticide usage. Integrating findings from my research, particularly the potential repercussions on insect pollinators, supports the adoption of Integrated Pest and Pollinator Management (IPPM) strategies (Egan et al., 2020, Prasad and Mathyam Prabhakar, 2012, Biddinger and Rajotte, 2015). Emphasis should be placed on minimal pesticide use through bio-control and resilient

crop varieties aligned with IPPM principles. The impacts of pesticides inputted for cocoa cultivation, on non-target organisms or beneficial insects need to be regularly assessed. Closely related to this, pesticide-free buffer zones or non-crop areas (Chreil and Maggi, 2023, Chreil and Maggi, 1977), such as the patches of natural forest or areas near river bodies, should be created in cocoa plantations to protect pollinators and other beneficial insects from direct contact with pesticides. As part of a multidimensional approach to promoting sustainable pesticide application, educational programs should be developed to raise awareness of the impacts of pesticides on pollinators among cocoa farmers. The program can be incorporated into the Cocoa Diseases and Pests Control Excise Committee (CODAPEC) popularly called the Mass Spraying (Abankwah et al., 2010). Mass Spraying is the program of the Government of Ghana initiated through the COCOBOD to fight the Black Pod disease and the capsid/mirid (Naminse et al., 2011, Abankwah et al., 2010). As part of the farmer's education and training for the cocoa farmers, Mass Spraying gangs and planning authorities, specialised training in alternative pest control methods and judicious use of pesticides should be provided (Pimentel and Burgess, 2014, Sagar, 1991, Smith, 2012). These policy directions, when implemented, will contribute to a sustainable, environmentally friendly approach to pesticide use, ensuring the protection of pollinators, and ecosystems, and maintaining cocoa productivity.

5.3.4 Quality concerns of pesticide residues in honey

Pesticide residues are expected to be within an acceptable MRL range to guarantee quality for human consumption (Dureja et al., 2015, Winter and Jara, 2015). However, during foraging, bees may pick contaminated pollen or nectar, interact with plants and soil sprayed with pesticides or encounter and consume contaminated water and transport it back into the beehive which may contaminate hive products (Bogdanov, 2006, Colin et al., 2004, Lambert et al., 2013). Furthermore, direct pesticide application to treat pest and disease infestation may also contaminate honey (Simon-Delso et al., 2014). Honey samples from different geographical regions of the world therefore are found to contain different residues of pesticides an indication of how pervasive pesticides abound in the environment (Meikle et al., 2022, Louca Christodoulou et al., 2015, López et al., 2014, Lin et al., 2021, Kumar et al., 2018). Consistent with this trend, I observed from the results in Chapter 3 that honey samples from fullsun cocoa,

agroforest cocoa and natural forest were contaminated with pesticide residues. Even though observed concentrations were found to be below MRL in Chapter 3 and therefore may not raise any safety concerns, it is acknowledged that the assessment of honey quality may not be limited to only the MRL but expanded to other contaminants. To prevent exposure of bee products to pesticides, I recommend research to identify and promote beekeeping practices that minimise pesticide exposure. In addition, beekeepers should be encouraged to mount beehives in designated pesticide-free zonal buffers in cocoa farms to prevent direct exposure of beehives or colonies to direct spraying.

5.4.5 Habitat conservation

Integrating habitat conservation and management into land use and development policies is imperative, and my research findings provide strong evidence in support of this approach (Chapter 4). An increasing proportion of natural forest was found to support the abundance of diverse groups of insects that visit flowers. Buffer zones may be established in natural forests to serve as essential habitats for bees and other flower visitors, serving as crucial areas for foraging and nesting (Kennedy et al., 2013). In addition, a systematic monitoring system for the health of bees should be established and must be informed by the findings presented in Chapters 3 and 4. This can focus on a period assessment of the impacts of pesticides on insect pollinators. This monitoring system will enable regular evaluations of the health of pollinator populations, thereby facilitating the timely detection and mitigation of emerging threats, ultimately addressing the issue of declining pollinator populations (Potts et al., 2010).

5.3.6 Research, innovations collaborations

Cocoa expansion may impact beekeeping through reduced floral resources through expansion and potential adverse effects of pesticides on honeybees (Chapters 2-4). Therefore, considerations of incorporating beekeeping into cocoa cultivation should first be integrated into land use planning processes. This should be supported with research which focuses on understanding the synergies between beekeeping and cocoa cultivation by identifying best practices and addressing challenges. At the heart of these, local and regional collaborative platforms that can bring together beekeepers, cocoa farmers, the COCOBOD, the Forestry Commission and the Cocoa Research

Institute must be established to facilitate regular communication, knowledge sharing and joint decision-making. This can foster an environment for the harmonious existence of beekeeping and cocoa farming, promoting sustainability, biodiversity, and the well-being of local communities.

5.4 Limitations and Challenges

This thesis attempted to explore the effect of cocoa cultivation on pollinator diversity and bee products. The overall aims and objectives set for the study were generally achieved. However, looking back I realise other aspects could have been taken into consideration to enhance the overall success or outcome of the study. First, the study was confined to cocoa landscapes. An inclusion of non-cocoa-producing districts as part of the study could have given a different dimension of pesticide residues and enhanced the generalizability of the outcomes of the study. Furthermore, the study primarily focused on ecological aspects with limited focus on the socio-economic dimensions of cocoa cultivation or beekeeping. An exploration of the socio-economic implications of changing land use on local communities, farmers, and other stakeholders could have added a gloss. Whilst the use of pan traps satisfied the study aim of Chapter 4, combining it with other methods such as malaise traps, could have been complementary. This would have facilitated a more thorough collection of flying insect specimens, allowing for a comprehensive assessment of pollinator diversity. Malaise traps, positioned strategically in the landscape, capture insects in flight passively, complementing the active trapping method of pan traps (Campbell and Hanula, 2007). Additionally, considering floral associations in conjunction with pollinator diversity could offer insights into the specific plant-pollinator relationships, contributing to a detailed understanding of the foraging behaviour and preferences of different pollinator species (Johnson, 2004).

Furthermore, in Chapter 4, bee identification was limited to morphological features focusing primarily on metrics such as species richness and abundance. Further analysis into genetic diversity, functional diversity, and ecosystem services could have provided more detailed insights into the genetic diversity within bee populations. However, no potential for misclassification due to morphological similarities was encountered with the bees identified in my study and therefore identification of

morphospecies proved very satisfactory. In addition, direct observations and measurements of pollination services, hive productivity, and cocoa yield could have been incorporated. These would provide a more holistic understanding of the bee communities and their contributions to ecosystem functioning and services. However, time constraints, exacerbated by the COVID-19 pandemic, meant I did not have a realistic time to incorporate these aspects within the specified duration as required. Nevertheless, this thesis lays a foundation for future research endeavours.

5.5 Future research directions

In this section, future research, based on the study outcomes and the implications of this thesis, is outlined.

In Chapter 4, I focused on the effect of decreasing proportion of natural forests due to cocoa expansion on insect pollinators. However, different stressors, including pesticides, may act individually or jointly to affect insect pollinators raising concerns about the long-term sustainability of both pollinator populations and the crucial pollination services they provide (Fürst et al., 2014, Woodcock et al., 2016, Harrison et al., 2018b, Potts et al., 2010). General circulation models point to the fact that deforestation in the tropics results in global warming on a par with fossil fuels burnt since 1850 and leaving significant drying in the tropics (Lawrence and Vandecar, 2015). Therefore, future research should focus on how decreased forests act jointly with shifts in temperature, pesticides, or other stressors to impact insect pollinators in cocoa landscapes. Outcomes from such a study can provide insight into the compounding challenges which pollinators face in a changing environment.

Chapter 3 of this thesis evaluated pesticide residues in honey in beehives located in cocoa and forest landscapes and gauged their sub-lethal effects on honeybees. Detected pesticide residues in the honey confirms bees' exposure to pesticides in the landscapes. However, how concentrations of pesticides relate to exposure levels in the landscapes was not evaluated. Besides, chronic exposure to bees has been found to impair natural foraging behaviour and increase worker mortality leading to a significant reduction (Gill et al., 2012). According to Dirilgen et al. (2023), there is a lack of comprehensive research on non-neonicotinoid insecticides and non-honey

bees which requires the expansion of research into other bee taxa. Interestingly, organophosphates, organochlorines and pyrethroids were found to be the three most detected classes of pesticides in cocoa-growing countries (Chapter 2). In light of these, future research should examine how the concentrations of the more detected pesticide residues in honey in cocoa-growing countries (e.g., organophosphate, organochlorines and pyrethroids) impact bees and other bee taxa due to exposure levels in the landscapes. How non-neonicotinoid pesticides impact bumblebee (*Bombus terrestris audax*) activity and their provision of pollen required for colony development has received attention in research (O'Reilly and Stanley, 2023). Future studies should therefore extend to other bees, including honeybees, and non-bee insects. Closely related to this, further research should also focus on the cumulative or long-term effects of pesticide exposure on honeybees and other flower-visiting insects in the landscapes.

Cocoa expansion may have the potential to isolate other crop types or form forest patches. Increasing distance from natural forests has been found to limit pollination services (Kremen et al., 2003, Ricketts, 2004). There may be implications for pollination services for other crop types which may be isolated from natural forests through cocoa expansion. Future research should therefore focus on how isolation limits pollination services in forest and cocoa landscapes. This can provide insight into what extent cocoa expansion limits pollination services to other crop types through forest loss or fragmentation.

Pesticide concentrations in honey sampled as revealed in Chapter 3 did not raise health concerns for human consumption. As pesticide residues are likely to find their way into other media, it is recommended that future results should investigate pesticide residues in other food products, water sources and soil in cocoa landscapes as well as the assessment of the potential health risks residents in the landscape may face. Such investigation will provide insight into the concentrations of pesticide residues in other food matrices.

I observed in Chapter 3 that pesticide residues detected in honey did not exceed maximum residue limits indicating the feasibility of integrating beekeeping into cocoa cultivation. With beekeeping having the potential to support local communities, future

research should investigate whether honey produced in landscapes with higher natural forest cover exhibits differences in flavour profiles, nutritional content, and potential bioactive compounds. This research can provide insights into the link between landscape features and honey characteristics and contribute to the sustainable development of beekeeping enterprises in the landscape.

Cocoa expansion is one of the leading causes of forest loss (Benefoh et al., 2018). However, my finding from Chapter 4 did not reveal any negative effects on bee abundance, species richness or diversity with changing proportion of natural vegetation. In future research, I propose research that is focused on the assessment of the foraging behaviour of honeybees in landscapes with varying degrees of natural forests. As part of this study, how changes in land use impact the availability of floral resources for honeybees and, consequently, the quality of honey produced should be evaluated. Findings from this research can inform the designing of effective landscape management strategies that can support honeybee foraging and honey production.

Outcomes of pesticide residues in honey as revealed in Chapter 3 are limited only to cocoa landscapes. These outcomes cannot therefore be generalised or extended to non-cocoa-growing landscapes. Future research should therefore focus on conducting regional or global studies to explore geographical variations in honey characteristics. In this regard, both cocoa and non-cocoa-producing districts or regions should receive attention. In addition, how environmental factors, climate, and land use patterns contribute to differences in honey properties, including colour, flavour, and nutritional content should be examined.

The contents in honey may reflect the chemical conditions of the environment and can therefore be used as a proxy to assess general ecosystem health (Chiesa et al., 2016, Hungerford et al., 2021). Pesticide residues in honey were evaluated in Chapters 2 and 3. Future research should focus on other hive matrices such as the chemical composition of propolis and royal jelly based on geographical locations and floral sources. As part of this study, there should be an investigation into the nutritional requirements of honeybees such as floral diversity, and pollen sources and how this impacts the nutritional content of honey. The outcome of such studies can guide land management practices to enhance honey nutrition and quality.

