Trace gas fluxes from soils and tree stems of rainforests and cacao agroforests in the Congo Basin, Cameroon

Dissertation zur Erlangung des Doktorgrades

der Fakultät für Forstwissenschaften und Waldökologie

der Georg-August-Universität Göttingen

vorgelegt von

NAJEEB AL-AMIN IDDRIS

geboren in Kumasi, Ghana

Göttingen, März 2020

1. Gutachter: Professor Dr. Edzo Veldkamp

2. Gutachter: Professor Dr. Alexander Knohl

Tag der mündlichen Prüfung: 26.05.2020

PhD supervisors: Professor Dr. Edzo Veldkamp and Dr. Marife D. Corre

Hasbunallahu Wa Ni'mal Wakeel

To my dad, **Iddris Issah**, who gave up his dreams so I could achieve mine, and to my supervisor, **Marife D. Corre**; "The scientist I am and hope to be I owe to her mentorship.

TABLE OF CONTENTS

Summary vi

Zusammenfassung viii

Chapter 1: General introduction 1

1.1. The role of the tropics in the global trace greenhouse gas budgets 2

1.2. Trees as conduits of N_2O and CH_4 fluxes 4

1.3. Effects of land-use change on trace gas fluxes 7

1.4. The Congo Basin, Cameroon 7

1.5. Aims and hypotheses 8

1.6. Study area and experimental set-up 9

1.7. References 13

Chapter 2: Stem and soil nitrous oxide fluxes from rainforest and cacao agroforest on highly weathered soils in the Congo Basin 20

2.1. Abstract 21

2.2. Introduction 22

2.3. Materials and methods 25

2.3.1. Study area and experimental design 25

2.3.2. Measurement of stem and soil N_2O fluxes 29

2.3.3. N_2O analysis and flux rate calculation 33

2.3.4. Soil and meteorological variables 34

2.3.5. Statistical analyses 37

2.4. Results 39

2.4.1. Stem N_2O emissions 39

2.4.2. Soil N_2O emissions 44

2.4.3. Soil biochemical characteristics 45

2.5. Discussion 45

2.5.1. Stem and soil N_2O emissions from the forest 45

2.5.2. Source of tree-stem N_2O emissions and their contribution to total (soil + stem) N_2O emissions 47

2.5.3. Factors controlling temporal variability of stem and soil N_2O fluxes 48

2.5.4. Land-use change effects on soil N_2O emissions 49

2.6. Implications 50

2.7. Acknowledgements 51

2.8. References 52

2.9. Appendix 58

Chapter 3: Tree stem and soil methane and carbon dioxide fluxes from rainforest and cacao agroforest on highly weathered soils in the Congo Basin 60
3.1. Abstract 61
3.2. Introduction 62
3.3. Materials and methods 65
3.3.1. Study area and experimental design 65
3.3.2. Measurement of stem and soil CH_4 fluxes and soil CO_2 fluxes 67
3.3.3. Trace greenhouse gas analysis and flux rate calculation 69
3.3.4. Soil and climatic variables 70
3.3.5. Statistical analysis 71
3.4. Results 73
3.4.1. Stem CH_4 fluxes 73
3.4.2. Soil CH_4 fluxes 78
3.4.3. Soil CO_2 fluxes 78
3.5. Discussion 79
3.5.1. Stem CH_4 emissions and their contribution to total (soil + stem) CH_4 emissions 79
3.5.2. Factors controlling temporal and spatial variability of soil fluxes 83
3.5.3. Effects of land-use change on soil CO_2 and CH_4 fluxes 85
3.6. Conclusions 88
3.7. Acknowledgement 89
3.8. References 89
3.9. Appendix 95
Chapter 4: Synthesis 97
4.1. Key findings of this thesis 98
4.2. Revising the African greenhouse gas budget 99
4.3. Tree stem emissions and implications for global greenhouse gas budgets 103
4.4. Outlook 104
4.5. References 105
Acknowledgement 107
Thesis declaration 109
Curriculum vitae ..... **Error! Bookmark not defined.**

SUMMARY

Tropical rainforests play a crucial role in biogeochemical cycles and global climate dynamics. Yet, research efforts to quantify the main sources and sinks of trace greenhouse gases lags behind that of other biomes. The African continent is among the least researched regions worldwide, and the effects of land-use change on trace greenhouse gases are identified as an important research gap in the greenhouse gas budget of Africa. Recent studies in wetland and temperate forests have provided evidence for tree stem nitrous oxide (N_2O) and methane (CH_4) emissions, but the magnitudes of tree contributions to total (soil + stem) N_2O and CH_4 emissions from tropical rainforests on heavily weathered soils remain unknown. Given these knowledge gaps, this thesis consists of two studies aimed at quantifying the changes in stem and soil N_2O and CH_4 fluxes, and soil carbon dioxide (CO_2) fluxes with forest conversion to cacao agroforestry. The study was conducted at three sites (villages) in central and southern Cameroon, all located on heavily weathered soils. To assess the impact of land-use change on stem and soil greenhouse gas fluxes, we studied two land-use systems at each site: the reference forest and the converted cacao agroforestry system. At each site, we selected four replicate plots (2500 m^2 each) for each land use. Soil and stem greenhouse gas fluxes were measured monthly using vented static chambers (4 chambers per plot) and stem chambers (6 trees per plot), respectively, from April 2017 to April 2018. On each measurement period, we also measured known soil and climatic controlling factors.

The aim of the first study was to quantify the changes in stem and soil N_2O fluxes with forest conversion to cacao agroforestry. Additionally, we conducted a ^{15}N tracing experiment at one of the sites as a follow-on study to elucidate the source of stem N_2O emissions. Our findings revealed that trees on well-drained, heavily weathered soils served as an important N_2O emission pathway, with the potential to overlook up to 38% of fluxes in the forests, and

up to 15% of fluxes in cacao agroforests, if tree stems are not considered in the ecosystem N_2O budget. ^{15}N-isotope tracing from soil mineral N to stem-emitted $^{15}N_2O$ suggest that emitted N_2O from stems originated predominantly from N_2O produced in the soil. Additionally, forest conversion to cacao agroforestry systems had no effect on stem and soil N_2O emissions, because of similarities in soil moisture and soil texture, absence of fertilizer application, and comparable presence of leguminous trees in both land uses, which can compensate for N export from harvest or other losses.

For our second study, we investigated the changes in stem and soil CH_4 fluxes and soil CO_2 fluxes with forest conversion to cacao agroforestry. Conversion of forest to cacao agroforestry had no effect on stem and soil CH_4 and CO_2 fluxes. The lack of differences may be due to the comparable soil texture and soil moisture content between the two land uses, which influences gas diffusivity into and out of the soil. All the studied trees emitted measurable CH_4 at some point during the study period. In both land uses, tree stems were net sources of CH_4, while the soils were net CH_4 sinks. Our upscaling suggests that tree stem emissions offset 3–18% of the annual soil CH_4 sink in both land uses.

This study provides the first year-round and spatially replicated quantifications of stem and soil trace gas fluxes for the Congo Basin, with key implications for improved estimates of trace gas budgets for Africa. Our results show for the first time that, N_2O and CH_4 emissions from tree stems on well-drained soils are apparently widespread and detectable in many tropical trees in Africa. As discussed in the synthesis chapter, even low stem trace gas emissions at the ecosystem level can upscale to significant fluxes globally. These findings emphasize the need for additional studies on tree stem fluxes in order to constrain their magnitudes and mechanisms, and to refine global greenhouse gas budgets.

ZUSAMMENFASSUNG

Tropische Regenwälder spielen eine entscheidende Rolle in biogeochemischen Kreisläufen und der globalen Klimadynamik. Dennoch bleiben die Forschungsbemühungen zur Quantifizierung der Hauptquellen und -senken von Treibhausgasen hinter denen anderer Biome zurück. Der afrikanische Kontinent gehört zu den am wenigsten erforschten Regionen weltweit, und die Auswirkungen von Landnutzungsänderungen auf Treibhausgase stellen eine wichtige Forschungslücke im Treibhausgasbudget Afrikas dar. Jüngste Studien in Feuchtgebieten und gemäßigten Wäldern haben Nachweise für die Distickstoffmonoxid- (N_2O) und Methan- (CH_4) Emissionen von Baumstämmen geliefert, aber die Größenordnung der Beiträge der Bäume zu den gesamten (Boden + Stamm) N_2O- und CH_4-Emissionen aus tropischen Regenwäldern auf stark verwitterten Böden bleibt unbekannt. Angesichts dieser Wissenslücken besteht diese Arbeit aus zwei Studien, die darauf abzielen, Veränderungen der N_2O- und CH_4-Flüsse in Stamm und Boden, sowie die Kohlenstoffdioxid (CO_2)-Flüsse im Boden bei der Umwandlung von Wald in Kakao-Agroforstwirtschaft zu quantifizieren. Die Studie wurde an drei Standorten (Dörfern) in Zentral- und Südkamerun durchgeführt, die alle auf stark verwitterten Böden liegen. Um die Auswirkungen von Landnutzungsänderungen auf die Treibhausgasflüsse von Stamm und Boden zu bewerten, untersuchten wir an jedem Standort zwei Landnutzungssysteme: den Referenzwald und das umgestellte Kakao-Agroforstsystem. An jedem Standort wählten wir für jede Landnutzung vier Wiederholungsflächen (je 2500 m^2) aus. Die Boden- und Stamm-Treibhausgasflüsse wurden von April 2017 bis April 2018 monatlich mit belüfteten statischen Hauben (4 Hauben pro Fläche) bzw. Stamm-Hauben (6 Bäume pro Fläche) gemessen. In jeder Messperiode wurden auch bekannte boden- und klimaregulierende Faktoren gemessen.

Das Ziel der ersten Studie war es, die Veränderungen der N_2O-Flüsse in Stamm und Boden bei der Umwandlung von Wald in Kakao-Agroforstwirtschaft zu quantifizieren. Zusätzlich führten wir als Folgestudie ein ^{15}N-Rückverfolgungsexperiment an einem der Standorte durch, um die Quelle der Stamm-N_2O-Emissionen ausfindig zu machen. Unsere Ergebnisse zeigten, dass Bäume auf gut entwässerten, stark verwitterten Böden als wichtiger N_2O-Emissionspfad dienten, mit dem Potenzial, bis zu 38% der Flüsse in den Wäldern und bis zu 15% der Flüsse in den Kakao-Agroforstwäldern zu übersehen, wenn die Baumstämme nicht im N_2O-Budget des Ökosystems berücksichtigt werden. Die Rückverfolgung des ^{15}N-Isotops vom mineralischen Bodenstickstoff auf das von den Stämmen emittierte $^{15}N_2O$ lässt vermuten, dass das von den Stämmen emittierte N_2O überwiegend aus dem im Boden produzierten N_2O stammt. Darüber hinaus hatte die Umstellung der Wälder auf Kakao-Agroforstwirtschaft keine Auswirkungen auf die N_2O-Emissionen von Stämmen und Böden aufgrund von Ähnlichkeiten in der Bodenfeuchte und Bodenbeschaffenheit, Abwesenheit von Düngemittel und vergleichbarer Präsenz von leguminosen Baumarten in beiden Landnutzungssystemen, was den Stickstoff- Export aus Ernte oder anderen Verlusten ausgleichen kann.

Für unsere zweite Studie untersuchten wir Veränderungen der CH_4-Flüsse in Stamm und Boden sowie die CO_2-Flüsse im Boden bei der Umwandlung von Wald in Kakao-Agroforstwirtschaft. Die Umwandlung von Wald in Kakao-Agroforstwirtschaft hatte keine Auswirkungen auf die CH_4- und CO_2-Flüsse von Stamm und Boden. Die Abwesenheit von Unterschieden könnte auf die vergleichbare Bodentextur und Bodenfeuchtigkeit zwischen beiden Landnutzungen zurückzuführen sein, welche das Diffusionsvermögen von Gasen in den Boden hinein und aus dem Boden heraus beeinflussen. Alle untersuchten Bäume emittierten irgendwann während der Untersuchungsperiode messbares CH_4. In beiden Landnutzungen waren die Baumstämme Nettoquellen von CH_4, während die Böden Netto-CH_4-Senken waren.

Unsere Hochskalierung deutet darauf hin, dass die Baumstammemissionen 3 bis 18% der jährlichen CH_4-Senkung des Bodens in beiden Landnutzungen ausgleichen.

Diese Studie liefert die ersten ganzjährigen und räumlich replizierten Quantifizierungen der Stamm- und Boden-Spurengasflüsse für das Kongobecken, mit entscheidenden Auswirkungen auf verbesserte Schätzungen der Spurengasbudgets für Afrika. Unsere Ergebnisse zeigen zum ersten Mal, dass N_2O- und CH_4-Emissionen von Baumstämmen auf gut entwässerten Böden offenbar weit verbreitet und bei vielen tropischen Bäumen in Afrika nachweisbar sind. Wie im Synthesekapitel erörtert, können selbst geringe Spurengasemissionen von Baumstämmen auf Ökosystemebene zu signifikanten Strömen weltweit führen. Diese Ergebnisse unterstreichen die Notwendigkeit zusätzlicher Studien über die Baumstamm-Flüsse, um ihre Größenordnung und Mechanismen zu begrenzen und die globalen Treibhausgasbudgets weiter zu verfeinern.