5.6 Conclusion

Cocoa has emerged as a very important cash crop which supports many West African countries with the income generated from cocoa export (ISSD, 2019, Fadji et al., 2021, Group, 2018). However, the attempt to increase production to meet global demand and the economic gains from cocoa cultivation must not be pursued at the expense of environmental sustainability. This is because, increased cocoa cultivation may lead to the conversion of more arable or forested lands for cocoa cultivation, as well as increased pesticide applications. A balance between cocoa expansion and biodiversity conservation will be essential. This thesis has explored the consequences of cocoa expansion on pollinator diversity and bee products and suggested policy directions on how issues could be addressed to ensure the sustainable production of cocoa. The investigation unfolded across three key dimensions namely land use changes, insect pollinators and pesticide use within cocoa landscapes. As the demand for cocoa products continues to rise, acknowledging the unexpected dynamics observed in this study becomes imperative. Conservation efforts should integrate the context-specific responses of insect communities, emphasizing the critical role of natural forests in sustaining biodiversity. In contemplating the policy and management aspects, the thesis recommends integrated landscape management approaches, sustainable beekeeping practices, and alternative pest management strategies. These policy directions aim to strike a balance between cocoa expansion and biodiversity conservation. As the whole world navigates the complex terrain of sustainable development goals, particularly Goal 15 on Life on Land, this research contributes vital insights into preserving biodiversity, fostering sustainable cocoa production ensuring the well-being of both ecosystems and communities. The overall aim of this study centres on the need to ensure cocoa expansion with considerations for the conservation of insect pollinators. It contributes to a wider discourse on sustainable development, pollinator health, and biodiversity conservation, offering transferable knowledge and implications for policymakers, conservationists, and researchers engaged in diverse ecosystems beyond cocoa landscapes. Moving forward, these findings advocate for a holistic approach, considering the dynamics between land use, pollinators, and biodiversity in the pursuit of sustainable cocoa cultivation. I hope the findings and policy recommendations expounded in this thesis will help us to become

more conscious of the need to protect the Earth knowing that “*The Earth does not belong to us: we belong to the Earth. Man did not weave the web of Life; he is merely a strand in it. Whatever he does to the web, he does to himself*” Chief Seattle.

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Appendix A

Cocoa-producing countries are categorised by the metric tons of cocoa beans they have produced. ¹

Ranking	Country	Cocoa Production (metric tons)	Pop. 2021
1	Côte d'Ivoire	2034000	27053.629
2	Ghana	883652	31732.129
3	Indonesia	659776	276361.783
4	Nigeria	328263	211400.708
5	Cameroon	295028	27224.265
6	Brazil	235809	213993.437
7	Ecuador	205955	17888.475
8	Peru	121825	33359.418
9	Dominican Republic	86599	10953.703
10	Colombia	56808	51265.844
11	Papua New Guinea	44504	9119.01
12	Uganda	31312	47123.531
13	Mexico	27287	130262.216
14	Venezuela	23349	28704.954
15	Togo	22522	8478.25
16	India	19000	1393409.038
17	Sierra Leone	14670	8141.343
18	Haiti	14173	11541.685
19	Guatemala	11803	18249.86
20	Madagascar	11010	28427.328
21	Guinea	10638	13497.244
22	Liberia	8552	5180.203
23	Tanzania	8548	61498.437
24	Philippines	7009	111046.913
25	Nicaragua	6600	6702.385
26	Bolivia	5518	11832.94
27	Solomon Islands	4940	703.996
28	Republic of the Congo	4000	5657.013
29	DR Congo	3758	92377.993
30	São Tomé and Príncipe	2778	223.368
31	Vanuatu	1813	314.464
32	Sri Lanka	1291	21497.31
33	Malaysia	1029	32776.194
34	Grenada	800	113.021
35	Honduras	751	10062.991

¹ Notably, in Chapter 2, results show that studies were conducted only in eight cocoa-producing countries.

36	Panama	662	4381.579
37	Costa Rica	545	5139.052
38	Samoa	479	200.149
39	Angola	442	33933.61
40	Guyana	429	790.326
41	Equatorial Guinea	413	1449.896
42	El Salvador	357	6518.499
43	Trinidad and Tobago	320	1403.375
44	Dominica	312	72.167
45	Jamaica	305	2973.463
46	Belize	244	404.914
47	Cuba	231	11317.505
48	Saint Vincent and the Grenadines	222	111.263
49	Gabon	187	2278.825
50	Timor-Leste	176	1343.873
51	Central African Republic	149	4919.981
52	Thailand	125	69950.85
53	Saint Lucia	51	184.4
54	Comoros	40	888.451
55	Micronesia	33	116.254
56	Fiji	12	902.906
57	Suriname	5	591.8
58	American Samoa	1	55.1

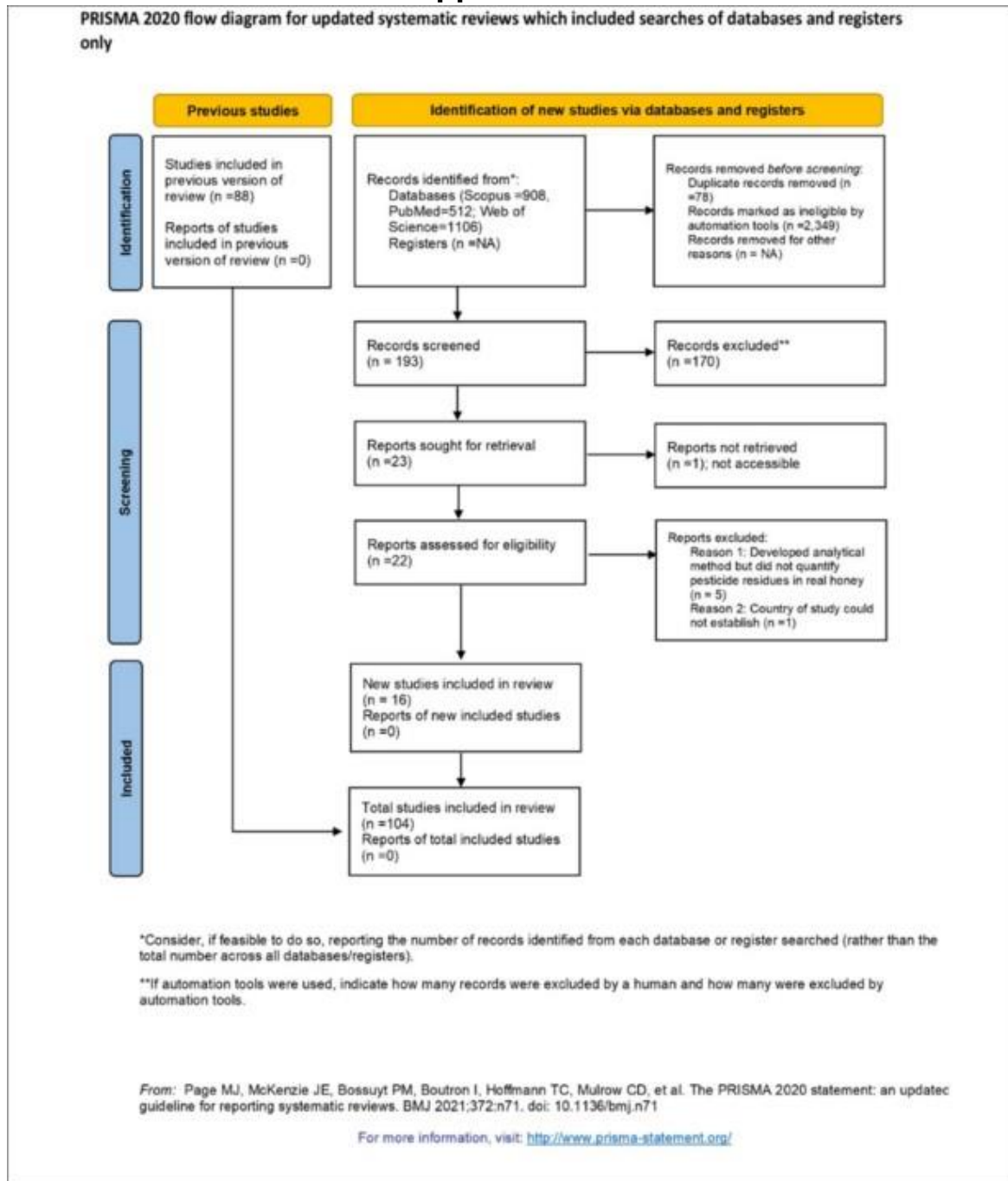
Sourced : (WPR, 2022)

Appendix B1

The search strings were used to retrieve articles from search engines. These search strings were created based on the key pesticides relevant to cocoa cultivation. The selection of these pesticides encompassed twenty-three insecticides, seventeen fungicides, and two herbicides, all of which have been approved for use in cocoa growing (See sections 2.3.2 and 2.3.2).

Search strings	Search engine	Targeted category
(cypermethrin OR capsaicin OR chlorpyrifos OR imidacloprid OR dimethoate OR deltamethrin OR thiamethoxam OR acetaprimid) AND (honey) (bifenthrin OR pyrethrum OR alpha-cypermethrin OR teflubenzuron OR "lambda cyhalothrin" OR indoxacarb OR chlorantraniliprole OR fipronil OR sulfoxaflor OR etofenprox OR pirimiphosmethyl OR promecarb) AND (honey)	Web of Science Core Collection, PubMed, and Scopus	Insecticides
("copper oxide" OR metalaxyl OR "cuprous hydroxide" OR mancozeb OR maned OR benalaxyl OR benomyl OR "Copper hydroxide" OR Metalaxyl-M OR "copper II hydroxide" OR mefenoxam OR "cupper (I) oxide" OR " <i>dicopper chloride trihydroxide</i> " OR dimethomorph OR fluazinam OR "cuprous hydroxide" OR "cupric hydroxide") AND (honey)	Web of Science Core Collection, PubMed, and Scopus	Fungicides
(glyphosate OR paraquat) AND (honey)	Web of Science Core Collection, PubMed, and Scopus	Herbicides

Appendix B2



The results of the updated data search performed to retrieve additional studies between November 2020 and November 2022. Sixteen publications were retrieved and subsequently added bringing the total papers to 104.

Appendix B3

This is the customised checklist designed to evaluate and appraise the papers that met the specified inclusion criteria. This carefully tailored checklist served as a comprehensive tool to assess the selected papers in a structured manner, ensuring that they align with the predefined criteria for inclusion and coverage.

The file can be assessed at the link below:

<https://doi.org/10.1371/journal.pone.0280175.s008>

Appendix B4

The scoring scheme was applied to evaluate the quality of the included studies (Adapted from (Kmet et al., 2004)). This system enabled a standardised and rigorous appraisal of research methodology and relevance, enhancing the overall reliability of the study findings.

Scoring: “yes”=2; “partial”=1 & “no”=0, “NA”=not applicable and not included in total score

How to summarise the total score per study

Total score = (number of “yes” scored “yes” * 2) + (number of “partials” * 1)

Summary score =total score/total possible score

(There are 11 criteria/questions, the total possible score is therefore 22)

Description of appraisal questions

1. Is the study objective clearly stated?

Yes: Easily identified in the abstract and the introduction.

Partial: Study objective not specific or incompletely described. Or where the whole paper must be read before understanding the aim of a study will amount to the study objective not being sufficiently described

No: Study objective not stated or inexplicable

2.0 Is the study design sufficiently described?

Y: The study design is clearly described in the method section and appropriate to answer the study objective

Partial: The study design not clearly identified or stated but is not appropriate to answer the study's aim

No: Study design not stated or cannot be identified

3.0 Are all honey samples from one specific country?

Yes: All samples taken from only one country

No. Where honey samples are from multiple countries or where the country name is not stated (where honey samples are not from one country study cannot be assigned to any specific country and is excluded for further analysis)

4.0 If pesticide residues were detected in honey, were the concentrations quantified in honey?

Yes: Concentrations of pesticide residues quantified and reported. This provides an opportunity for further analysis

Partial: If detected pesticides are not quantified. Detections of pesticide residues were reported but their concentrations were not reported in results or abstract.

No: Where only blank honey is analysed but no actual concentrations of pesticides are necessarily quantified in real honey. Where no concentrations of pesticides are quantified in actual honey, the study is excluded for further analysis.