CHAPTER 1

GENERAL INTRODUCTION

1.1. The role of the tropics in the global trace greenhouse gas budgets

Carbon dioxide (CO_2), nitrous oxide (N_2O) and methane (CH_4) constitute the most important long-lived greenhouse gases (GHG) in the atmosphere. CO_2 has a longer atmospheric lifetime (5–200 years) than both N_2O (114 years) and CH_4 (12 years) (Forster *et al.*, 2007), and the absolute quantity of emitted CO_2 exceeds that of N_2O and CH_4 by several orders of magnitude (Oertel *et al.*, 2016). However, N_2O and CH_4 causes 263 and 32 times more radiative forcing, respectively, than CO_2 by mass over a century (Neubauer & Megonigal, 2015), making these gases equally relevant to climate studies. Despite the high vulnerability of biogeochemical cycles in tropical ecosystems to climatic changes, trace gas budgets remain poorly constrained for these important ecosystems.

Tropical soils are one of the largest natural source of CO_2, contributing *ca.* 58 Pg C yr^{-1} to the estimated global soil respiration of about 91 Pg C yr^{-1} (Hashimoto *et al.*, 2015), although previous estimates suggest lower global CO_2 effluxes of between 68 and 78 Pg C yr^{-1} (Raich & Potter, 1995; Raich & Schlesinger, 1998; Hashimoto, 2012). This efflux of respiratory carbon from the soil to the atmosphere largely offsets global atmospheric CO_2 uptake by terrestrial plants (Beer *et al.*, 2010; Richardson *et al.*, 2019). Net soil CO_2 flux is largely a product of heterotrophic (soil microbial respiration) and autotrophic (root respiration) respiration processes (Luo & Zhou, 2006). The proximal controlling factors of soil CO_2 efflux are soil temperature and moisture, but are also influenced by spatial differences in soil texture, substrate availability and vegetation type (Raich & Schlesinger, 1998; Luo & Zhou, 2006).

Soil processes are considered to be the most important natural source of global N_2O, with fluxes from natural and agricultural soils accounting for 56–70% of global N_2O emissions (Syakila & Kroeze, 2011). Using ground-based, bottom-up approaches, recent estimates of N_2O emissions from tropical rainforest soils come up with lower values of 1.1 Tg N_2O-N yr^{-1}

(Stehfest & Bouwman, 2006) and 1.3 Tg N_2O-N yr^{-1} (Werner *et al.*, 2007) than earlier best estimates of 2.3 Tg N2O-N yr^{-1} (Bouwman *et al.*, 1995) and 3.5 Tg N_2O-N yr^{-1} (Breuer *et al.*, 2000). Although a wealth of microbial metabolic pathways and abiotic processes can produce N_2O in the soil, the contrasting microbial processes of nitrification and denitrification forms the most dominant processes of soil N_2O production, contributing *ca.* 70% of global N_2O emissions (Syakila & Kroeze, 2011). The activities of these nitrifying/denitrifying bacterial communities are affected by proximal environmental factors such as nitrogen (N) availability, soil moisture, soil temperature and soil pH (Davidson *et al.*, 2000a; Kesik *et al.*, 2006; Butterbach-Bahl *et al.*, 2013).

Tropical forest soils also constitute one of the largest biogenic sink of atmospheric CH_4 (Dutaur & Verchot, 2007). CH_4 flux at the soil-atmosphere interface is a net result of the simultaneous activities of methanogens (CH_4 producers under anaerobic conditions) and methanotrophs (CH_4 consumers under aerobic conditions). For well-drained soils, CH_4 oxidation by methanotrophic bacteria exceeds CH_4 production, resulting in a net uptake of 20 to 45 Tg CH_4-C yr^{-1} at the global scale (Dutaur & Verchot, 2007; Kirschke *et al.*, 2013; Schlesinger & Bernhardt, 2013). Soil CH_4 fluxes are largely controlled by soil moisture, which influences gas diffusivity into and out of the soil (Verchot *et al.*, 2000; Veldkamp *et al.*, 2013; Matson *et al.*, 2017), and soil N availability, through its influence on the activities of methanotrophs (Bodelier & Laanbroek, 2004).

Tropical ecosystems continue to play an important role in biogeochemical cycles and global climate, yet, research efforts to quantify the main sources and sinks of trace GHG lags behind that of other biomes, with the African continent among the most under researched region worldwide (Kim *et al.*, 2016b). Presently, trace gas budgets from the African continent are poorly constrained due to the lack of data on biogenic fluxes of trace GHG (Bombelli *et al.*,

2009; Ciais *et al.*, 2011; Valentini *et al.*, 2014). Africa may be a small carbon sink (−0.04 Pg C yr^{-1}; Fisher et al., 2013), nevertheless, the emissions of N_2O and CH_4 may turn the continent into a net source of GHG (Valentini *et al.*, 2014). Paradoxically, for several decades now, plant productivity and biomass in African tropical forests have reportedly increased due to increasing atmospheric CO_2 concentrations, resulting in net carbon gains (Cao *et al.*, 2001; Lewis *et al.*, 2009; Ciais *et al.*, 2011). However, recent findings suggest a potential slowdown in the carbon sink strength of African tropical forests during the last decade (Hubau *et al.*, 2020), due to increasing tree mortality and reduced tree growth, as a result of heat stress and extreme drought events, among other limiting factors (Allen *et al.*, 2010; Hubau *et al.*, 2020). Hubau et al. (2020) went on to predict that the carbon sink strength of African tropical forests might decline by 14% by the year 2039. Conversely, the prediction by Hubau *et al.* (2020) is in stark contrast to model projections of continuous high carbon uptake by Africa tropical forests up to the year 2100 (Huntingford *et al.*, 2013). Such inconsistencies underline the need to pursue field research efforts aimed at improving trace gas budget estimations for the African continent. In the recent study on a greenhouse gas budget for Africa, one of the key uncertainties mentioned was: "Non-CO_2 greenhouse gas emissions are poorly studied across the various African ecosystems (...) The lack of such information hinders the understanding of the African methane budget (…) and insight on the natural sources of nitrous oxide" (Valentini *et al.*, 2014, pg. 400).

1.2. Trees as conduits of N_2O and CH_4 fluxes

For some decades, plants have been shown to contribute to GHG emissions by acting as conduits for trace gases, facilitating the transport between the soil, where gases are produced or consumed by microbial activity, and the atmosphere. Here, trace GHG emissions may originate from root uptake of dissolved gases produced in the soil, and then conveyed to the atmosphere via aerenchyma tissue (Cicerone & Shetter, 1981; Butterbach-Bahl *et al.*, 1997) or transpiration

stream (Chang *et al.*, 1998). Earlier studies investigating the role of plants as conduits for soil-produced trace gases focused on herbaceous species, where the contribution of plant-mediated trace gas emissions were reported to make up to 90% of the total (plant + soil) emission (Singh & Singh, 1995; Butterbach-Bahl *et al.*, 1997; Yu *et al.*, 1997; Chen *et al.*, 2002).

Trace gas emissions from trunks of woody trees were initially suggested by *Schütz et al.* (1991), but actual data on CH_4 and N_2O emissions from trees were first reported for seedlings of black alder (*Alnus glutinosa*), a tree species that typically grows in European wetlands (Rusch & Rennenberg, 1998). Later studies also reported mangrove trees and tropical swamp trees to emit trace gases (Gauci *et al.*, 2010; Pangala *et al.*, 2013; Terazawa *et al.*, 2015). These trees, which are adapted to wetlands, have aerenchyma tissue which facilitates egress of soil-produced CH_4 via gas transport through the tree, and exchange with the atmosphere appears to happen predominantly through lenticels in stems (Buchel & Grosse, 1990). The described stem emission pathway has mostly been demonstrated in the field for CH_4 (Pangala *et al.*, 2014; Terazawa *et al.*, 2015). Also, N_2O can be transported through the aerenchyma system; however, preferential transport mechanism appears to be through dissolution in xylem sap flow and exchange with the atmosphere through stomata or the stem surface (Machacova *et al.*, 2013; Wen *et al.*, 2017). Accordingly, N_2O emissions have also been observed in seedlings from trees that have no aerenchyma, like *Fagus sylvatica* (Machacova *et al.*, 2013).

Tree-stem trace gas fluxes have been found to be largely controlled by tree physiology and traits of wood anatomy. For example, it has been shown in tropical peatlands that small trees and trees with a low wood specific density are correlated with high CH_4 emissions (Pangala *et al.*, 2013). The density of stem lenticels also correlated positively with stem CH_4 emissions (Pangala *et al.*, 2014), while stem N_2O and CH_4 emissions varied significantly among species in both upland (Pitz & Megonigal, 2017; Wen *et al.*, 2017; Welch *et al.*, 2019) and

wetland forests (Pangala *et al.*, 2015; Pitz *et al.*, 2018). Additionally, soil moisture content, temperature, and soil trace gas concentrations have all been found to correlate with stem emissions and thus may control them (Machacova *et al.*, 2013; Pangala *et al.*, 2015; Wen *et al.*, 2017).

Until now, it is unknown whether trees on heavily weathered soils in lowland tropical forests, such as in our study sites, contribute to N_2O and CH_4 emissions. However, some factors suggest that emissions through stems are possible: high N_2O concentrations in the soil are common in lowland tropical forest soils especially during the wet season when values as high as 4 to 8 ppm N_2O (compared to atmospheric concentration of 0.32 ppm N_2O) have been measured (i.e. Brazil: Perez *et al.*, 2000). Additionally, Welch et al. (2019) measured high tree-stem N_2O and CH_4 emissions in humid tropical forests in Panama. Over a short measurement campaign (2 weeks), annual tree-stem emissions were found to contribute up to 18% to total forest emissions (Machacova *et al.*, 2016). In another study, the inclusion of tree-stem fluxes from floodplain trees in bottom-up CH_4 inventories closed the Amazon CH_4 budget (Pangala *et al.*, 2017). Despite the evidence for tree stem emissions, estimations of global trace gas budgets generally assumes soils to be the only active surfaces emitting trace gases, thereby excluding the contributions of trees (Syakila & Kroeze, 2011; Hashimoto *et al.*, 2015; Saunois *et al.*, 2016). It is possible that tree stem emissions may be the "missing" emission pathways needed to explain the mismatches in trace gas estimates between ground-based, bottom-up models and top-down modelling and atmospheric inversion methods (Werner *et al.*, 2007; Thompson *et al.*, 2014; Saunois *et al.*, 2016). Given the extensive coverage of well-drained tropical forests relative to tropical wetlands, it is imperative that tree stem emissions in tropical upland forests are measured over sufficient spatial and temporal variability in order to provide insights on stem flux magnitudes and underlying mechanisms, and their role in global trace gas budgets.

1.3. Effects of land-use change on trace gas fluxes

Although the number of studies on trace gas fluxes from tropical land uses is still limited, it has become clear that forest conversion and agricultural intensification contribute to the increasing trace gas emissions from soils (Veldkamp & Keller, 1997). The current pattern of deforestation in Africa is similar to the rest of the tropics, with an estimated 3.4 million ha of forest converted to agricultural lands yearly in Africa (Kim *et al.*, 2016b). Consequently, a study on the GHG budget of Africa reported land-use change to be the dominant source of trace gas emissions in Africa, resulting in an estimated emission of 0.32 ± 0.05 Pg C yr^{-1} (Valentini *et al.*, 2014). This estimated budget was found to be even higher than emissions from fossil fuels, which is unique for the African continent.

Tropical forest conversion to other land uses affects trace gas fluxes due to changes in physicochemical properties of soil (Veldkamp *et al.*, 2008; Hassler *et al.*, 2015, 2017). For example, changes in soil CO_2 fluxes following forest conversion have been related to changes in root mass (Bae *et al.*, 2013), litter input and soil organic carbon stocks (Hassler *et al.*, 2015). Land use associated changes in soil N_2O fluxes are predominantly controlled by changes in soil N availability and soil water content (Davidson *et al.*, 2000a), whereas changes in soil CH_4 fluxes have been linked to differences in gas diffusivity due to soil compaction (Corre *et al.*, 2006; Veldkamp *et al.*, 2008). How land use affect non-CO_2 greenhouse gas fluxes was identified as a research gap in the recent greenhouse gas budget for Africa (Valentini *et al.*, 2014).

1.4. The Congo Basin, Cameroon

The Congo Basin forest is the second largest intact tropical rainforest in the world after the Amazon, making it an important repository of biodiversity and other ecosystem services. It is home to about 20,000 plant species of which 8,000 are endemic (Billand, 2012). The Congo

Basin is estimated to store *ca.* 57 billion t C, representing 21% of the total C stored in tropical forests globally (FAO, 2011). It is also important to global precipitation patterns, as it has the highest amount of rainfall during the transition seasons (Washington *et al.*, 2013). These signify the Basin's significance to terrestrial carbon cycling and global climate. While Africa has been underrepresented in trace gas flux research, studies from the Congo Basin are almost absent, possibly due to chronic political instability and limited logistical support (Verbeeck *et al.*, 2011).