NA: Not applicable is entered if pesticide residues were not detected. Where no detections were made and therefore no quantification was required, scoring for this response is not included in the total sum of scoring

5.0 Is the limit of detection (LOD) below the maximum residues (MRL) of studied compounds?

Yes. If all LODs of detected pesticide residues are below the specified MRL. On this occasion, the analytical technique is deemed sufficiently sensitive to detect compounds being studied

Partial: Where the study reports detection but does not give LOD

Partial: If some LOD is below with others being higher the EU MRL

No: If all LODs are higher than EU MRL

6.0 Is the limit of quantification (LOQ) below the maximum residue limit (MRL).

Yes. If all LODs of detected pesticide residues are below the specified EU MRL. On this occasion, the analytical technique is deemed sufficiently sensitive to quantify compounds being studied

Partial: If some LOQ is below with others being higher the MRL

No: If all LODs are higher than MRL

7.0 Is the extraction technique clearly described /appropriate?

Yes: The extraction technique clearly defined. Very appropriate based on scientifically acceptable or standard procedures.

Partial: Vaguely defined or incompletely described. Or where the whole paper must be read before understanding the procedure applied. Or where extraction technique is not applied to analyse residues of pesticides in actual honey.

No: Where extraction procedure is not described or not based on standard procedures.

8.0 Is the analytical technique clearly described/appropriate?

Yes: Analytical technique clearly defined. Very appropriate based on scientifically acceptable or standard procedures

Partial: Vaguely defined or incompletely described. Or where the whole paper must be read before understanding the procedure applied

No: Where analytical technique is not described or not based on standard procedures

9.0 Is the type of source of honey (raw or commercial) indicated?

Yes: If the honey is indicated as being either raw honey or commercial honey

No: if the types of honey samples analysed are not indicated

10.0 Are study results reported in sufficient detail?

Yes: All major outcomes and any secondary outcomes are reported

Partial: Quantitative results reported for some outcomes. Or difficult to assess as the study question/objective is not fully described even though results may seem appropriate

No: Results reported for some samples or pesticides studied or reported proportion does not account for the entire study sample.

11.0 Are the conclusions supported by the results?

Yes: All the conclusions are supported by data. Conclusions based on results relevant to the study questions whether negative or positive

Partial: Some of the major conclusions are supported by the data but others are not.

No: Either no or a very small minority of the major conclusions are supported by the data. Or conclusions missing

Appendix B5

This is the dataset for the systematic literature review contained in chapter two. It comprises a list of 104 papers which satisfied both inclusion criteria and quality assessment. This encompasses papers retrieved from both the original search and the subsequent updated search.

The file can be assessed at the link below:

<https://doi.org/10.1371/journal.pone.0280175.s009>

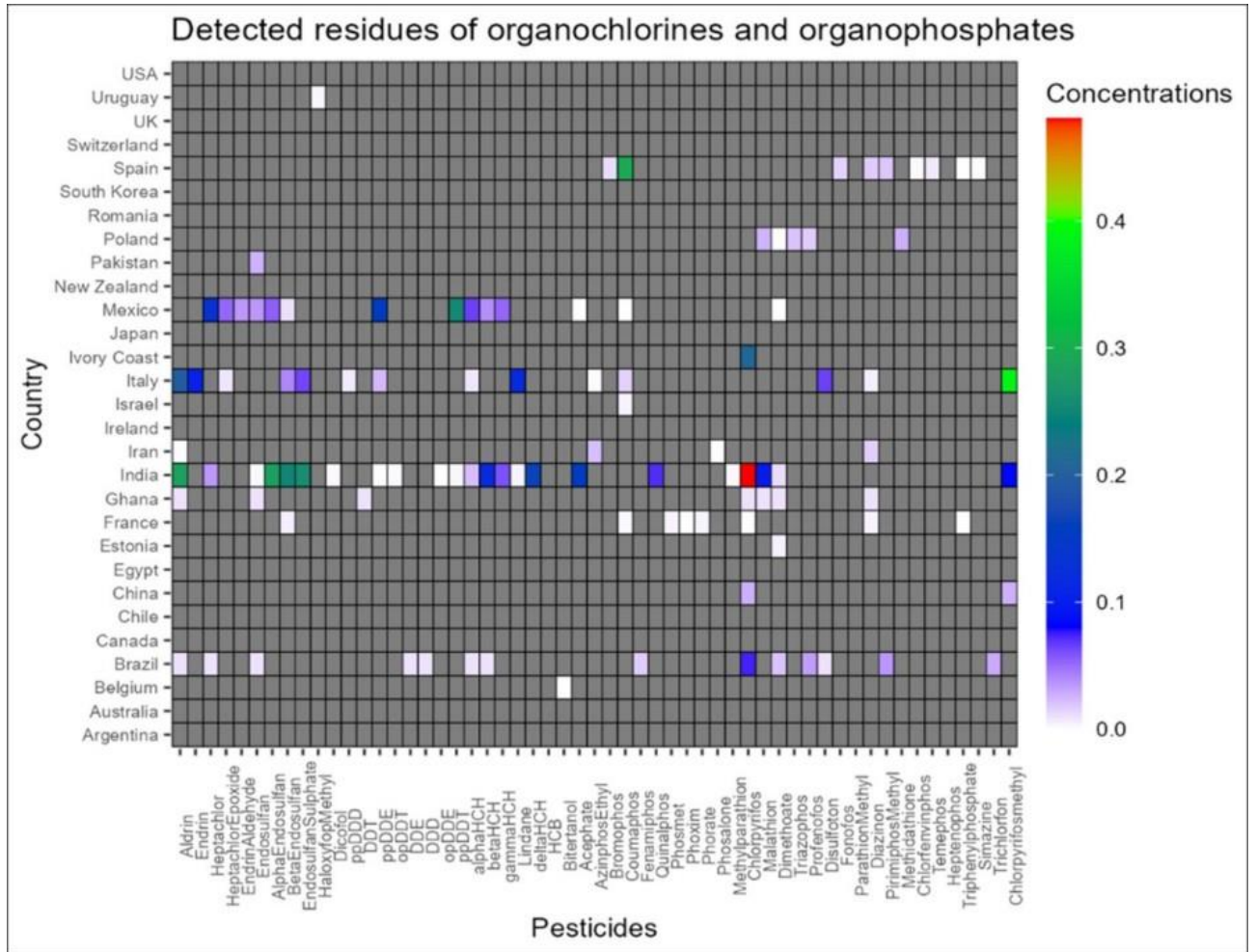
Appendix B6

This is an Excel document containing the detailed inventory of the included papers, featuring their respective aims, study designs, key findings, summarised content and key results.

The file can be accessed at the link below:

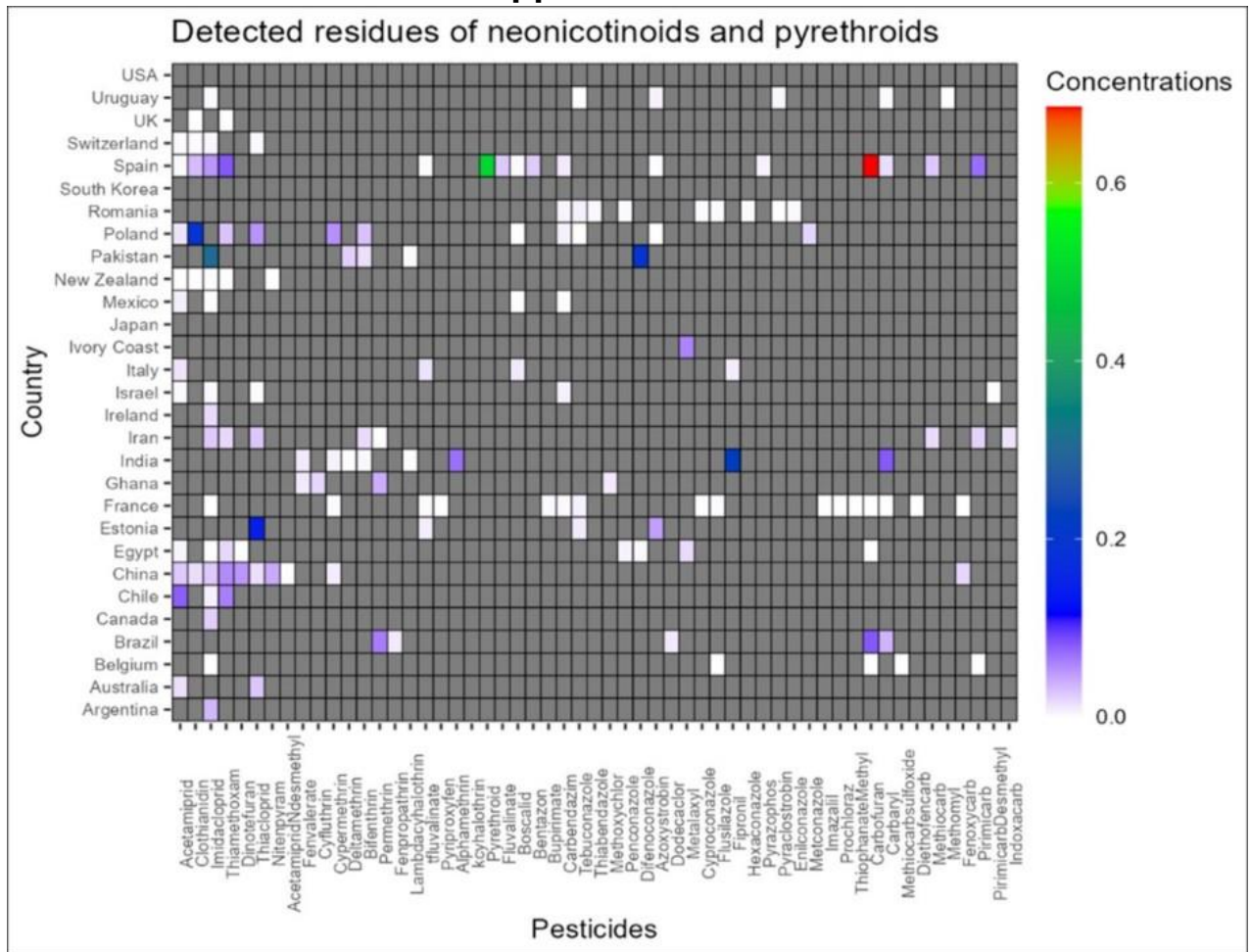
<https://doi.org/10.1371/journal.pone.0280175.s010>

Appendix B7



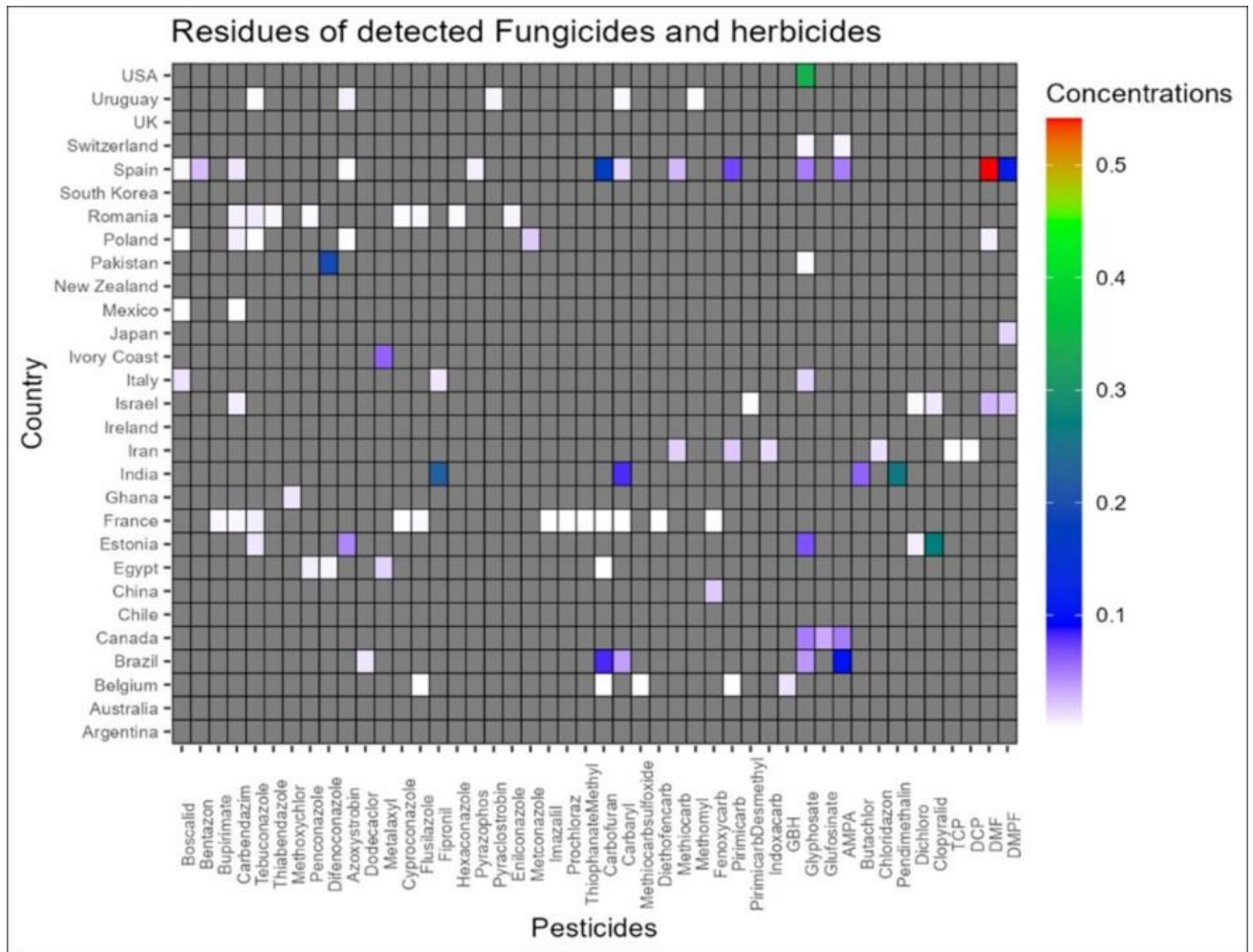
A heatmap with a colour scale on the right, illustrating the presence of studied organochlorines and organophosphates over time (x-axis) across various countries (y-axis). The concentrations of each detected pesticide were averaged per country to generate a single value for each pesticide in each country for this visualization.

Appendix B8



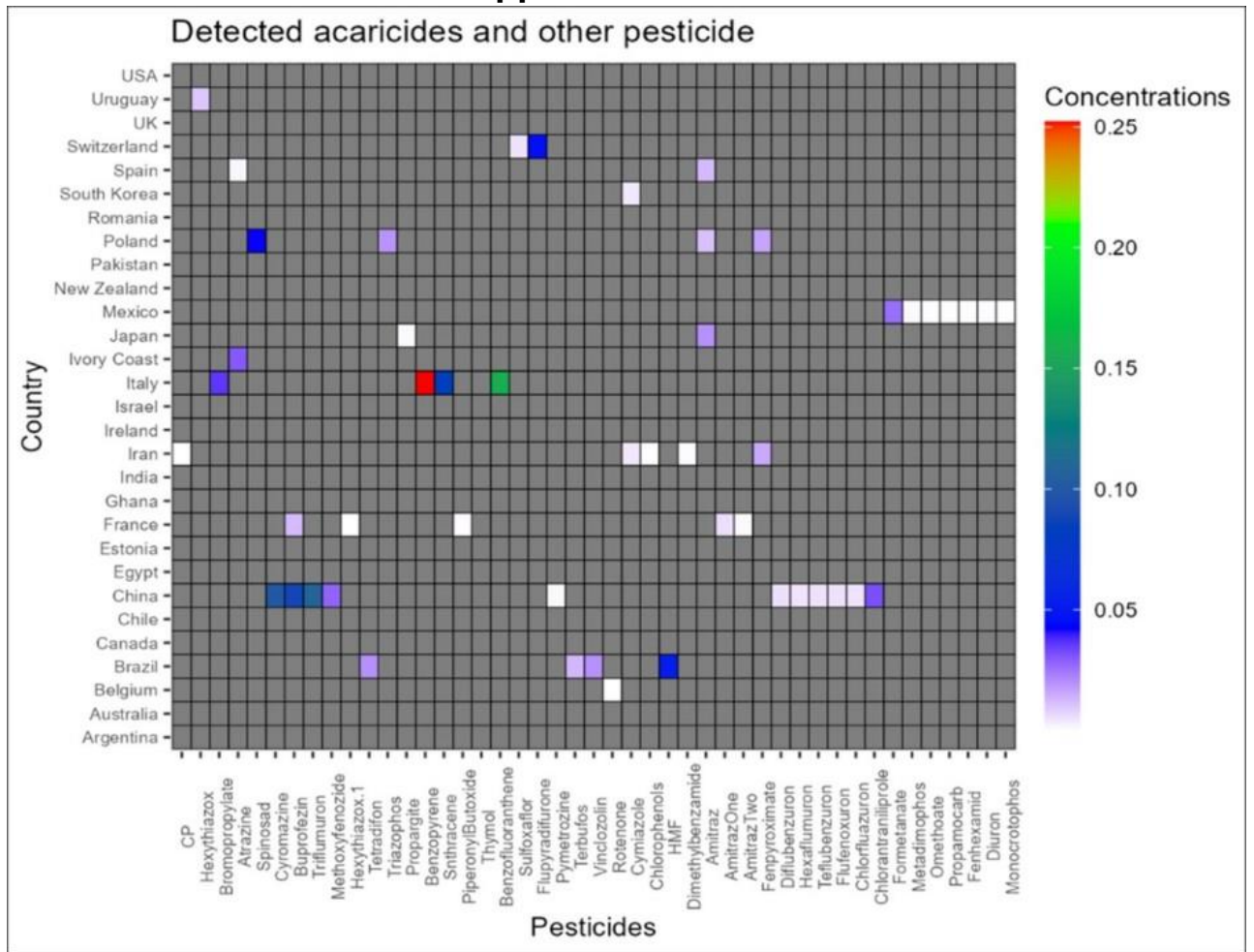
A heatmap featuring a colour scale on the right, portraying the presence of neonicotinoids, pyrethroids, and carbamates (x-axis) studied over time, and their corresponding occurrences in various countries (y-axis).

Appendix B9



A heatmap with a colour scale on the right, depicting the analysis of fungicides and herbicides (x-axis) across time, and their occurrences in different countries (y-axis).

Appendix B10



A heat map, accompanied by a colour scale on the right, illustrating the exploration of acaricides and other pesticides (x-axis) across time and their identification in various countries (y-axis).

Appendix B11

This Excel document contains the number of pesticide residues that were detected across different studies conducted in cocoa-growing countries.

The file can be accessed at the link below:

<https://doi.org/10.1371/journal.pone.0280175.s012>

Appendix B12

An overview of the number and concentrations of pesticide residues detected in countries where multiple studies took place. Generally, the concentrations of these pesticides exhibited fluctuations in different studies. It was only in Estonia where azoxystrobin was detected at an identical concentration in two separate studies.

Detected Pesticides	Concentration mg/kg	Country	Reference
Coumaphos	0.0051	Spain	(Gomez-Perez et al., 2012)
Coumaphos	0.004	Spain	(Juan-Borras et al., 2016)
Coumaphos	0.28	Spain	(Ostiguy and Eitzer, 2014)
Coumaphos	0.036	Spain	(Lozano et al., 2019)
Clothianidin	0.01	Spain	(Sanchez-Hernandez et al., 2016)
Clothianidin	0.045	Spain	(Valverde et al., 2018)
Imidacloprid	0.05	Spain	(Sanchez-Hernandez et al., 2016)
Imidacloprid	0.002	Spain	(Juan-Borras et al., 2016)
Thiamethoxam	0.01	Spain	(Sanchez-Hernandez et al., 2016)
Thiamethoxam	0.144	Spain	(Valverde et al., 2018)
Clothianidin	0.0013	Switzerland	(Mitchell et al., 2017)
Clothianidin	0.00042	Switzerland	(Kammoun et al., 2019)
Imidacloprid	0.0068	Switzerland	(Kammoun et al., 2019)
Imidacloprid	0.00035	Switzerland	(Mitchell et al., 2017)
Thiamethoxam	0.0025	Switzerland	(Kammoun et al., 2019)
Thiamethoxam	0.00029	Switzerland	(Mitchell et al., 2017)
Thiacloprid	0.014	Estonia	(Raimets et al., 2020)
Thiacloprid	0.13	Estonia	(Laaniste et al., 2016)
Tebuconazole	0.009	Estonia	(Karise et al., 2017)
Tebuconazole	0.005	Estonia	(Raimets et al., 2020)
Azoxystrobin	0.031	Estonia	(Raimets et al., 2020)
Azoxystrobin	0.031	Estonia	(Karise et al., 2017)
Glyphosate	0.062	Estonia	(Raimets et al., 2020)
Glyphosate	0.009	Estonia	(Karise et al., 2017)
2,4-D	0.009	Estonia	(Raimets et al., 2020)
2,4-D	0.002	Estonia	(Karise et al., 2017)
Acetamiprid	0.068	China	(Song et al., 2018)
Acetamiprid	0.0088	China	(Hou et al., 2019)
Imidacloprid	0.072	China	(Song et al., 2018)
Imidacloprid	0.0012	China	(Muhammad et al., 2018)
Thiacloprid	0.042	China	(Song et al., 2018)
Thiacloprid	0.0047	China	(Hou et al., 2019)
Chlorpyrifos	0.024	Brazil	(Salami and Queiroz, 2013)
Chlorpyrifos	0.1	Brazil	(de Pinho et al., 2010)
Thiamethoxam	0.202	Poland	(Barganska et al., 2013)
Thiamethoxam	0.0252	Poland	(Bargańska et al., 2018)

Bifenthrin	0.145	Poland	(Barganska et al., 2013)
Bifenthrin	0.0152	Poland	(Bargańska et al., 2018)
Spinosad	0.0206	Poland	(Barganska et al., 2013)
Spinosad	0.02126	Poland	(Bargańska et al., 2018)
Imidacloprid	0.055	Pakistan	(Farooqi et al., 2017)
Imidacloprid	0.736	Pakistan	(Yaqub et al., 2020)

Appendix B13

PRISMA Checklist.

Section and Topic	Item #	Checklist item	The location where the item is reported
TITLE: Honey contamination from plant protection products approved for cocoa (<i>Theobroma cacao</i>) cultivation: A systematic review of existing research and methods			
Title	1	Identify the report as a systematic review.	1
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	1
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	Introduction
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	Introduction
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	Method
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	Method
Search strategy	7	Present the full search strategies for all databases, registers, and websites, including any filters and limits used.	Method
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	Method
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	Method
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g., for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	Method
	10b	List and define all other variables for which data were sought (e.g., participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	Method
Study risk of bias assessment	11	Specify the methods used to assess the risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	n/a
Effect measures	12	Specify for each outcome the effect measure(s) (e.g., risk ratio, mean difference) used in the synthesis or presentation of results.	n/a
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g., tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	Method
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	Method
	13c	Describe any methods used to tabulate or visually display the results of individual studies and syntheses.	Method

Section and Topic	Item #	Checklist item	The location where the item is reported
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	n/a
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g., subgroup analysis, meta-regression).	n/a
	13f	Describe any sensitivity analyses conducted to assess the robustness of the synthesised results.	n/a
Reporting bias assessment	14	Describe any methods used to assess the risk of bias due to missing results in a synthesis (arising from reporting biases).	n/a
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	n/a
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	Results & supplementary Information
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	Method & Results
Study characteristics	17	Cite each included study and present its characteristics.	Results, & Appendix B5
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	n/a
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimates and its precision (e.g., confidence/credible interval), ideally using structured tables or plots.	n/a
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	n/a
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g., confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	n/a
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	n/a
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesised results.	n/a
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	n/a
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	n/a
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	Discussion
	23b	Discuss any limitations of the evidence included in the review.	Discussion
	23c	Discuss any limitations of the review processes used.	Discussion
	23d	Discuss implications of the results for practice, policy, and future research.	Discussion
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	n/a
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	n/a