Cameroon, which shares the Congo Basin, is the second highest deforested country behind the Democratic Republic of Congo (Dkamela, 2010). Forest clearing for small-scale agriculture has been found to be the dominant cause of deforestation in the region, accounting for more than 90% of forest cover loss (Tyukavina *et al.*, 2018). Most of the cleared forest areas are used to establish cacao agroforests, especially in densely populated areas such as central and south Cameroon. And like many other African countries where cacao agroforests dominate agricultural production, the conversion of forest for the establishment of cacao farms have mostly being unselective. Nevertheless, most of these small-scale cacao farms, presently estimated to be *ca.* 400,000 hectares, are hand planted under the shade of forests' remnant trees with no fertilizer inputs (Kotto *et al.*, 2002; Saj *et al.*, 2013), making these cacao agroforests one of the most sustainable land-use systems in Central and West Africa forest zones.

1.5. Aims and hypotheses

Despite disparity of estimates for African trace gas budget between bottom-up and top-down approaches, no study has concurrently quantified soil and stem trace gas emissions from Africa. The research presented in this thesis aimed to provide a systematic comparison between a reference land use and a converted system for quantifying land-use change effects on stem and soil trace gas fluxes, which are virtually lacking for the Congo Basin, and thus an important

contribution in the improvement of greenhouse gas budget of Africa. This study therefore provides the first year-round, multiple site quantifications for forests and cacao agroforestry systems in the Congo Basin, including 23 tree species that have not been measured before.

This thesis consists of two studies carried out at three sites across central and southern Cameroon. The aims of the first study were to quantify the changes in stem and soil N_2O fluxes with forest conversion to cacao agroforestry, and to determine the temporal and spatial controls of stem and soil N_2O fluxes. In this study, we hypothesized that: (i) stem and soil N_2O fluxes from these extensively managed CAF systems will be comparable to the natural forests, and (ii) the seasonal pattern of stem emissions will parallel that of soil N_2O emissions and both will have similar soil and climatic controlling factors.

In the second study, we quantified changes in stem and soil CH_4 and soil CO_2 fluxes with forest conversion to CAF, and determined the temporal and spatial controls of stem and soil CH_4 and CO_2 fluxes. The following hypotheses were tested: (i) stem and soil CH_4 fluxes from these extensively managed CAF systems will be comparable to the natural forests, (ii) trees from tropical forests and cacao agroforestry emit CH_4 from stems, and (iii) stem emissions will offset a considerable fraction of the net CH_4 consumption by soils.

1.6. Study area and experimental set-up

Our research was conducted at three sites located in southern and central regions of Cameroon, where natural forest conversion into cacao agroforestry systems is common. Sites in the southern region were located around the villages of Aloum and Biba Yezoum, and the third site was located around the village of Tomba. To investigate the effects of land-use change on trace gas fluxes, we examined two land-use systems at each site: the reference forest and the converted cacao agroforestry system, each represented by four replicate plots (Fig. 1.1). In total,

we measured stem and soil trace gas fluxes in 24 plots (3 sites × 2 land uses × 4 replicate plots) all located on relatively flat topography. All sites were located on heavily weathered soils which are classified as Ferralsols (IUSS Working Group WRB, 2015).

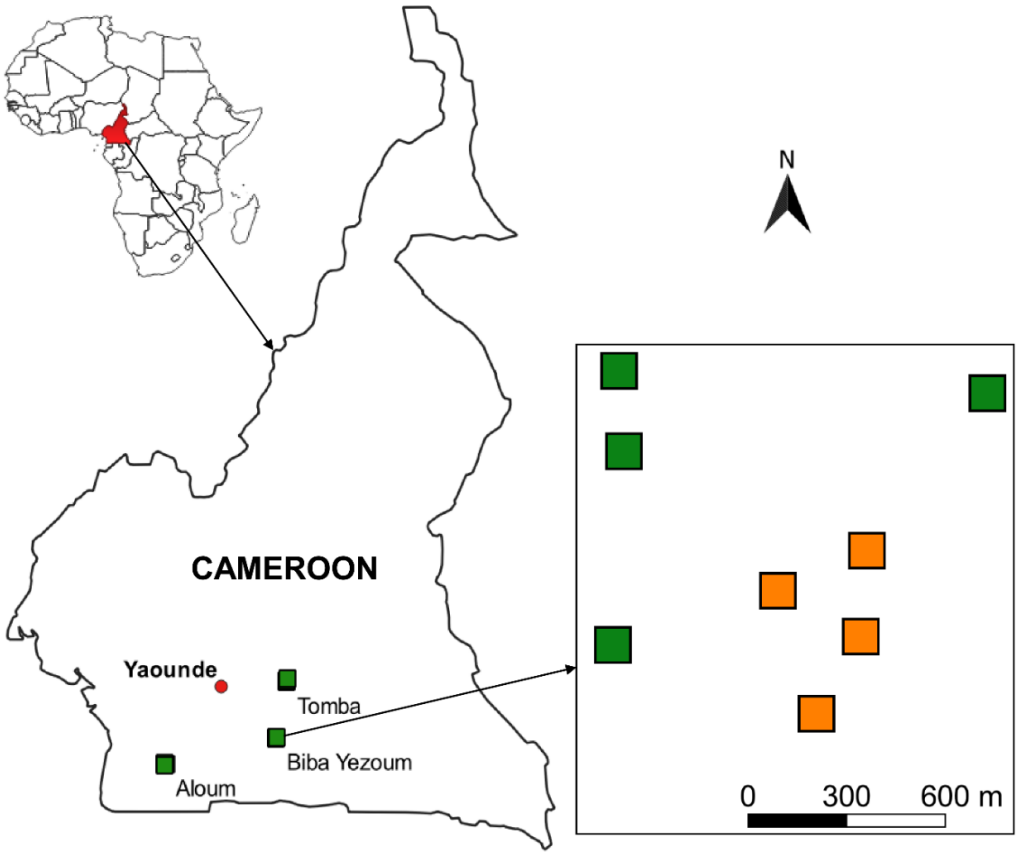

Figure 1. 1. Location of the study sites in Cameroon, showing the four replicate plots per land use (green for forests and orange for cacao agroforestry) at one site.

All the study sites are characterised by significant rainfall in most months of the year, spanning an annual precipitation from 1576 mm yr^{-1} in the centre to 2064 mm yr^{-1} in the south of Cameroon (Climate-Data.org, 2019). In all of the sites, precipitation occurs in a bimodal pattern, with typical wet seasons occurring from March to June and September to November (Fig. 1.2). The mean annual temperature across the three sites is 23.5 °C (Fig. 1.2; Climate-Data.org, 2019).

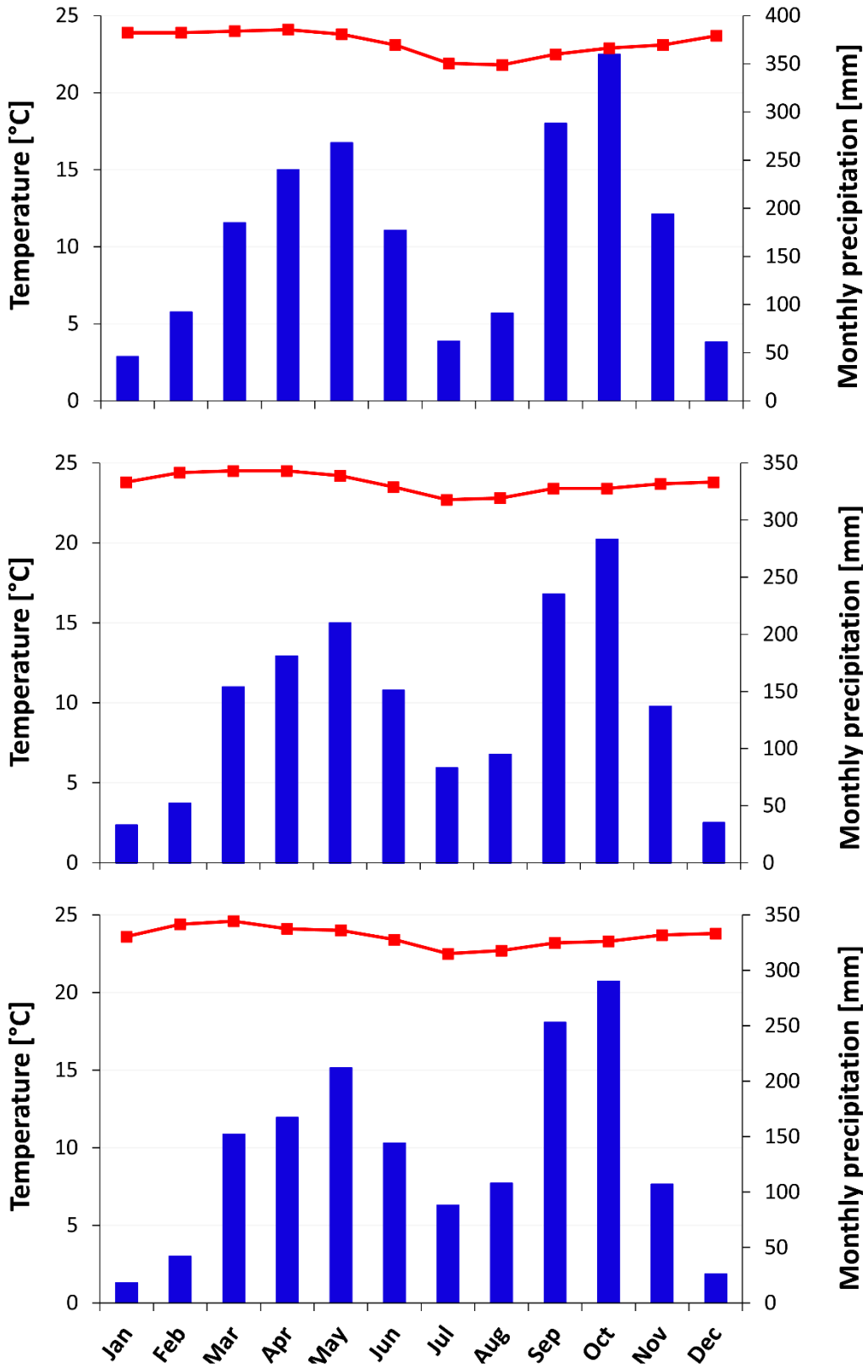

Figure 1. 2. Mean monthly temperature and precipitation (from 1982 to 2012) for Aloum (top panel), Biba Yezoum (centre panel), and Tomba (bottom panel) in southern and central regions of Cameroon (Data source: (Climate-Data.org, 2019).

Prior to stem and soil trace gas flux measurements, we conducted a tree inventory in all the forests and cacao plots (Fig. 1.3) where all stems including cacao trees with a diameter at breast height (DBH) ≥ 10 cm were identified and measured for DBH. We identified 135 tree species belonging to 118 genera and 45 families in the natural forests. In the cacao agroforestry, we identified 89 shade tree species belonging to 77 genera and 33 families. The high number of species in the cacao agroforests signifies the high diversity and sustainability of these extensively managed farms.

Figure 1. 3. Natural forests (left) and cacao agroforestry (right) in the Congo Basin, Cameroon.

For measurements of stem N_2O and CH_4 fluxes, we selected six cacao trees per replicate plot in the CAF, and six trees representing the most dominant species within each replicate plot in the forest. For soil trace gas flux measurements, we installed four permanent chamber bases per replicate plot, which were randomly distributed within the plot. Concurrent to the stem and soil N_2O-flux measurements, we measured soil temperature, soil water content, and extractable mineral N in the top 5-cm depth. We also sampled soil-air gas concentrations at 50-cm depth from permanently installed stainless-steel probes located at ~1 m from the measured trees. We conducted trace gas flux measurements, soil and meteorological parameters in the inner 40-m

x 40-m area within each plot to minimize edge effects (Fig. 1.4). Details on study area and experimental design are given in Chapters 2 and 3.