Section and Topic	Item #	Checklist item	The location where the item is reported
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	n/a
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	n/a
Competing interests	26	Declare any competing interests of review authors.	The authors have no conflict of interest to declare
Availability of data, code, and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	n/a

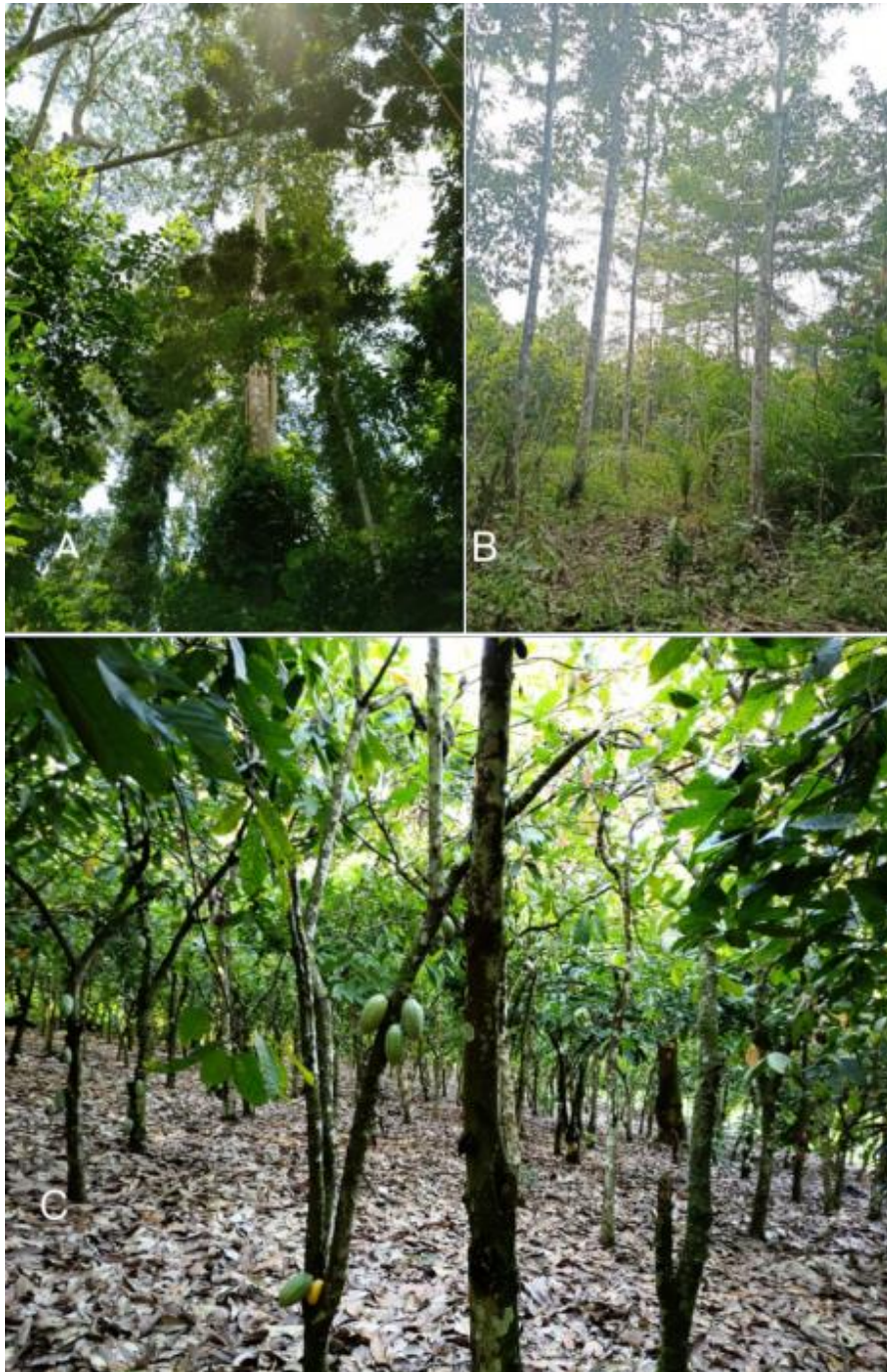
Appendix C1

This is an Excel document which contains a summary of the frequency of detection rates and average concentration per analyte per hive (mg/kg), geographic information and output of landscape analysis. ND denotes not detected, <LOQ denotes pesticide concentration quantified to be below individual analyte's Limit of Quantification (LOQ) and <MRL denotes pesticide concentration quantified to be above LOQ but below Method Reporting Limit (MRL) of 0.1 mg/kg concentration. B2, B4 B5 & BN3 BN4 sample from natural forest; CH7, CH12, CH21, CH24 CH25 samples from fullsun cocoa and CH9, CH16, CH17, CH18 and CH29 samples from agroforestry cocoa.

The file, labelled as "Appendix C1_Detection_Summaries" can be accessed at the link below:

[\[https://docs.google.com/spreadsheets/d/1StxA6JPJiiDHwR_2m4ynTjA6iyb9DVh/edit?usp=sharing&oid=112683485687372806168&rtpof=true&sd=true \]](https://docs.google.com/spreadsheets/d/1StxA6JPJiiDHwR_2m4ynTjA6iyb9DVh/edit?usp=sharing&oid=112683485687372806168&rtpof=true&sd=true)

Appendix C2



Samples of landscapes studied. A) Natural forest inside Bia Conservation Area with rising tree canopies; B) Understorey of fullsun cocoa plantation devoid of any trees and C) Agroforest showing the integration of tree planting in cocoa plantations. (Photo Credit: Richard Boakye).

Appendix C3



Images showing beehive mounting, and honey sample collection. A) A beehive being constructed for field experiments. Specifically, the beehives were top bar beehives of dimensions: base: 91.44 cm x 30.48 cm x 22.85 cm; Lid: 55.88 cm x 93.98 cm; top bars: 48cm x 3.2cm and placed on a metal stand. The beehives constructed were of the same design as those used by the beekeepers. Ten beehives were constructed in July 2020 and mounted to bait for honeybee colonies during the swarming season from August to December 2020. B) Staff of Bia Conservation Area assisting in carrying beehives 2 Km into Bia Conservation Area; C) Beekeepers monitoring and bating their beehives on their farms during the baiting season; D) Monitoring and baiting of a beehive mounted inside Bia North Forest Reserve; E) A colonised beehive located inside BCA as part of the field experiment and F) Honey sample being scooped directly from the honeycomb. (Photo Credit: Richard Boakye).

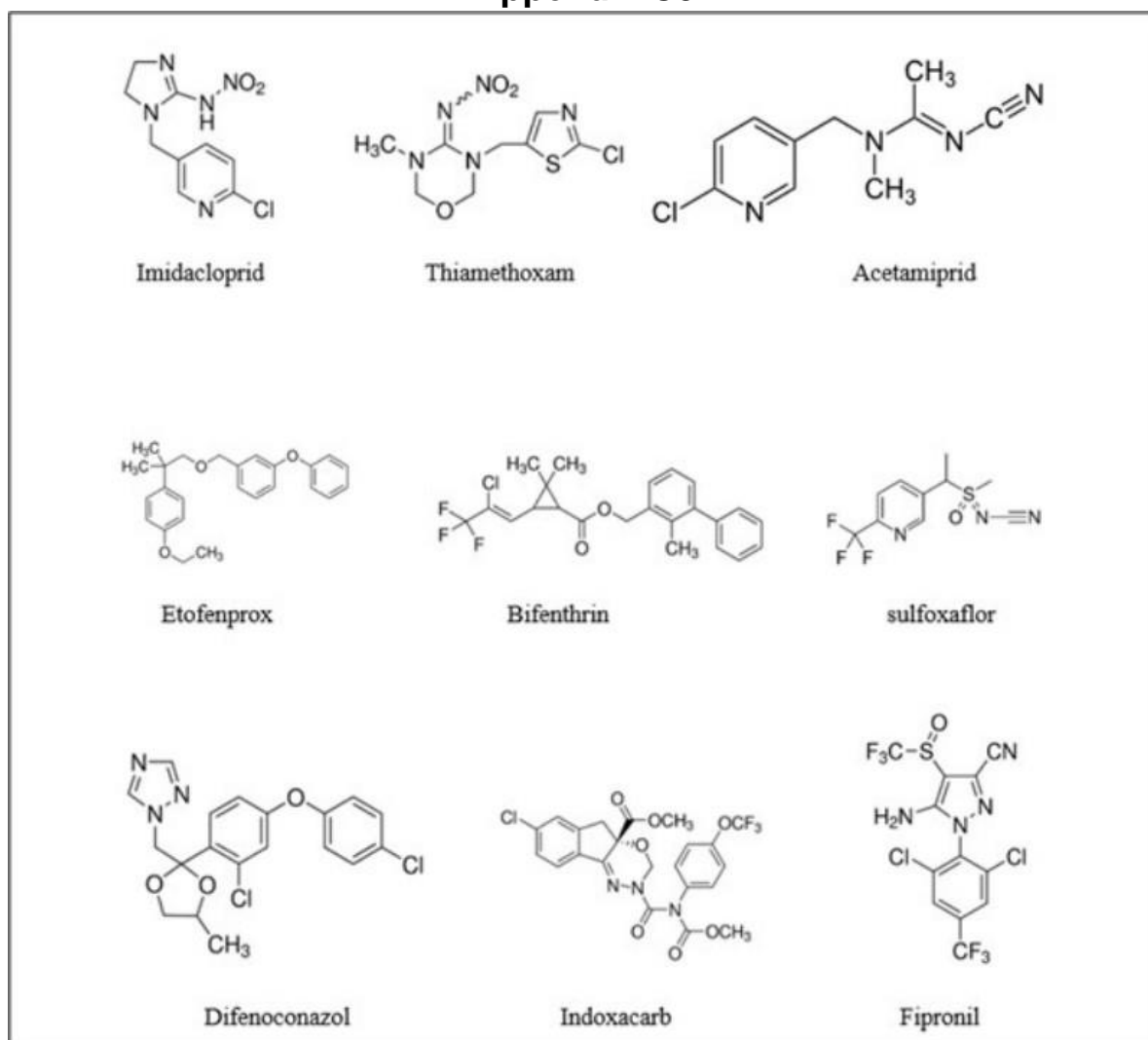
Appendix C4

List of pesticides mostly applied for cocoa cultivation in Bia West District. The list was provided to CODAPEC and distributed to cocoa farmers from 2018 to 2020 as part of the Cocoa Mass Spraying program. Capsaicin was excluded from this study as it is freely occurring in nature as an active ingredient of chilli pepper (Rains and Bryson, 1995).

No	Products (insecticides)	Active ingredient	Cartons of products received and distributed	Year
1	Regent	Fipronil	250	Not indicated
2	Akate Star	Bifenthrin	Unavailable	24/05/2018
3	AF confidence	Capsaicin	130	07/07/2018
4	AF confidence	Capsaicin	80	30/07/2018
5	Confidor	Imidacloprid	200	01/08/2018
6	Akate master	Bifenthrin	150	22/11/2018
7	Akate master	Bifenthrin	100	07/08/2019
8	AF confidence	Capsaicin	167	05/05/2021
9	Akatewura	Thiamethoxam	480	04/04/2020
10	Akate master	Bifenthrin	266	30/04/2021
11	AF confidence	Capsaicin	Unavailable	10/05/2021
12	Viper Super	Indoxacarb +Acetamiprid	1,060	30/07/2020
13	Transfrom Akati	Sulfoxaflor	136	30/07/2020
14	Inspire	Difenoconazole	50	08/09/2020
15	Transfrom Akati	Sulfoxaflor	80	21/07/2020
	Akatewura	Thiamethoxam	200	07/10/2020
17	Akatewura	Thiamethoxam	200	17/08/2021
18	Okum Akate	Thiamethoxam	100	13/09/2021
19	Akate Kaptain (pyrethroid derivative)	Etofenprox	159	16/09/2021
20	Akate Asa	Bifenthrin	250	20/09/2018
21	Akate Asa	Bifenthrin	1,060	17/05/2021

Source: CODAPEC (Cocoa Disease and Pest Control) office at Bia West District. Supplied by Christopher Adu-Gyamfi the District Cocoa Officer and Patrick Asare-Mensah the District Extension Coordinator of CODAPEC.