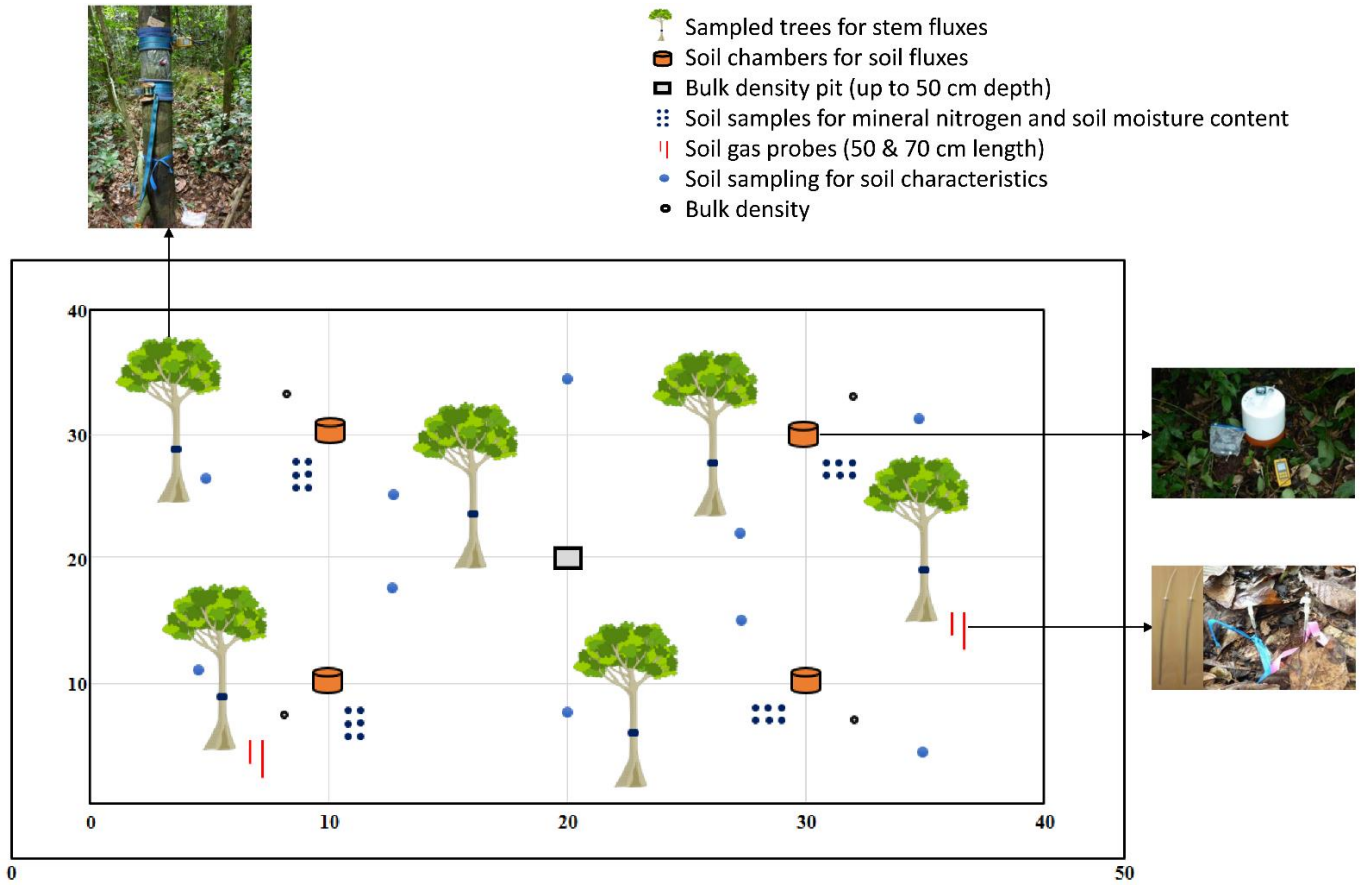

Figure 1. 4. Experimental layout of the stem and soil flux measurements in one of the replicate plots in the Congo Basin, Cameroon.

1.7. References

Allen, C. D., Macalady, A. K., Chenchouni, H., Bachelet, D., McDowell, N., Vennetier, M., et al. (2010). A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *Forest Ecology and Management*, *259*(4), 660–684. https://doi.org/10.1016/j.foreco.2009.09.001

Bae, K., Lee, D. K., Fahey, T. J., Woo, S. Y., Quaye, A. K., & Lee, Y. K. (2013). Seasonal variation of soil respiration rates in a secondary forest and agroforestry systems. *Agroforestry Systems*, *87*(1), 131–139. https://doi.org/10.1007/s10457-012-9530-8

Beer, C., Reichstein, M., Tomelleri, E., Ciais, P., Martin, J., Carvalhais, N., et al. (2010). Terrestrial Gross Carbon Dioxide Uptake: Global Distribution and Covariation with Climate. *Science*, *329*(August), 834–838. https://doi.org/https://doi.org/10.1126/science.1184984

Billand, A. (2012). Biodiversity in Central African forests: an overview of knowledge, main challenges and conservation measures. In C. de Wasseige, P. de Marcken, N. Bayol, l F. Hiol Hio, P. Mayaux, B. Desclée, et al. (Eds.), *The forests of the Congo Basin—state of the forest 2010* (p. 276). Publications Office of the European Union. Luxembourg. https://doi.org/10.2788/47210

Bodelier, P. L. E., & Laanbroek, H. J. (2004). Nitrogen as a regulatory factor of methane oxidation in soils and sediments. *FEMS Microbiology Ecology*, *47*(3), 265–277. https://doi.org/10.1016/S0168-6496(03)00304-0

Bombelli, A., Henry, M., Castaldi, S., Adu-Bredu, S., Arneth, A., De Grandcourt, A., et al. (2009). An outlook on the Sub-Saharan Africa carbon balance. *Biogeosciences*, *6*(10), 2193–2205. https://doi.org/10.5194/bg-6-2193-2009

Bouwman, A. F., Van Der Hoek, K. W., & Olivier, J. G. J. (1995). Uncertainties in the global source distribution of nitrous oxide. *Journal of Geophysical Research*, *100*(D2), 2785–2800. https://doi.org/10.1029/94JD02946

Breuer, L., Papen, H., & Butterbach-Bahl, K. (2000). N2O emission from tropical forest soils of Australia. *Journal of Geophysical Research Atmospheres*, *105*(D21), 26353–26367. https://doi.org/10.1029/2000JD900424

Buchel, H. B., & Grosse, W. (1990). Localization of the porous partition responsible for pressurized gas transport in Alnus glutinosa (L.) Gaertn. *Tree Physiology*, *6*(3), 247–256.

Butterbach-Bahl, K., Papen, H., & Rennenberg, H. (1997). Impact of gas transport through rice cultivars on methane emission from rice paddy fields. *Plant, Cell and Environment*, *20*(9), 1175–1183. https://doi.org/10.1046/j.1365-3040.1997.d01-142.x

Butterbach-Bahl, K., Baggs, E. M., Dannenmann, M., Kiese, R., & Zechmeister-Boltenstern, S. (2013). Nitrous oxide emissions from soils: How well do we understand the processes and their controls? *Philosophical Transactions of the Royal Society B: Biological Sciences*, *368*(1621). https://doi.org/10.1098/rstb.2013.0122

Cao, M., Zhang, Q., & Shugart, H. H. (2001). Dynamic responses of African ecosystem carbon cycling to climate change. *Climate Research*, *17*(2 SPECIAL 8), 183–193. https://doi.org/10.3354/cr017183

Chang, C., Janzen, H. H., Nakonechny, E. M., & Cho, C. M. (1998). Nitrous Oxide Emission through Plants. *Soil Science Society of America Journal*, *62*(1), 35–38. https://doi.org/10.2136/sssaj1998.03615995006200010005x

Chen, X., Cabrera, M. L., Zhang, L., Wu, J., Shi, Y., Yu, W. T., & Shen, S. M. (2002). Nitrous oxide emission from upland crops and crop-soil systems in northeastern China. *Nutrient Cycling in Agroecosystems*, *62*(3), 241–247. https://doi.org/10.1023/A:1021202114354

Ciais, P., Bombelli, A., Williams, M., Piao, S. L., Chave, J., Ryan, C. M., et al. (2011). The carbon balance of Africa: Synthesis of recent research studies. *Philosophical*

Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences, *369*(1943), 2038–2057. https://doi.org/10.1098/rsta.2010.0328

Cicerone, R. J., & Shetter, J. D. (1981). Sources of atmospheric methane: Measurements in rice paddies and a discussion. *Journal of Geophysical Research*, *86*(8 C), 7203–7209. https://doi.org/10.1029/jc086ic08p07203

Climate-Data.org. (2019). Cameroon climate. Retrieved May 21, 2019, from https://en.climate-data.org/africa/cameroon-142/

Corre, M. D., Dechert, G., & Veldkamp, E. (2006). Soil nitrogen cycling following montane forest conversion in Central Sulawesi, Indonesia. *Soil Science Society of America Journal*, *70*(2), 359–366. https://doi.org/10.2136/sssaj2005.0061

Davidson, E. A., Keller, M., Erickson, H. E., Verchot, L. V., & Veldkamp, E. (2000). Testing a Conceptual Model of Soil Emissions of Nitrous and Nitric Oxides. *BioScience*, *50*(8), 667. https://doi.org/10.1641/0006-3568(2000)050[0667:tacmos]2.0.co;2

Dkamela, G. P. (2010). *The context of REDD+ in Cameroon: Drivers, agents and institutions* (Occasional). CIFOR, Bogor, Indonesia.

Dutaur, L., & Verchot, L. V. (2007). A global inventory of the soil CH4 sink. *Global Biogeochemical Cycles*, *21*(4), 1–9. https://doi.org/10.1029/2006GB002734

FAO. (2011). *The State of Forests in the Amazon Basin, Congo Basin and Southeast Asia. A report prepared for the Summit of the Three Rainforest Basins Brazzaville, Republic of Congo*. Rome, Italy, Italy.

Fisher, J. B., Sikka, M., Sitch, S., Ciais, P., Poulter, B., Galbraith, D., et al. (2013). African tropical rainforest net carbon dioxide fluxes in the twentieth century. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *368*(1625), 13–17. https://doi.org/10.1098/rstb.2012.0376

Forster, P., Ramaswamy, V., Artaxo, P., Berntsen, T., Betts, R., Fahey, D. W., et al. (2007). Changes in Atmospheric Constituents and in Radiative Forcing. In: Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change [Solomon, S., D. Qin, M. Ma. Cambridge, United Kingdom and New York, NY, USA, United Kingdom and New York, NY, USA: Cambridge University Press.

Gauci, V., Gowing, D. J. G., Hornibrook, E. R. C., Davis, J. M., & Dise, N. B. (2010). Woody stem methane emission in mature wetland alder trees. *Atmospheric Environment*. https://doi.org/10.1016/j.atmosenv.2010.02.034

Hashimoto, S. (2012). A new estimation of global soil greenhouse gas fluxes using a simple data-oriented model. *PLoS ONE*, *7*(8), 1–7. https://doi.org/10.1371/journal.pone.0041962

Hashimoto, S., Carvalhais, N., Ito, A., Migliavacca, M., Nishina, K., & Reichstein, M. (2015). Global spatiotemporal distribution of soil respiration modeled using a global database. *Biogeosciences*, *12*(13), 4121–4132. https://doi.org/10.5194/bg-12-4121-2015

Hassler, E., Corre, M. D., Tjoa, A., Damris, M., Utami, S. R., & Veldkamp, E. (2015). Soil fertility controls soil-atmosphere carbon dioxide and methane fluxes in a tropical

landscape converted from lowland forest to rubber and oil palm plantations. *Biogeosciences Discussions*, *12*(12), 9163–9207. https://doi.org/10.5194/bgd-12-9163-2015

Hassler, E., Corre, M. D., Kurniawan, S., & Veldkamp, E. (2017). Soil nitrogen oxide fluxes from lowland forests converted to smallholder rubber and oil palm plantations in Sumatra, Indonesia. *Biogeosciences*, *14*(11), 2781–2798. https://doi.org/https://doi.org/10.5194/bg-14-2781-2017

Hubau, W., Lewis, S. L., Phillips, O. L., Affum-Baffoe, K., Beeckman, H., Cuní-Sanchez, A., et al. (2020). Asynchronous carbon sink saturation in African and Amazonian tropical forests. *Nature*, *579*(7797), 80–87. https://doi.org/10.1038/s41586-020-2035-0

Huntingford, C., Zelazowski, P., Galbraith, D., Mercado, L. M., Sitch, S., Fisher, R., et al. (2013). Simulated resilience of tropical rainforests to CO2 -induced climate change. *Nature Geoscience*, *6*(4), 268–273. https://doi.org/10.1038/ngeo1741

IUSS Working Group WRB. (2015). *World Reference Base for Soil Resources 2014, update 2015 International soil classification system for naming soils and creating legends for soil maps. World Soil Resources Reports No. 106.* FAO, Rome.

Kesik, M., Blagodatsky, S., Papen, H., & Butterbach-Bahl, K. (2006). Effect of pH, temperature and substrate on N2O, NO and CO 2 production by Alcaligenes faecalis p. *Journal of Applied Microbiology*, *101*(3), 655–667. https://doi.org/10.1111/j.1365-2672.2006.02927.x

Kim, D. G., Thomas, A. D., Pelster, D. E., Rosenstock, T. S., & Sanz-Cobena, A. (2016). Greenhouse gas emissions from natural ecosystems and agricultural lands in sub-Saharan Africa: Synthesis of available data and suggestions for further research. *Biogeosciences*, *13*(16), 4789–4809. https://doi.org/10.5194/bg-13-4789-2016

Kirschke, S., Bousquet, P., Ciais, P., Saunois, M., Canadell, J. G., Dlugokencky, E. J., et al. (2013). Three decades of global methane sources and sinks. *Nature Geoscience*, *6*(10), 813–823. https://doi.org/10.1038/ngeo1955

Kotto, J. S., Moukam, A., Njomgang, R., Tiki-Manga, T., Tonye, J., Diaw, C., et al. (2002). *Alternatives to slash-and-burn in Indonesia: summary report & synthesis of phase II in Cameroon*. *ASB-Indonesia report*. Nairobi. Kenya.

Lewis, S. L., Lopez-Gonzalez, G., Sonké, B., Affum-Baffoe, K., Baker, T. R., Ojo, L. O., et al. (2009). Increasing carbon storage in intact African tropical forests. *Nature*, *457*(7232), 1003–1006. https://doi.org/10.1038/nature07771

Luo, Y., & Zhou, X. (2006). *Soil Respiration and the Environment*. Amsterdam, Netherlands, Netherlands: Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-088782-8.X5000-1

Machacova, K., Papen, H., Kreuzwieser, J., & Rennenberg, H. (2013). Inundation strongly stimulates nitrous oxide emissions from stems of the upland tree Fagus sylvatica and the riparian tree Alnus glutinosa. *Plant and Soil*, *364*(1–2), 287–301. https://doi.org/10.1007/s11104-012-1359-4

Machacova, K., Bäck, J., Vanhatalo, A., Halmeenmäki, E., Kolari, P., Mammarella, I., et al. (2016). Pinus sylvestris as a missing source of nitrous oxide and methane in boreal

forest. *Scientific Reports*, *6*(March), 1–8. https://doi.org/10.1038/srep23410

Matson, A. L., Corre, M. D., Langs, K., & Veldkamp, E. (2017). Soil trace gas fluxes along orthogonal precipitation and soil fertility gradients in tropical lowland forests of Panama. *Biogeosciences*, *14*(14), 3509–3524. https://doi.org/10.5194/bg-14-3509-2017

Neubauer, S. C., & Megonigal, J. P. (2015). Moving Beyond Global Warming Potentials to Quantify the Climatic Role of Ecosystems. *Ecosystems*, *18*(6), 1000–1013. https://doi.org/10.1007/s10021-015-9879-4

Oertel, C., Matschullat, J., Zurba, K., Zimmermann, F., & Erasmi, S. (2016). Greenhouse gas emissions from soils—A review. *Chemie Der Erde*, *76*(3), 327–352. https://doi.org/10.1016/j.chemer.2016.04.002

Pangala, S. R., Moore, S., Hornibrook, E. R. C., & Gauci, V. (2013). Trees are major conduits for methane egress from tropical forested wetlands. *New Phytologist*, *197*(2), 524–531. https://doi.org/10.1111/nph.12031

Pangala, S. R., Gowing, D. J. G., Hornibrook, E. R. C., & Gauci, V. (2014). Controls on methane emissions from Alnus glutinosa saplings. *New Phytologist*, *201*(3), 887–896. https://doi.org/10.1111/nph.12561

Pangala, S. R., Hornibrook, E. R. C., Gowing, D. J. G., & Gauci, V. (2015). The contribution of trees to ecosystem methane emissions in a temperate forested wetland. *Global Change Biology*, *21*(7), 2642–2654. https://doi.org/10.1111/gcb.12891

Pangala, S. R., Enrich-Prast, A., Basso, L. S., Peixoto, R. B., Bastviken, D., Hornibrook, E. R. C., et al. (2017). Large emissions from floodplain trees close the Amazon methane budget. *Nature*, *552*(7684), 230–234. https://doi.org/10.1038/nature24639

Perez, T., Trumbore, S. E., Tyler, S. C., Davidson, E. A., Keller, M., & de Camargo, P. B. (2000). Isotopic variability of N2O emissions from tropical forest soils. *Global Biogeochemical Cycles*, *14*(2), 525–535.

Pitz, S. L., & Megonigal, J. P. (2017). Temperate forest methane sink diminished by tree emissions. *New Phytologist*, *214*(4), 1432–1439. https://doi.org/10.1111/nph.14559

Pitz, S. L., Megonigal, J. P., Chang, C. H., & Szlavecz, K. (2018). Methane fluxes from tree stems and soils along a habitat gradient. *Biogeochemistry*, *137*(3), 307–320. https://doi.org/10.1007/s10533-017-0400-3

Raich, J. W., & Potter, C. S. (1995). Global patterns of carbon dioxide emissions from soils. *Global Biogeochemical Cycles*, *9*(1), 23–36. https://doi.org/10.1029/94GB02723

Raich, J. W., & Schlesinger, W. H. (1998). The global carbon dioxide flux in soil respiration and its relationship to vegetation and climate. *Tellus B*, *44B*, 81–99.

Richardson, A. D., Hollinger, D. Y., Shoemaker, J. K., Hughes, H., Savage, K., & Davidson, E. A. (2019). Six years of ecosystem-atmosphere greenhouse gas fluxes measured in a sub-boreal forest. *Scientific Data*, *6*(1), 117. https://doi.org/10.1038/s41597-019-0119-1

Rusch, H., & Rennenberg, H. (1998). Black alder (Alnus glutinosa (L.) Gaertn.) trees mediate methane and nitrous oxide emission from the soil to the atmosphere. *Plant and Soil*, *201*(1), 1–7. https://doi.org/10.1023/A:1004331521059

Saj, S., Jagoret, P., & Todem Ngogue, H. (2013). Carbon storage and density dynamics of associated trees in three contrasting Theobroma cacao agroforests of Central Cameroon. *Agroforestry Systems*, *87*(6), 1309–1320. https://doi.org/10.1007/s10457-013-9639-4

Saunois, M., Bousquet, P., Poulter, B., Peregon, A., Ciais, P., Canadell, J. G., et al. (2016). The global methane budget 2000-2012. *Earth System Science Data*, *8*(2), 697–751. https://doi.org/10.5194/essd-8-697-2016

Schlesinger, W. H., & Bernhardt, E. S. (2013). *Biogeochemistry: an analysis of global change*. San Diego, CA, USA, CA, USA: Academic Press.

Schütz, H., Schröder, P., & Rennenberg, H. (1991). Role of plants in regulation the methane flux to the atmosphere. In T. D. Sharkey, E. A. Holland, & A. H. Mooney (Eds.), *In Trace Gas Emissions by Plants* (pp. 29–63). San Diego, CA, USA: Academic Press.

Singh, S., & Singh, J. S. (1995). Plants as conduits for methane in wetlands. *Proc. National Academy of Sciences India*, *65 (B)*(II), 147–157.

Stehfest, E., & Bouwman, L. (2006). N2O and NO emission from agricultural fields and soils under natural vegetation: Summarizing available measurement data and modeling of global annual emissions. *Nutrient Cycling in Agroecosystems*, *74*(3), 207–228. https://doi.org/10.1007/s10705-006-9000-7

Syakila, A., & Kroeze, C. (2011). The global nitrous oxide budget revisited. *Greenhouse Gas Measurement and Management*, *1*(1), 17–26. https://doi.org/10.3763/ghgmm.2010.0007

Terazawa, K., Yamada, K., Ohno, Y., Sakata, T., & Ishizuka, S. (2015). Spatial and temporal variability in methane emissions from tree stems of Fraxinus mandshurica in a cool-temperate floodplain forest. *Biogeochemistry*, *123*(3), 349–362. https://doi.org/10.1007/s10533-015-0070-y

Thompson, R. L., Chevallier, F., Crotwell, A. M., Dutton, G., Langenfelds, R. L., Prinn, R. G., et al. (2014). Nitrous oxide emissions 1999 to 2009 from a global atmospheric inversion. *Atmospheric Chemistry and Physics*, *14*(4), 1801–1817. https://doi.org/10.5194/acp-14-1801-2014

Tyukavina, A., Hansen, M. C., Potapov, P., Parker, D., Okpa, C., Stehman, S. V., et al. (2018). Congo Basin forest loss dominated by increasing smallholder clearing. *Science Advances*, *4*(11). https://doi.org/10.1126/sciadv.aat2993

Valentini, R., Arneth, A., Bombelli, A., Castaldi, S., Cazzolla Gatti, R., Chevallier, F., et al. (2014). A full greenhouse gases budget of africa: Synthesis, uncertainties, and vulnerabilities. *Biogeosciences*, *11*(2), 381–407. https://doi.org/10.5194/bg-11-381-2014

Veldkamp, E., & Keller, M. (1997). Nitrogen oxide emissions from a banana plantation in the humid tropics. *Journal of Geophysical Research*, *102*(D13), 15889–15898.

Veldkamp, E., Purbopuspito, J., Corre, M. D., Brumme, R., & Murdiyarso, D. (2008). Land use change effects on trace gas fluxes in the forest margins of Central Sulawesi, Indonesia. *Journal of Geophysical Research: Biogeosciences*, *113*(2), 1–11. https://doi.org/10.1029/2007JG000522

Veldkamp, E., Koehler, B., & Corre, M. D. (2013). Indications of nitrogen-limited methane uptake in tropical forest soils. *Biogeosciences*, *10*(8), 5367–5379.

https://doi.org/10.5194/bg-10-5367-2013

Verbeeck, H., Boeckx, P., & Steppe, K. (2011). Tropical forests : include Congo basin. *Nature*, *479*(179), 2011. https://doi.org/https://doi.org/10.1038/479179b

Verchot, L. V., Davidson, E. A., Cattânio, J. H., & Ackerman, I. L. (2000). Land-use change and biogeochemical controls of methane fluxes in soils of eastern Amazonia. *Ecosystems*, *3*(1), 41–56. https://doi.org/10.1007/s100210000009

Washington, R., James, R., Pearce, H., Pokam, W. M., & Moufouma-Okia, W. (2013). Congo basin rainfall climatology: Can we believe the climate models? *Philosophical Transactions of the Royal Society B: Biological Sciences*, *368*(1625). https://doi.org/10.1098/rstb.2012.0296

Welch, B., Gauci, V., & Sayer, E. J. (2019). Tree stem bases are sources of CH4 and N2O in a tropical forest on upland soil during the dry to wet season transition. *Global Change Biology*, *25*(1), 361–372. https://doi.org/10.1111/gcb.14498

Wen, Y., Corre, M. D., Rachow, C., Chen, L., & Veldkamp, E. (2017). Nitrous oxide emissions from stems of alder, beech and spruce in a temperate forest. *Plant Soil*. https://doi.org/10.1007/s11104-017-3416-5

Werner, C., Butterbach-Bahl, K., Haas, E., Hickler, T., & Kiese, R. (2007). A global inventory of N2O emissions from tropical rainforest soils using a detailed biogeochemical model. *Global Biogeochemical Cycles*, *21*(3). https://doi.org/10.1029/2006GB002909

Yu, K. W., Wang, Z. P., & Chen, G. X. (1997). Nitrous oxide and methane transport through rice plants. *Biology and Fertility of Soils*, *24*(3), 341–343. https://doi.org/10.1007/s003740050254

CHAPTER 2

STEM AND SOIL NITROUS OXIDE FLUXES FROM RAINFOREST AND CACAO AGROFOREST ON HIGHLY WEATHERED SOILS IN THE CONGO BASIN

Under review in EGU Biogeosciences

Najeeb A. Iddris[1], Marife D. Corre[1], Martin Yemefack[2,3], Oliver van Straaten[1,4], Edzo Veldkamp[1]

[1]Soil Science of Tropical and Subtropical Ecosystems, University of Goettingen, Goettingen, Germany

[2] International Institute of Tropical Agriculture, Yaoundé, Cameroon

[3] Now at: Sustainable Tropical Solutions (STS), Yaoundé, Cameroon

[4] Now at: Northwest German Forest Research Institute, Goettingen, Germany

2.1. Abstract

Although tree stems act as conduits for greenhouse gases (GHG) produced in the soil, the magnitudes of tree contributions to total (soil + stem) nitrous oxide (N_2O) emissions from tropical rainforests on heavily weathered soils remain unknown. Moreover, soil GHG fluxes are largely understudied in African rainforests, and the effects of land-use change on these gases are identified as an important research gap in the global GHG budget. In this study, we quantified the changes in stem and soil N_2O fluxes with forest conversion to cacao agroforestry. Stem and soil N_2O fluxes were measured monthly for a year (2017–2018) in four replicate plots per land use at three sites across central and southern Cameroon. Tree stems consistently emitted N_2O throughout the measurement period, and were positively correlated with soil N_2O fluxes. ^{15}N-isotope tracing from soil mineral N to stem-emitted $^{15}N_2O$ as well as correlations between temporal patterns of stem N_2O emissions, soil-air N_2O concentration, soil N_2O emissions, and vapor pressure deficit suggest that N_2O emitted by the stems originated predominantly from N_2O produced in the soil. Forest conversion to extensively managed, mature (>20 years old) cacao agroforestry had no effect on stem and soil N_2O fluxes. The annual total N_2O emissions were 1.55 ± 0.20 kg N ha^{-1} yr^{-1} from the forest and 1.15 ± 0.10 kg N ha^{-1} yr^{-1} from cacao agroforestry, with tree N_2O emissions contributing 11 to 38% for forests and 8 to 15% for cacao agroforestry. These substantial contributions of tree stems to total N_2O emissions highlight the importance of including tree-mediated fluxes in ecosystem GHG budgets. Taking into account that our study sites' biophysical characteristics represented two-thirds of the humid rainforests in the Congo Basin, we estimated a total N_2O source strength for this region of 0.18 ± 0.05 Tg N_2O-N yr^{-1}.