Appendix C5



Chemical structures of the investigated pesticides. The selected pesticides consisted of 8 insecticides and 1 fungicide with the following CAS numbers: acetamiprid (AC463930000), imidacloprid (138261-41-3), thiamethoxam (153719-23-4), bifenthrin (99267-18-2), Indoxacarb (144171-61-9), Difenoconazole (119446-68-3), Fipronil (120068-37-3) and Sulfoxaflor (946578-00-3). Chemical structures of studied pesticides were sourced individually at Merck Life Science Ltd (2022).

Appendix C6

Exclusion of etofenprox from analysis

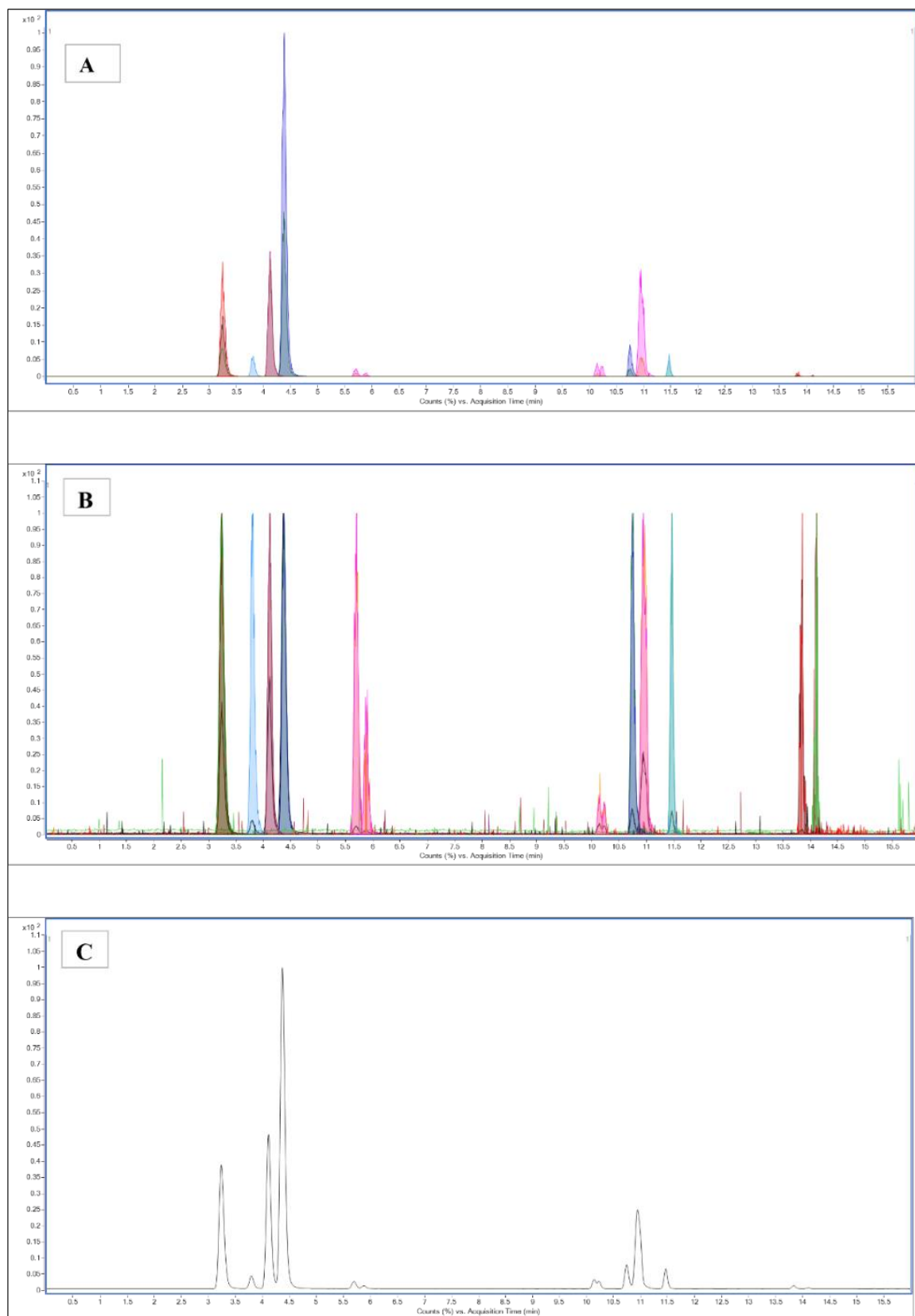
Etofenprox was persistently detected with concentrations which repeatedly exceeded MRL in all the honey samples across all the landscape categories. This pointed to the contamination of the samples themselves. Cross-contamination between analyses was therefore investigated and excluded as a possibility. However, the wood used for the construction of beehives was purchased from the local markets and etofenprox is used as a wood preservative. Therefore, as a product that is used as a wood preservative, contamination from wood treated with etofenprox is a possibility but could not be ascertained from our study. Due to this uncertainty regarding the source of detection and concentration levels of etofenprox, we decided to exclude the results for etofenprox from our analysis. Therefore, the pesticide residues eventually reported in our results were those strictly applied as pesticides on farms without recourse to additional functions as wood preservatives. Consequently, the pesticide residues reported in our results solely pertain to those applied as pesticides on farms and not as a result of any additional use as wood preservatives. This approach ensured that our analysis remains focused on the direct application of pesticides and avoids any potential confounding factors from wood treatment with etofenprox.

Appendix C7

Chromatographic parameters and MS/MS (tandem mass spectrometric) detection for compounds analysed by LC-MS/MS.

Name	RT (min)	Precursor > Product Ion				
		Quantifier Ion	CE	Qualifier Ion	CE	Ion Polarity
Acetamiprid	4.538	223.2 > 126.1	20	223.2 > 56.1	20	ESI+
Bifenthrin	14.075	439.9 > 181.1	16	440.2 > 181.1	16	ESI+
				442.1 > 181.1		
Difenoconazole	11.036	406.1 > 251.0	28	406.1 > 111.1	28	ESI+
Etofenprox	13.797	394.3 > 177.0	15	394.3 > 106.9	15	ESI+
Fipronil	10.833	434.9 > 330.0	17	434.9 > 250.0	17	ESI-
Imidacloprid	4.217	256.2 > 175.2	25	256.0 > 209.0	25	ESI+
Indoxacarb	11.585	528.1 > 203.0	40	528.1 > 150.0	40	ESI+
Sulfoxaflor	5.804	278.3 > 174.0	8	278.3 > 154.0	8	ESI+
Thiamethoxam	3.378	292.0 > 211.1	15	292.0 > 181.0	15	ESI+

Appendix C8



Sample MRM chromatogram separation of all targeted analytes. Based on their retention times from left to right: thiamethoxam 3.378 min, Imidacloprid 4.217 min, acetamiprid 4.538 min, sulfoxaflor 5.804 min, Fipronil 10.833 min, Difenoconazole

11.036 min, Indoxacarb 11.585 min, Etofenprox 13.797 min, bifenthrin 14.075 min; B). sample MRM chromatogram separation of all the analytes (scaled to the largest peak); C) Sample TIC MRM chromatogram separation of all the analytes. Each colour depicts one specific analyte based on its MRM transition ion with details of its retention times, quantifier, and qualifier ions.

Appendix C9

The current applicable Maximum Residue Limits (MRL) of targeted pesticides in honey. The MRL for acetamiprid (0.3 mg kg⁻¹) is the newly proposed with the current applicable MRL being 0.05 under the Reg. (EU) 2019/88.

Substance	MRL (mg kg⁻¹)	Source/Remarks
Acetamiprid	0.3	SANTE/11278/2021 Annex II
Imidacloprid	0.05	Reg. (EU) 2021/1881 Annex II
Thiamethoxam	0.05	Reg. (EU) 2017/671 Annex II
Indoxacarb	0.05	Reg. (EU) 2015/845 Annex II
Sulfoxaflor	0.05	Reg. (EU) 2018/832 Annex II
Bifenthrin	0.05	Reg. (EU) 2018/687 Annex II
Etofenprox	0.05	Reg. (EU) 2021/590 Annex II
Difenoconazole	0.05	Reg. (EU) 2019/552 Annex IIIA
Fipronil	0.005	Reg. (EU) 2019/1792 Annex V

Sourced at <https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/start/screen/mrls> on 06/11/05/2022

Appendix C10

Method validation

Linear range, linearity, limit of detection (LOD) and limit of quantification (LOQ) were determined for all traces analysed. The linearity of the internal calibration was determined using a six-point calibration which ranged from 0.1 to 50 $\mu\text{g mL}^{-1}$ by spiking blank honey samples before extraction was conducted. The correlation coefficient (r^2) in the blank honey samples was evaluated and was found to be 0.999 for eight of the eight analytes but 0.99 for bifenthrin. The injection volume of 20 μL used in this study had previously been applied (Kavanagh et al., 2021, Mitchell et al., 2017) and has been shown to provide efficient sensitivity with no overlapping of chromatography peaks with the elution programme (Campillo et al., 2013). The applied method was appropriate for a multi-residue analysis and allowed for the simultaneous detection and quantification of the analytes. The separation of the eight targeted pesticide residues was achieved within acceptable LODs and LOQs. The LODs for the targeted pesticide residues ranged from 0.001 to 0.022 mg/kg with the LOD for each analyte being lower than its specified MRL (Appendix C9) indicating the method's high sensitivity. Similarly, all LOQs for the pesticide residues were lower than the specified MRLs except bifenthrin whose LOQ (0.068mg/kg) exceeded its specified MRL.

Linear range, linearity, LOD and LOQ for all the targeted pesticides using solvent standard calibration using LC-MS/MS.

No.	Pesticides	Linear Range	Linearity (r^2)	Limit of Detection (ng mL^{-1})	Limit of Quantification (ng mL^{-1})
		(ng mL^{-1})			
1	Acetamiprid	0.1-50	0.999	0.005	0.014
2	Bifenthrin	0.1-50	0.99	0.022	0.068
3	Difenoconazole	0.1-50	0.999	0.001	0.002
4	Etofenprox	0.1-50	0.999	0.004	0.012
5	Fipronil	0.1-50	0.999	0.003	0.01
6	Imidacloprid	0.1-50	0.999	0.001	0.003
7	Indoxacarb	0.1-50	0.999	0.001	0.002
8	Sulfoxaflor	0.1-50	0.999	0.001	0.003

9	Thiamethoxam	0.1-50	0.999	0.002	0.007
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Prior to a full scale of batch analyses of targeted analytes, recovery experiments were conducted by fortifying blank honey samples at three concentrations: at the reporting limit (RL) which is 0.1 ug L⁻¹; then at 5xRL and finally at 10xRL in three replicates. This ensured an assessment of the recovery efficiencies over a range of concentration levels using a t-test to assess the significant difference between the mean of the analytes' recoveries. The matrix effects (%) were calculated by comparing the peak area of the matrix-matched standard to the peak area of the solvent standard. Mean recoveries lying in the range of 70%-120% as specified in SANTE/2019/12682 by (Commission, 2019) with associated repeatability relative standard deviation (RSDr) ≤ 20% are deemed acceptable. Recovery rates between 60% and 140% in the routine analysis are however also regarded as satisfactory (Commission, 2019). The recovery rates obtained for 89% of the targeted analytes ranged from 73.9%-111.7% except for bifenthrin whose minimum and maximum recovery percentages were 41.2% and 84.1% respectively. A significant matrix effect (-145) was detected for bifenthrin. The validation of the precision, represented as the RSDr is presented in Appendix C11.

Appendix C11

Recoveries, RSD% and matrix effect (ME) of all the targeted pesticides in the blank honey samples using LC-MS/MS ($n = 3$). ME (%) = (Peak area of matrix-matched standard-peak area of solvent standard) x100/Peak area of matrix-matched standard ME (%) with +values are signal enhancement, -values are signal suppression. Values between +20% and -20% are considered to represent low matrix effects, values between +20% and +50% represent a medium matrix effect, and values less than -50% or higher than +50% represent high matrix effects.