Keywords: Africa, cacao agroforest, Congo Basin, Ferralsol, land-use change, nitrous oxide, Oxisol, soil N_2O emissions, stem N_2O emissions, tropical rainforest

2.2. Introduction

The trace gas nitrous oxide (N_2O) has become the main stratospheric ozone depleting substance produced by human activities (Ravishankara *et al.*, 2009), and is after carbon dioxide and methane (CH_4) the most important anthropogenic greenhouse gas (GHG) (Denman *et al.*, 2007). Humid tropical soils are considered one of the most important global N_2O sources (Denman *et al.*, 2007; Werner *et al.*, 2007a), with tropical rainforests alone estimated to contribute between 0.9 to 4.5 Tg N_2O-N yr^{-1} to the global N_2O source of about 16 Tg N_2O-N yr^{-1} (Bouwman *et al.*, 1995; Breuer *et al.*, 2000; Werner *et al.*, 2007a). However, ground-based, bottom-up N_2O emission estimates appear to be in stark contrast to the high emissions estimated from top-down approaches such as modelling and global N_2O atmospheric inversions (Huang *et al.*, 2008; Thompson *et al.*, 2014). Nevertheless, there exists considerable uncertainty in both approaches (Davidson & Kanter, 2014), especially for the tropics (Valentini *et al.*, 2014). Recent studies suggest two possible reasons for large uncertainties in bottom-up approaches: "missing" emission pathways such as trees (Welch *et al.*, 2019), and a strong geographic bias of measured N_2O fluxes from tropical forests.

Most of the studies on soil N_2O fluxes from tropical ecosystems were conducted in South and Central America (Davidson & Verchot, 2000; Neill *et al.*, 2005; Wolf *et al.*, 2011; Matson *et al.*, 2017), tropical Asia (Purbopuspito *et al.*, 2006; Verchot *et al.*, 2006; Werner *et al.*, 2006; Veldkamp *et al.*, 2008; Hassler *et al.*, 2017) and Australia (Breuer *et al.*, 2000; Kiese *et al.*, 2003). Africa remains the continent with the least published field studies on soil N_2O fluxes from the tropical forest biome. After the pioneering work by Serca *et al.* (1994), very few field studies have been conducted, most of which were either not replicated with independent plots or only with short measurement campaigns (Werner *et al.*, 2007b; Castaldi *et al.*, 2013; Gütlein *et al.*, 2018; Wanyama *et al.*, 2018). The remaining studies were based on laboratory incubations, which cannot be translated to actual field conditions. Consequently,

field-based studies with sufficient spatial and temporal coverage are critical for improving the highly uncertain N_2O sink and source estimates for Africa (Valentini *et al.*, 2014; Kim *et al.*, 2016b).

The Congo Basin is the second largest intact tropical forest in the world and constitutes one of the most important carbon (C) and biodiversity reservoirs globally. Behind the DR Congo, Cameroon is the second highest deforested country in the Congo Basin with about 75% of its forest being subject to pressure from other land uses including agroforestry (Dkamela, 2010). Conversion of forests to traditional cacao agroforestry (CAF) systems have well been documented in Cameroon (Zapfack *et al.*, 2002; Sonwa *et al.*, 2007; Abada Mbolo *et al.*, 2016). Presently, an estimated 400,000 hectares is under CAF on small family farms of approximately one to three hectares (Kotto *et al.*, 2002; Saj *et al.*, 2013). These CAF systems are commonly established under the shade of the forests' remnant trees, and are characterised by absence of fertilizer inputs and low yields of up to 1 t cacao beans ha^{-1} (Saj *et al.*, 2013).

Changes in land use have been found to affect soil N_2O emissions due to changes in soil N availability (Corre *et al.*, 2006), vegetation (Veldkamp *et al.*, 2008) and management practices such as N fertilization (Hassler *et al.*, 2017). In particular, unfertilized agroforestry and agricultural systems have been found to have comparable N_2O fluxes as those from the reference forests (Hassler *et al.*, 2017), whereas N-fertilized systems tend to have higher N_2O fluxes than the previous forest due to elevated soil mineral N following fertilization (Verchot *et al.*, 2006). This is in line with postulations of the conceptual hole-in-the-pipe (HIP) model, which suggest that the magnitude of N_2O emissions from the soil are largely controlled first by soil N availability and second by soil water content (Davidson *et al.*, 2000a). As the number of studies on soil GHG fluxes from agricultural land uses in Africa is still limited, the effect of

land-use change on GHG fluxes is identified as an important research gap in the GHG budget of Africa (Valentini *et al.*, 2014).

Tree stems have been found to act as conduits for soil N_2O in wetlands, mangroves and well-drained forests (Rusch & Rennenberg, 1998; Kreuzwieser *et al.*, 2003; Welch *et al.*, 2019), facilitating the transport from the soil, where N_2O are produced or consumed by microbial nitrification and denitrification processes, to the atmosphere. Findings of strong declines in N_2O emissions with increasing stem height (Díaz-Pinés *et al.*, 2016; Wen *et al.*, 2017; Barba *et al.*, 2019b) suggest that N_2O is mainly emitted through the stems and less likely through the leaves. Trees adapted to wetlands and mangroves have aerenchyma systems through which N_2O can be transported from the soil into the tree by both gas diffusion and transpiration stream, with exchange to the atmosphere predominantly through the stem lenticels (Rusch & Rennenberg, 1998; Wen *et al.*, 2017). However, for trees on well-drained soils, a different transport mechanism appears to be dominant: transpiration causes the xylem sap flow in which dissolved N_2O is transported from the soil to the tree and emitted to the atmosphere through the stem surface and stomata (Machacova *et al.*, 2013; Wen *et al.*, 2017). Recent evidence shows that trees can also act as N_2O sinks (Machacova *et al.*, 2017; Barba *et al.*, 2019b), highlighting the need for further research of the stem N_2O flux magnitudes and their mechanisms.

The most important soil parameters found to influence tree-stem N_2O fluxes include soil water content (Rusch & Rennenberg, 1998; Machacova *et al.*, 2016), soil N_2O fluxes (Díaz-Pinés *et al.*, 2016; Wen *et al.*, 2017), soil temperature (Machacova *et al.*, 2013) and soil-air N_2O concentration within the rooting zone (Wen *et al.*, 2017). These studies also reported environmental parameters, such as air temperature and vapour pressure deficit (VPD), to drive stem N_2O fluxes due to their influence on transpiration (O'Brien *et al.*, 2004). For temperate forests on a well-drained soil, annual stem N_2O fluxes have been found to contribute up to 10%

of the ecosystem N_2O emissions (Wen *et al.*, 2017). However, until now, there is no ground-based spatial extrapolation of the contribution of stem N_2O emissions from tropical forests on well-drained soils. Hence, there is a need for concurrent quantifications of the contributions of stem and soil N_2O fluxes so as to provide insights on the source strengths of N_2O emissions from tropical African land uses and to improve estimates of N_2O emissions from the region.

Our present study addresses these knowledge gaps by providing year-round measurements of stem and soil N_2O fluxes from forests and converted CAF systems with spatially replicated plots in the Congo Basin as well as stem N_2O fluxes of 23 tree species that have not been measured before. Our study aimed to (i) assess whether trees in tropical rainforests and CAF are important conduits of N_2O, (ii) quantify changes in soil-atmosphere N_2O fluxes with forest conversion to CAF, and (iii) determine the temporal and spatial controls of stem and soil N_2O fluxes. We hypothesized that (i) stem and soil N_2O fluxes from these extensively managed CAF systems (unfertilized and manual harvest) will be comparable to the natural forests, and (ii) the seasonal pattern of stem emissions will parallel that of soil N_2O emissions and both will have similar soil and climatic controlling factors.

2.3. Materials and methods

2.3.1. Study area and experimental design

Our study was conducted at three study sites located in southern and central Cameroon, where natural forests are predominantly converted to CAF (Sonwa *et al.*, 2007). Sites in the southern region were located around the villages of Aloum (2.813°N, 10.719°E; 651 m above sea level, asl) and Biba Yezoum (3.158°N, 12.292°E; 674 m asl), and the third site was located around the village of Tomba (3.931°N, 12.430°E; 752 m asl) in the central region (Fig. 1.1). The mean annual air temperature across the three sites is 23.5°C (Climate-Data.org, 2019), and the soil temperature ranged from 21.6–24.4 °C during our measurement period from May 2017 to April

2018. The study sites span an annual precipitation from 1576 mm yr^{-1} in the centre to 2064 mm yr^{-1} in the south of Cameroon (Table S2.1; Climate-Data.org, 2019). Precipitation occurs in a bimodal pattern, with two dry seasons (< 120 mm monthly rainfall) occurring from July to August and December to February (Fig. 1.2). All sites are situated on heavily weathered soils classified as Ferralsols (IUSS WRB, 2015). Geologically, Tomba and Biba Yezoum are underlain by middle to superior Precambrian basement rocks, made up of metamorphic schists, phyllites and quartzites, whereas Aloum site is situated on inferior Precambrian basement rocks, made up of inferior gneiss and undifferentiated gneiss (Gwanfogbe *et al.*, 1983).

At each site, we studied two land-use systems: the reference forest and the converted CAF system. Additional information on vegetation and site characteristics are reported in Table S2.1. These CAF sites were established right after clearing the natural forests, where remnant forest trees were retained by farmers to provide shade for understorey cacao trees (*Theobroma cacao*). Cacao planting and localised weeding were all done manually using hand tools. Surveys from farm owners indicated that there had been no mineral fertilization in any of the CAF sites. The ages of the CAF since conversion varied between 22 and ~45 years.

We selected four replicate plots (50 m x 50 m each with a minimum distance of 100 m between plots) per land-use type within each site (Fig. 1.1), totalling to 24 plots that were all located on relatively flat topography. Within each plot, all stems including cacao trees with a diameter at breast height (DBH) ≥ 10 cm were identified and measured for DBH and height. We conducted N_2O flux measurements, soil and meteorological parameters in the inner 40-m × 40-m area within each plot to minimize edge effects. To check that soil conditions were comparable between the reference forests and converted CAF, we compared a land-use-independent soil characteristic, i.e. clay content at 30−50 cm depth, between these land uses at each site. Since we did not find significant differences in clay contents between the forest and

CAF at each of the sites (Table 2.1), we inferred that land-use types within each site had comparable initial soil characteristics prior to conversion and any differences in N_2O fluxes and soil controlling factors can be attributed to land-use conversion.

For measurements of stem N_2O fluxes, we selected six cacao trees per replicate plot in the CAF, and six trees representing the most dominant species within each replicate plot in the forest, based on their importance value index (IVI) (Table S2.1). The species IVI is a summation of the relative density, relative frequency and relative dominance of the tree species (Curtis & McIntosh, 1951). For a given species, the relative density refers to its total number of individuals in the four forest plots at each site; the relative frequency refers to its occurrence among the four forest plots; and the relative dominance refers to its total basal area in the four forest plots, all expressed as percentages of all species. These 24 trees measured at each site (6 trees × 4 forest plots) included nine species in Aloum site, seven species in Biba Yezoum site, and 10 species in Tomba site (species are specified in Fig. 2.1). The trees were measured for stem N_2O fluxes at 1.3 m height above the ground at monthly interval from May 2017 to April 2018. Furthermore, we assessed the influence of tree height on stem N_2O fluxes by conducting additional measurements on 16 individual trees per land use in May 2018; these trees were included in the monthly measurements but were additionally measured at three stem heights (1.3 m, 2.6 m and 3.9 m from the ground) per tree in the forest, and at two heights (1.3 m and 2.6 m) per tree in the CAF due to the limited height of the cacao trees.

For soil N_2O flux measurements, we installed four permanent chamber bases per replicate plot, which were randomly distributed within the inner 40-m × 40-m area. We conducted monthly measurements of soil N_2O fluxes from May 2017 to April 2018 as well as meteorological and soil variables known to control N_2O emission (see below).

Table 2.1. Mean (±SE, n = 4) soil biochemical characteristics in the top 50-cm† depth in forest and cacao agroforestry (CAF) within each site in the Congo Basin, Cameroon. Means followed by different lowercase letters indicate significant differences between land-use types within each site and different capital letters indicate significant differences among the three sites within a land-use type (Anova with Fisher's LSD test or Kruskal-Wallis ANOVA with multiple comparison extension test at $p \leq 0.05$).