No.	Pesticides	Honey						
		MRL (0.1 ng mL ⁻¹)		5xMRL (0.5 ng mL ⁻¹)		10xMRL (1 ng mL ⁻¹)		ME (%)
		Rec.	RSD%	Rec.	RSD%	Rec.	RSD%	
1	Acetamiprid	93	30.4	100.3	12.7	107.1	9.8	-8
2	Bifenthrin	41.2	5.7	66.5	21.6	84.1	7.3	-143
3	Difenoconazole	92.4	24.7	97.1	11.6	101	19.5	-8
4	Etofenprox	95.6	19.4	97.8	9.5	95.3	15.7	-5
5	Fipronil	86.2	11.4	96.8	10.6	85.7	10.6	-16
6	Imidacloprid	106.7	16.3	96.9	15.4	102.7	3.2	6
7	Indoxacarb	79.8	33.6	98.2	13.6	97.1	5.3	-25
8	Sulfoxaflor	73.9	8.9	93.9	4.6	94.1	1.16	-35
9	Thiamethoxam	99.6	22.8	111.7	18.3	100.5	13.7	-0.4

Appendix C12

This document contains included papers found eligible and used for cross-referencing to evaluate sub-lethal effects.

The file, labelled as “Appendix C12_Sublethal” can be accessed at the link below :

[\[https://docs.google.com/spreadsheets/d/1mF0uVY91-dqtlqJZmj1WlIKuuofajkJ/edit?usp=sharing&oid=112683485687372806168&rtpof=true&sd=true \]](https://docs.google.com/spreadsheets/d/1mF0uVY91-dqtlqJZmj1WlIKuuofajkJ/edit?usp=sharing&oid=112683485687372806168&rtpof=true&sd=true)

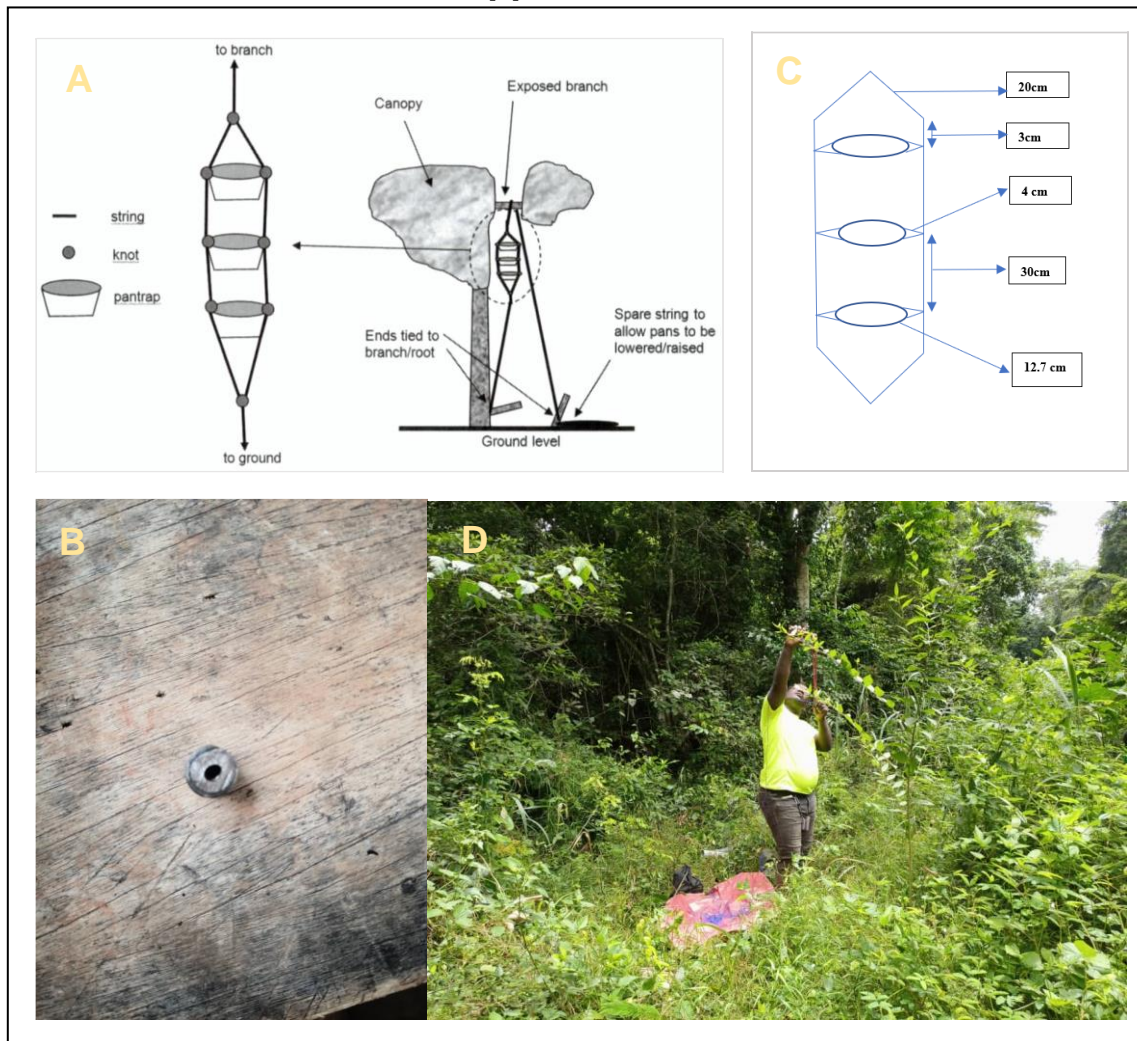
Appendix D2

The total number of candidate sites which were subjected to principal component analysis leading to the final selection of the 18 sampled sites. The per cent cover of habitat types within 2km around both total sites evaluated. The column labelled NF connotes the sum of open and closed forests. The colour-shaded areas are the selected and sampled sites based on PCA performed which have been compiled separately in Tab2.

The file, labelled as “Appendix D2” can be accessed at the link below:

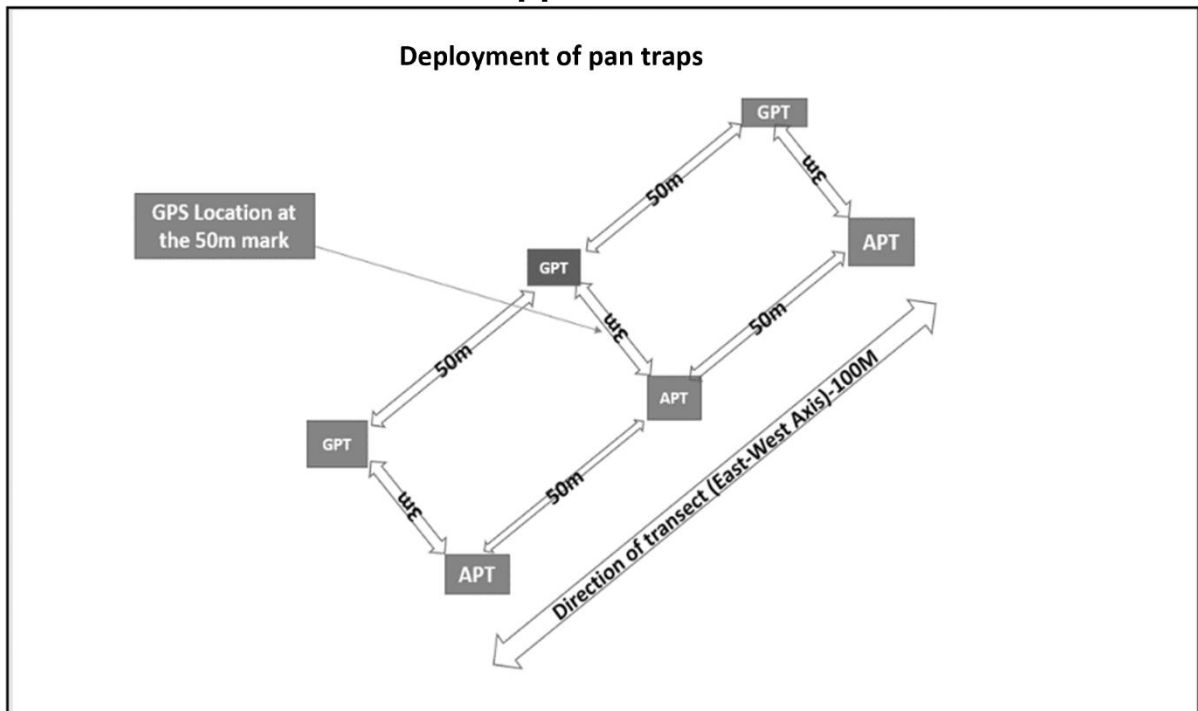
[\[https://docs.google.com/spreadsheets/d/1kxmyPgkj_p_lqUgg_bPm0yAXByOfTVEk/edit?usp=sharing&oid=112683485687372806168&rtpof=true&sd=true \]](https://docs.google.com/spreadsheets/d/1kxmyPgkj_p_lqUgg_bPm0yAXByOfTVEk/edit?usp=sharing&oid=112683485687372806168&rtpof=true&sd=true)

Appendix D3



A) The diagrammatic representation of how the aerial pan traps were guided into the tree canopies [Sourced: Nuttman et al. (2011)]. We made a slight modification to this design. In our study, we used a metal frame/seat to construct the bowl seats as opposed to the recommended string for the seat (Nuttman et al., 2011). This provided a more stable seat for the bee bowls when raising them into the tree canopies; B) Metal projectile. A hole is drilled through the metal. The nylon fishing line is tied to it by passing it through the drilled hole. The projectile helps carry the nylon rope over the emergent tree branch; C) Dimensions for metal frame for aerial pan trap. The bowls used to make the pan traps were of 6" diameter at the tip, 5" deep and 4" at the base and D) Metal projectile being fired using a catapult into the tree canopies.

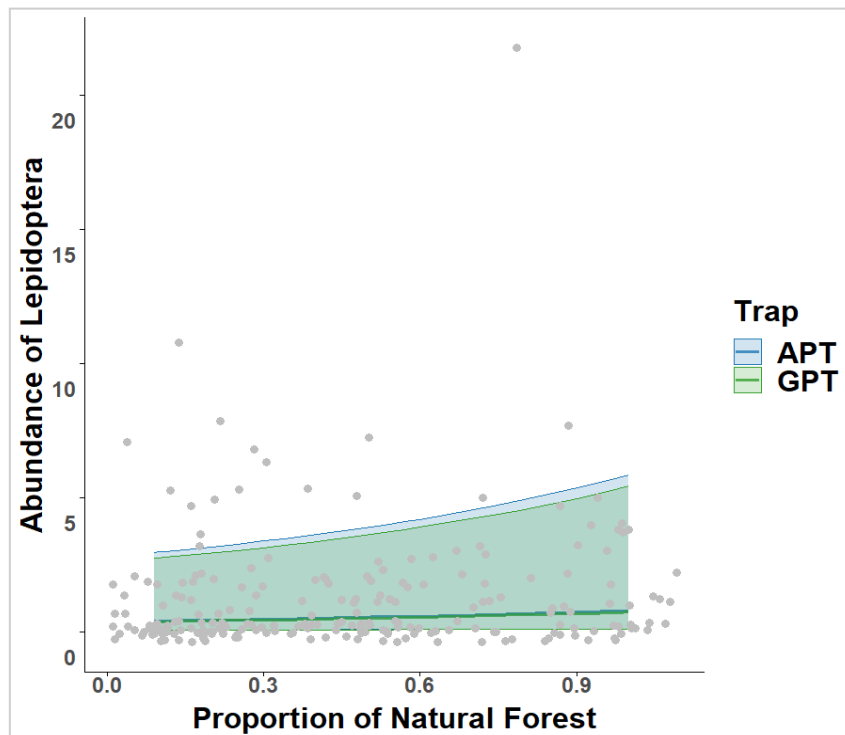
Appendix D4



The outlay of transects at study sites. A 100 m transect was placed at each selected study site along an East-West axis with the GPS coordinate of the site placed exactly at the 50m mark on the transect. 3 GPTs (Ground pan traps) and 3 APTs (Aerial pan traps) were placed on the transect.