Soil characteristics	Aloum site		Biba Yezoum site		Tomba site	
	Forest	**CAF**	**Forest**	**CAF**	**Forest**	**CAF**
Clay (30-50 cm) (%)	$66.0 \pm 2.4^{a,A}$	$59.3 \pm 6.1^{a,A}$	$32.8 \pm 9.4^{a,B}$	$39.5 \pm 0.9^{a,B}$	$55.3 \pm 0.5^{a,AB}$	$51.8 \pm 1.1^{a,AB}$
Bulk density ($g\ cm^{-3}$)	$1.2 \pm 0.1^{a,A}$	$1.2 \pm 0.1^{a,A}$	$1.2 \pm 0.1^{a,A}$	$1.2 \pm 0.1^{a,A}$	$1.2 \pm 0.1^{a,A}$	$1.2 \pm 0.1^{a,A}$
pH (1:4 H_2O)	$3.7 \pm 0.0^{b,A}$	$4.1 \pm 0.1^{a,A}$	$3.7 \pm 0.1^{b,A}$	$4.6 \pm 0.2^{a,A}$	$3.6 \pm 0.0^{b,A}$	$4.5 \pm 0.2^{a,A}$
^{15}N natural abundance (‰)	$8.4 \pm 0.2^{b,A}$	$10.2 \pm 0.1^{a,A}$	$8.6 \pm 0.2^{a,A}$	$9.1 \pm 0.2^{a,B}$	$8.8 \pm 0.1^{a,A}$	$8.8 \pm 0.1^{a,B}$
Soil organic C ($kg\ C\ m^{-2}$)	$12.1 \pm 0.4^{a,A}$	$6.7 \pm 0.2^{b,A}$	$7.2 \pm 0.9^{a,B}$	$5.6 \pm 0.7^{a,A}$	$9.8 \pm 0.2^{a,AB}$	$7.1 \pm 0.4^{b,A}$
Total N ($kg\ N\ m^{-2}$)	$1.1 \pm 0.1^{a,A}$	$0.7 \pm 0.0^{b,A}$	$0.7 \pm 0.1^{a,A}$	$0.5 \pm 0.0^{a,B}$	$0.9 \pm 0.0^{a,A}$	$0.7 \pm 0.0^{b,A}$
ECEC ($mmol_c\ kg^{-1}$)	$57.5 \pm 3.9^{a,A}$	$33.9 \pm 2.8^{b,A}$	$49.1 \pm 11.3^{a,A}$	$41.1 \pm 7.2^{a,A}$	$58.5 \pm 2.0^{a,A}$	$46.8 \pm 4.7^{a,A}$
Exch. bases ($mmol_c\ kg^{-1}$)	$3.5 \pm 0.3^{b,B}$	$8.7 \pm 1.7^{a,B}$	$8.5 \pm 1.1^{b,A}$	$31.0 \pm 8.5^{a,A}$	$9.3 \pm 0.8^{b,A}$	$30.4 \pm 7.6^{a,A}$
Exchangeable Al ($mmol_c\ kg^{-1}$)	$47.3 \pm 3.1^{a,A}$	$20.9 \pm 3.5^{b,A}$	$32.9 \pm 8.9^{a,A}$	$5.4 \pm 1.2^{b,B}$	$39.2 \pm 2.3^{a,A}$	$12.3 \pm 2.7^{b,AB}$

† Values are depth-weighted average, except for clay content (30-50 cm) and stocks of soil organic C and total N, which are sum of the entire 50-cm depth. Abbreviations: ECEC, effective cation exchange capacity; Exch. bases: sum of exchangeable Ca, Mg, K, Na.

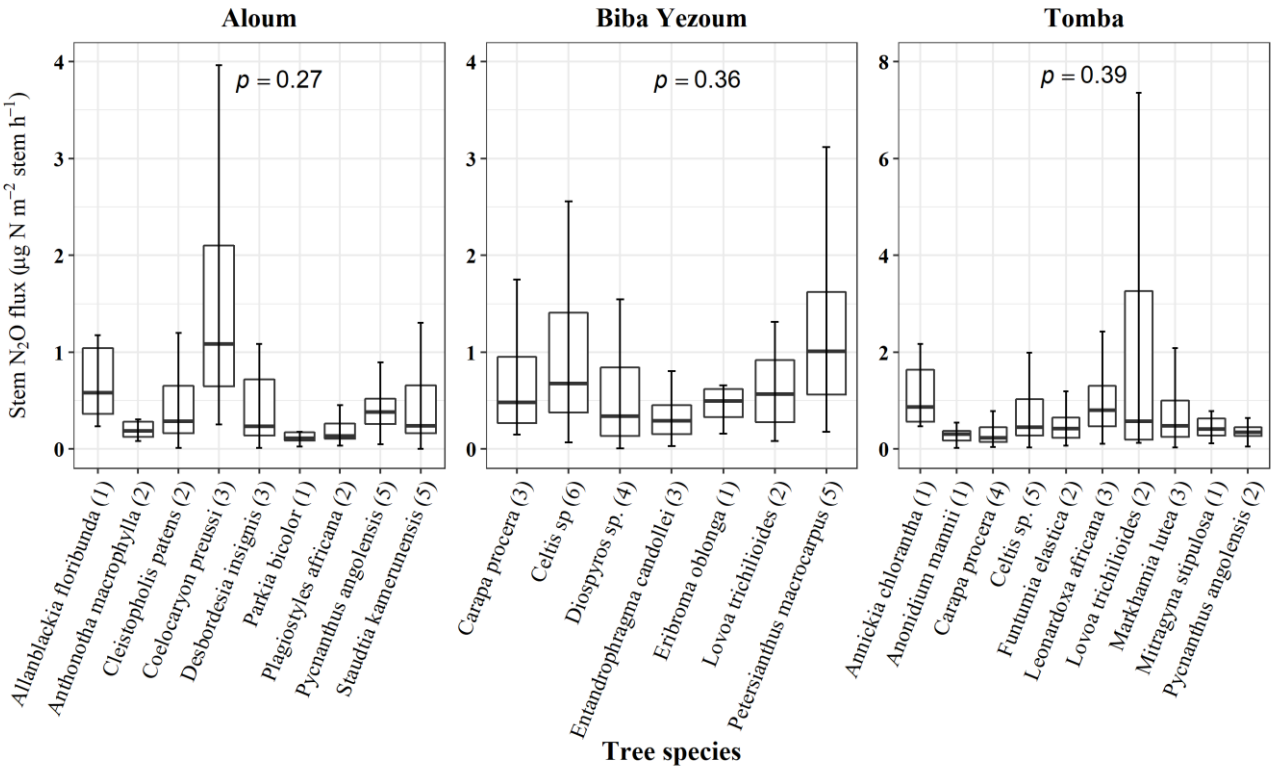

Figure 2.1. Stem N_2O fluxes from 22 tree species at three forest sites (Aloum, Biba Yezoum and Tomba) across central and south Cameroon in the Congo Basin. Boxes (25th, median and 75th percentile) and whiskers (1.5 × interquartile range) are based on N_2O fluxes measured monthly from May 2017 to April 2018 for each tree species, and the values in parentheses represent the number of trees measured per species. There were no differences in N_2O fluxes among species (linear mixed-effect models with Tukey's HSD at $p > 0.27$).

2.3.2. Measurement of stem and soil N_2O fluxes

We measured in-situ stem N_2O fluxes using stem chambers made from transparent polyethylene-terephthalate foil, as described by Wen *et al.* (2017). One month prior to measurement, we applied 1-cm wide silicone sealant strips (Otto Seal ® S110, Hermann Otto GmbH, Fridolfing, Germany) 20 cm apart around the surface of the tree stems (between 1.2-m and 1.4-m heights from the ground) that stayed permanently to ensure that all the stem chambers had air-tight seals. As many of the measured trees have buttresses (rendering stem chambers

impossible to attach at low stem height, e.g. Fig. 2.2), we chose the measurements at an average of 1.3-m height (or between 1.2-1.4 m), congruent to the standard measurement of DBH. Since chamber installation is quick, chambers were newly installed on each sampling date, using the silicone sealant strips as a mark to ensure that the same 0.2-m length stem section was measured. We wrapped a piece of foil (cut approximately 50 cm longer than the measured stem circumference and fitted with a Luer-lock sampling port) around each stem. Using a gas-powered heat-gun, we "shrank" the top and bottom part of the foil to fit closely onto the silicone strips, leaving 0.2-m length between the top and bottom silicone strips, which served as the chamber for collecting gas samples (Fig. 2.2). We then wrapped strips of polyethylene foam around the edges of the foil and adjusted the foam tightly using lashing straps equipped with ratchet tensioners (two straps at the top and two at the bottom). The lashing straps adjusted the flexible foam and the foil (on top of the silicone strips) to any irregularities on the bark and ensured an airtight fitting.

After installation, we completely evacuated the air inside the stem chamber using a syringe fitted with a Luer-lock one-way check valve. Afterwards, we used a manual hand pump to refill the stem chamber with a known volume of ambient outside air for correct calculation of stem N_2O flux. A 25-mL air sample was taken with syringe through the Luer-lock sampling port immediately after refilling the stem chamber with ambient air, and then again after 20, 40 and 60 minutes. Each air sample was immediately stored in pre-evacuated 12 mL Labco exetainers with rubber septa (Labco Limited, Lampeter, UK), maintaining an overpressure.

Figure 2.2. Sampling set-up for stem nitrous oxide (N_2O)-flux measurement at three stem heights in a rainforest in the Congo Basin, Cameroon.

In May 2018, we conducted a ^{15}N tracing experiment at the Tomba site as a follow-on study to elucidate the source of stem N_2O emissions. The tracing was conducted in three replicate plots per land use, where one tree was selected in each plot. Around each selected tree, 290 mg ^{15}N (in the form of $(^{15}NH_4)_2SO_4$ with 98% ^{15}N) dissolved in 8 L distilled water was applied evenly onto the soil surface of 0.8 m^2 around the tree using a watering can (equivalent to 10 mm of rain). The water-filled pore space (WFPS) in the top 5-cm depth was 49 ± 1% and 52 ± 2% for the forest and CAF, respectively, which were similar to the monthly averages of these plots during this period (Fig. 2.4). Based on the monthly average soil mineral N concentrations in this site, the applied ^{15}N was only 20% of the extant mineral N in the top 10 cm soil (resulting to a starting enrichment of 17% ^{15}N), such that we only minimally changed the substrate which could influence N_2O flux, similar to that described by Corre *et al.* (2014). Stem and soil $^{15}N_2O$ fluxes were measured one day, seven days and 14 days following ^{15}N application, and on each sampling day gas samples were taken at 0, 30, and 60 minutes after

chamber closure. The gas samples were stored in new pre-evacuated glass containers (100 mL) with rubber septa and transported to the University of Goettingen, Germany for analysis. We also stored $^{15}N_2O$ standards in similar 100-mL glass containers, which were brought to Cameroon and back to Germany, to have the same storage duration as the gas samples in order to check for leakage; we found no difference in $^{15}N_2O$ with the original standard at our laboratory.

We measured soil N_2O fluxes using vented, static chambers made from polyvinyl chloride that were permanently inserted ~0.02 m into the soil at least one month prior to the start of measurements, as described in our earlier studies (e.g., Koehler *et al.*, 2009b; Corre *et al.*, 2014; Müller *et al.*, 2015). On each sampling day, we covered the chamber bases with vented, static polyethylene hoods (0.04 m^2 in area and ~11 L total volume) equipped with Luer-lock sampling ports. Soil N_2O fluxes were then determined by taking four gas samples (25 mL each) at 2, 12, 22 and 32 minutes after chamber closure. The samples were taken with a syringe and immediately injected into pre-evacuated 12 mL exetainers as described above.

Concurrent to the stem and soil N_2O-flux measurements, we sampled soil-air N_2O concentrations at 50-cm depth from permanently installed stainless-steel probes (1-mm internal diameter) located at ~1 m from the measured trees. The stainless steel probes were installed one month prior to the start of measurements. Luer-locks were attached to the probes, and on each sampling day the probes were first cleared of any previous accumulation of N_2O concentration by removing 5-mL air volume using a syringe and discarding it. We then took 25-mL gas samples and stored them in pre-evacuated 12-mL exetainers as described above.

2.3.3. N_2O analysis and flux rate calculation

The N_2O concentrations in the gas samples were analysed using a gas chromatograph equipped with an electron capture detector, a make-up gas of 5% CO_2 – 95% N_2 (SRI 8610C, SRI Instruments Europe GmbH, Bad Honnef, Germany), and an autosampler (AS-210, SRI Instruments). $^{15}N_2O$ was analysed on an isotope ratio mass spectrometer (IRMS) (Finnigan Deltaplus XP, Thermo Electron Corporation, Bremen, Germany). We calculated N_2O fluxes from the linear change in concentrations over time of chamber closure, and adjusted the fluxes with air temperature and atmospheric pressure, measured at each replicate plot on each sampling day. We included zero and negative fluxes in our data analysis.

We up-scaled the measured stem N_2O fluxes (considering trees ≥ 10 cm DBH) to annual values on a ground area in the following steps: (1) the relationship between stem N_2O fluxes and stem heights was modelled from the 16 individual trees per land use (see above) that were measured at multiple heights, from which we observed decreases in stem N_2O fluxes with increasing stem heights. A linear function was statistically the best fit characterizing these decreases in stem N_2O fluxes with height. (2) Using this linear function and considering the stem surface area as a frustum with 20-cm increment, the tree-level N_2O fluxes on each sampling day was calculated for the regularly measured six trees per plot. (3) The annual tree-level N_2O fluxes from these regularly measured six trees per plot were calculated using a trapezoidal interpolation between the tree-level N_2O fluxes (step 2) and measurement day intervals from May 2017 to April 2018. (4) The annual tree-level N_2O fluxes were then extrapolated on a ground-area basis for each replicate plot as follows:

Annual stem N_2O flux (kg N_2O-N ha^{-1} yr^{-1}) = $\{ \Sigma [((X_{1\text{-}24} \div DBH_{1\text{-}24})/24) * DBH_n]\} \div A$

where: $X_{1\text{-}24}$ and $DBH_{1\text{-}24}$ are the corresponding annual tree-level N_2O flux (kg N_2O-N yr^{-1} of each tree; step 3) and DBH (cm) of each of the 24 measured trees (6 trees x 4 plots) per land

use at each site; DBH_n is the individual tree DBH (cm) measured for all trees (with ≥10 cm DBH) present within the inner 40-m x 40-m area of each plot (Table S2.1); Σ is the sum of the annual N_2O fluxes of all trees within each plot (kg N_2O-N yr^{-1}); A is the plot area (0.16 ha). For step 4 of the CAF plots, the annual stem N_2O flux was the sum of the cacao and shade trees (Table S2.1); as these shade trees were remnants of the original forest, we used the average annual tree-level N_2O flux of the measured trees in the corresponding paired forest plots multiplied by the actual DBH of the shade trees in the CAF plots. This spatial extrapolation based on trees' DBH of each plot was also supported by the fact that there were no significant differences in stem N_2O fluxes among tree species (Fig. 2.1).