Appendix D5

Variations were observed in the abundance of Lepidoptera between traps, with 138 individuals in APT and 128 in GP. Out of the total abundance collected, 250 individuals were observed during the dry season, but only 16 individuals were recorded during the rainy season. The abundance of the Lepidoptera was not predicted by the forest ($\chi^2=3.42$, $df=1$, $p=0.06$); the trap type ($\chi^2=0.38$, $df=1$, $p=0.54$) or their interaction term. It must however be noted that pan traps are not standard method for sampling lepidoptera which are better sampled using malaise traps, light traps, pheromone traps, netting among others (Freitas et al., 2021, Brehm and Axmacher, 2006). It was not surprising that Lepidoptera was the lowest represented in in our study using pan traps. The underrepresentation of Lepidoptera in our study aligns with observations by Vrdoljak and Samways (2012) and Csanády et al. (2021) who noted Lepidoptera's lower abundance in pan trap samplings. This discrepancy could be linked to the colour preferences of Lepidopterans, as pan traps are typically painted yellow, white, and blue, while Lepidoptera may exhibit specific preferences related to their association with flower shapes and patterns (Kristensen, 2003, Haverkamp et al., 2016).



The graph above shows how the abundance of Lepidoptera is related to the changing proportion of natural forests.

Appendix D6

This dataset consists of the abundances of the different insect orders collected during field sampling. Also included are the abundances of the different bee species collected. Insect abundances are linked to study sites, trap or bowl colour used, the season and sampling rounds as well as the habitat types and geographic coordinates.

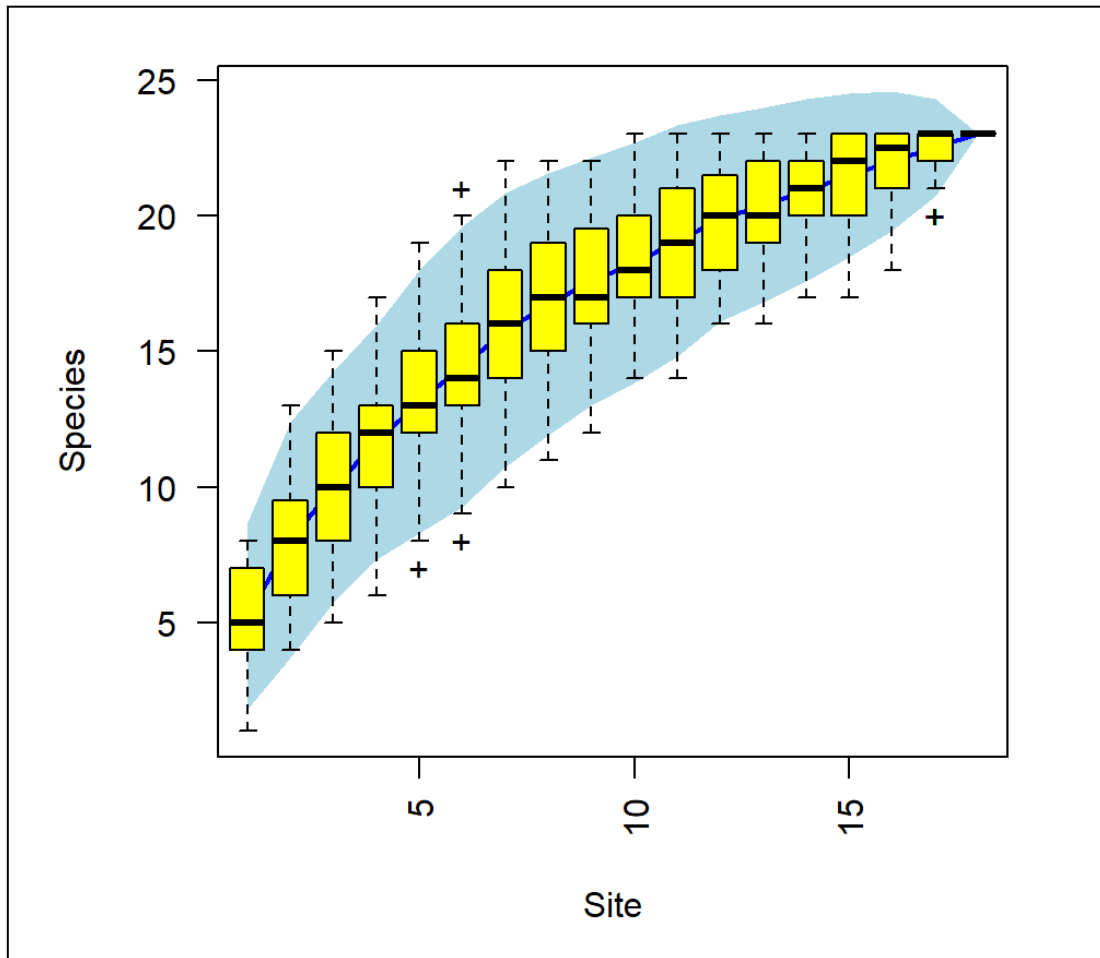
The file labelled as “Appendix D6_Dataset” can be accessed at the link below:

[\[https://docs.google.com/spreadsheets/d/1Z9B4YLxj9j1Boys8eAkozmA4hRN3yLtl/edit?usp=sharing&ouid=112683485687372806168&rtpof=true&sd=true\]](https://docs.google.com/spreadsheets/d/1Z9B4YLxj9j1Boys8eAkozmA4hRN3yLtl/edit?usp=sharing&ouid=112683485687372806168&rtpof=true&sd=true)

Appendix D7

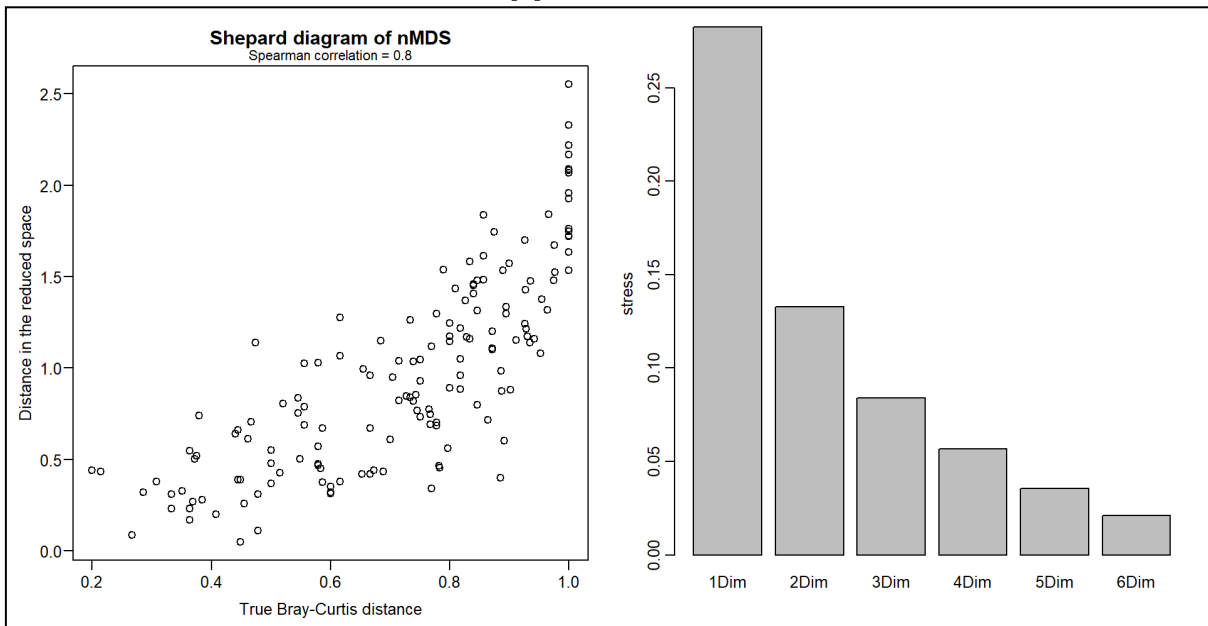
Common Name	Family	Genus	Species	Abbreviations	Abundance per season		Abundance per trap		Total
					Dry	Rainy	APT	GPT	
Honeybees	Apidae	<i>Apis</i>	<i>Apis mellifera adansonii</i>	Apis	57	23	42	38	80
Stingless bees	Apidae	<i>Meliponula</i>	<i>Meliponula (Axestotrigona) ferruginea</i> (Lepeletier, 1836)	MeliF	34	3	20	17	17
			<i>Meliponula bocandei</i> (Spinola, 1853)	MeliB	2	0	1	1	2
	Apidae	<i>Hypotrigona</i>	<i>Hypotrigona gribodoi</i> (Magretti, 1884)	Hypo	2	0	1	1	2
	Apidae	<i>Dactylurina</i>	<i>Dactylurina staudingeri</i> (Gribodo, 1893)	Dacty	3	6	6	3	9
Solitary bees	Apidae	<i>Compsomelissa</i>	<i>Compsomelissa nigrinervis</i> (Cameron, 1905)	Comps	7	86	18	75	93
	Apidae	<i>Braunsapis</i>	<i>Braunsapis facialis</i> (Gerstaecker, 1858),	Brau	54	0	18	36	54
	Apidae	<i>Ceratina</i>	<i>Ceratina calcarata</i> Vachal (1903)	Cere	0	1	0	1	1
	Apidae	<i>Xylocopa</i>	<i>Xylocopa nigrita</i> (Fabricius, 1775),	Nigrita	12	7	10	9	19
	Apidae		<i>Xylocopa varipes</i> Smith (1854)	Varipes	1	1	1	1	2
	Apidae		<i>Xylocopa hottentotta</i> Smith (1854)	XyloH	1	0	0	1	1
	Apidae		<i>Xylocopa imitator</i> Smith (1854)	XyloIm	1	0	0	1	1
	Apidae		<i>Xylocopa flavonifa</i> (De Gees, 1778)	Dbees	0	1	1	0	1
	Apidae	<i>Amegilla</i>	<i>Amegilla albocaudata</i> (Dours, 1869)	Albo	9	0	5	4	9
	Apidae		<i>Amegilla nila</i> Eardley (1994)	Nilla	2	2	2	2	4
	Apidae		<i>Amegilla acraensis</i> (Fabricius, 1793)	Acree	3	0	0	3	3
	Megachilidae	<i>Lithurgus</i>	<i>Lithurgus pullatus</i> (Vachal, 1903)	Lith	3	0	3	0	3
	Halictidae	<i>Halictus</i>	<i>Halictus jucundus</i> Smith 1853	Hali	3	0	1	2	3
	Halictidae	<i>Lipotriches</i>	<i>Lipotriches cirrita</i> (Vachal, 1903)	Cribo	0	2	1	1	2
	Halictidae		<i>Lipotriches orientalis</i> (Friese, 1909)	Lipsp	2	0	2	0	2
	Halictidae	<i>Pseudapis</i>	<i>Pseudapis squamata</i> (Morawitz, 1895)	Pseu	1	0	1	0	1
	Halictidae	<i>Lasioglossum</i>	<i>Lasioglossum duponti</i> (Vachal, 1903)	Lasio	0	1	1	0	1
Halictidae	<i>Nomia</i>	<i>Nomia bouyssoui</i> Vachal (1903)	Nom	2	0	1	1	2	

Appendix D8



A randomised species accumulation curve, showing the cumulative number of species observed across the entire study area. The curve reflects the increasing count of unique species as sampling efforts intensify. The boxplots represent the distribution and variability in species richness at each sampling site. The yellow boxes and blue borders showcase the interquartile range, while the horizontal lines within the boxes signify the median species count. This visualization offers a comprehensive overview of the richness patterns and sampling variability throughout the study, aiding in the interpretation of biodiversity dynamics.

Appendix D9



Shepard Diagram evaluating the NMDS goodness-of-fit of the relationship between species composition and environmental variable. Left) Sheperd diagram showing the distances in the 2-dimension of the ordination of the original distance. Right) The Possible dimension of representation is based on the Bray-Curtis distance. The bars represent the total stress based on the stress of the solution of the dimension selected. A 2-dimension was applied in this study which achieved a good presentation of the community com