Annual soil N_2O fluxes from each plot were calculated using the trapezoidal rule to interpolate the measured fluxes from May 2017 to Apr. 2018, as employed in our earlier studies (e.g., Koehler *et al.*, 2009b; Veldkamp *et al.*, 2013). Finally, the annual N_2O fluxes from each replicate plot were represented by the sum of the stem and soil N_2O fluxes.

2.3.4. Soil and meteorological variables

We measured soil temperature, WFPS, and extractable mineral N in the top 5-cm depth concurrent to stem and soil N_2O flux measurements on each sampling day. The soil temperature was measured ~1 m away from the soil chambers using a digital thermometer (GTH 175, Greisinger Electronic GmbH, Regenstauf, Germany). We determined soil WFPS and extractable mineral N by pooling soil samples from four sampling locations within 1 m from each soil chamber in each replicate plot. Gravimetric moisture content was determined by oven-drying the soils at 105 °C for 24 h and WFPS was calculated using a particle density of 2.65 g cm^{-3} for mineral soil and our measured soil bulk density (Table 2.1). Soil mineral N (NO_3^- and NH_4^+) was extracted in the field by putting a subsample of soil into a pre-weighed bottle containing 150 mL 0.5 M K_2SO_4. The bottles were weighed and then shaken for 1 hour, and

the solution was filtered through pre-washed (with 0.5 M K_2SO_4) filter papers. The extracts were immediately frozen and later transported to the University of Goettingen, where NH_4^+ and NO_3^- concentrations were analysed using continuous flow injection colorimetry (SEAL Analytical AA3, SEAL Analytical GmbH, Norderstedt, Germany) (described in details by Hassler et al., 2015). The dry mass of soil extracted for mineral N was calculated using the measured gravimetric moisture content.

During each measurement day, we set up a portable weather station in each site to record relative humidity, air temperature and solar irradiance over the course of each sampling day at 15-minute interval. We calculated VPD as the difference between saturation vapour pressure (based on its established equation with air temperature) and actual vapour pressure (using saturation vapour pressure and relative humidity; Allen, Pereira, Raes, & Smith, 1998).

Soil biochemical characteristics were measured in April 2017 at all 24 plots. We collected soil samples from the top 50-cm depth, where changes in soil biochemical characteristics resulting from land-use changes have been shown to occur (van Straaten *et al.*, 2015; Tchiofo Lontsi *et al.*, 2019). In each plot, we collected ten soil samples from the top 0-10 cm, and five soil samples each from 10-30 and 30-50 cm depths; in total, we collected 480 soil samples from the 24 plots. The soil samples were air-dried, 2-mm sieved and transported to the University of Goettingen, where they were dried again at 40 °C before analysis. Soil pH was analysed from 1:4 soil-to-distilled water ratio. Soil texture for each plot was determined using the pipette method after iron oxide and organic matter removal (Kroetsch & Wang, 2008). Effective cation exchange capacity (ECEC) and exchangeable cation concentrations (Ca, Mg, K, Na, Al, Fe, Mn) were determined by percolating the soil samples with unbuffered 1 M NH_4Cl , and the extracts analysed using inductively coupled plasma-atomic emission spectrometer (ICP-AES; iCAP 6300 Duo VIEW ICP Spectrometer, Thermo Fischer Scientific GmbH,

Dreieich, Germany). Soil subsamples were ground and analysed for total organic C and N using a CN analyser (vario EL cube; Elementar Analysis Systems GmbH, Hanau, Germany), and the soil ^{15}N natural abundance signatures were determined using IRMS (Delta Plus; Finnigan MAT, Bremen, Germany). Soil organic carbon (SOC) and total N stocks were calculated for the top 50 cm in both land uses. We used the bulk density of the reference forest for calculating the SOC and total N stocks of the converted CAF in order to avoid overestimations of element stocks resulting from increases in soil bulk densities following land-use conversion (Veldkamp, 1994; van Straaten *et al.*, 2015).

To evaluate the representativeness of our study area with the rest of the Congo Basin forest, we estimated the proportion of the Congo rainforest area which have similar biophysical conditions (elevation, precipitation ranges and soil type) as our study sites (Table S2.1). Using the FAO's Global Ecological Zone map for the humid tropics, we identified the areal coverage of (*i*) Ferralsols (FAO Harmonized World Soil Database; FAO/IIASA/ISRIC/ISS-CAS/JRC, 2012) with (*ii*) elevation ≤ 1000 m asl (SRTM digital elevation model; Jarvis *et al.*, 2008) and (*iii*) precipitation range between 1,500 and 2,100 mm yr^{-1} (WorldClim dataset; Hijmans *et al.*, 2005) within the six Congo rainforest countries (Fig. 2.3). This analysis was conducted using QGIS version 3.6.3.

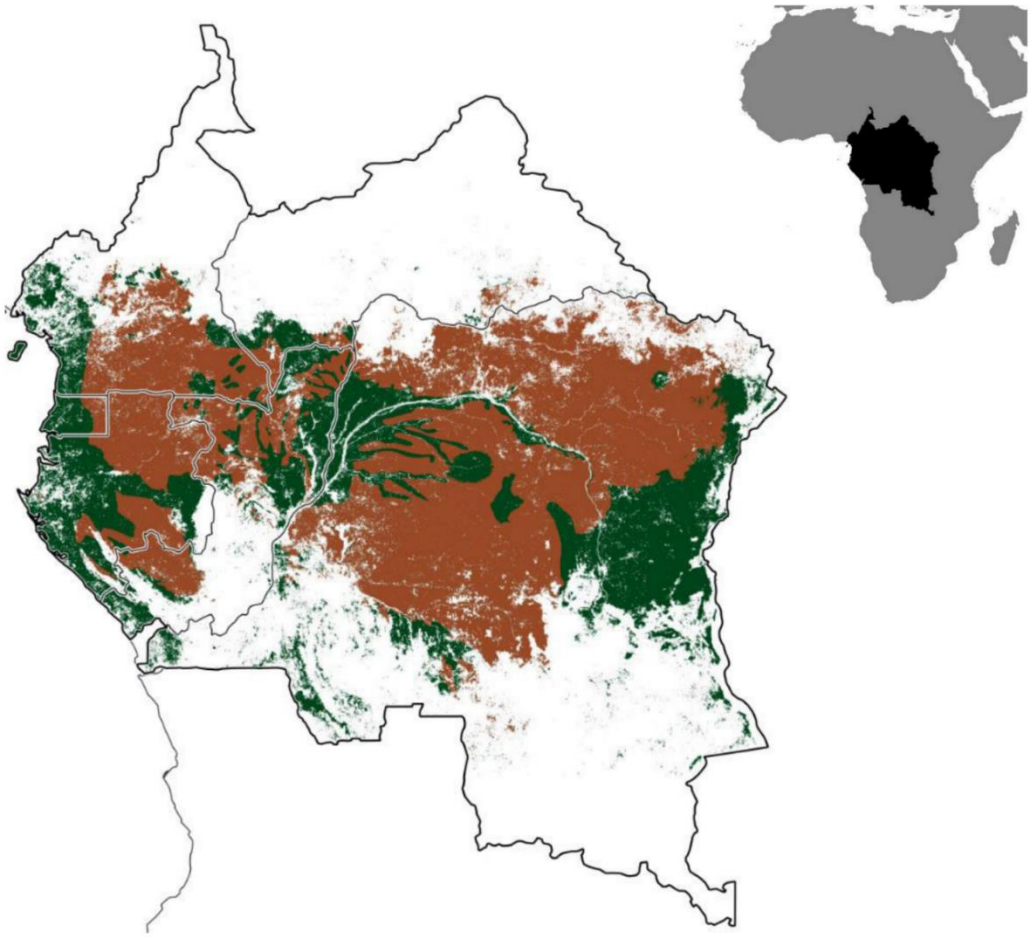

Figure 2.3. Map of the Congo Basin rainforest (green) spanning across the six major Congo Basin countries. Brown shaded area represents the proportion of the Congo rainforest with similar biophysical conditions as our study sites (Ferralsol soils, ≤ 1000 m elevation, and 1500-2100 mm yr^{-1} precipitation).

2.3.5. Statistical analyses

Statistical comparisons between land uses or among sites for stem and soil N_2O fluxes were performed on the monthly measurements and not on the annual values as the latter are trapezoidal interpolations. As the six trees and four chambers per plot were considered subsamples representing each replicate plot, we conducted the statistical analysis using the means of the six trees and of the four chambers on each sampling day for each replicate plot

(congruent to our previous studies, e.g. Koehler *et al.*, 2009b; Veldkamp *et al.*, 2013; Matson *et al.*, 2017). We tested each parameter for normal distribution (Shapiro-Wilk's test) and homogeneity of variance (Levene's test), and applied a logarithmic or square root transformation when these assumptions were not met. For the repeatedly measured parameters, i.e. stem and soil N_2O fluxes and the accompanying soil variables (temperature, WFPS, NH_4^+ and NO_3^- concentrations), differences between land-use types for each site or differences among sites for each land-use type were tested using linear mixed effect (LME) models with land use or site as fixed effect and replicate plots and sampling days as random effects. We extended the LME model to include either (1) a variance function that allows different variances of the fixed effect, and/or (2) a first-order temporal autoregressive process, which assumes that correlation between sampling days decreases with increasing time difference, if this improved the relative goodness of the model fit based on the Akaike information criterion (Crawley, 2009). Using diagnostic plots, the model residuals were checked for normality and homoscedasticity, and the data were log- or square root-transformed when necessary. We assessed significant differences between land uses or sites using analysis of variance (ANOVA) with Fisher's least significant difference (LSD) test.

We also analysed if there were differences in stem N_2O fluxes among tree species across four forest plots at each site as well as across the three sites. Similar LME analysis was carried out with tree species as fixed effect, and the random effects were trees belonging to each species and sampling days; only for this test, we used individual trees as random effect because most of the tree species (selected based on their IVI; see section 2.1) were not present in all plots, which is typical in species-diverse tropical forest. For soil biochemical characteristics that were measured once (Table 2.1), one-way ANOVA with Fisher's LSD test was used to assess the differences between land uses or sites for the variables with normal distribution and

homogenous variance; if otherwise, we applied Kruskal-Wallis ANOVA with multiple comparison extension test.

To determine the temporal controls of soil and meteorological variables (temperature, WFPS, NH_4^+ and NO_3^- concentrations, soil-air N_2O concentration, VPD) on stem and soil N_2O fluxes, we conducted Spearman's Rank correlation tests using the means of the four replicate plots for each land use on each sampling day. For each land use, the correlation tests were conducted across sites and sampling days (n = 33, from 3 sites × 11 monthly measurements).

To determine the spatial controls of soil biochemical characteristics (which were measured once, Table 2.1) on stem and soil N_2O fluxes, we used the plots' annual N_2O emissions and tested with Spearman's Rank correlation across land uses and sites (n = 24, from 3 sites × 2 land uses × 4 replicate plots). The statistical significance for all the tests were set at $p \leq 0.05$. All statistical analyses were conducted using the open source software R 3.5.2 (R Core Team, 2018).

2.4. Results

2.4.1. Stem N_2O emissions

Stem N_2O emissions neither differed between forest and CAF at each site (p = 0.15–0.76; Table 2.2) nor among the three sites for each land use (p = 0.16–0.78; Table 2.2). There were also no differences in stem N_2O emissions among tree species in forest plots at each site as well as across the three sites (p = 0.06–0.39; Fig. 2.1). For the forests, stem N_2O emissions exhibited seasonal pattern with larger fluxes in the wet season than in the dry season at all sites (all $p <$ 0.01; Fig. 2.4, Table S2.2). However, for the CAF, we observed seasonal differences only at Aloum site ($p < 0.01$; Fig. 2.4, Table S2.3). Contributions of annual stem N_2O emissions reached up to one-third of the total (soil + stem) N_2O emissions from the forests (Table 2.2).

Table 2.2. Mean (±SE, $n = 4$) stem and soil N_2O emission as well as annual stem, soil, and total (soil + stem) N_2O fluxes from forest and cacao agroforestry (CAF) within each site in the Congo Basin, Cameroon. Means followed by different lowercase letters indicate significant differences between land-use types within each site and different capital letters indicate significant differences among the three sites within a land-use type (linear mixed-effect models with Tukey's HSD at $p \leq 0.05$).

Site/ Land-use type	Stem N_2O fluxes ($\mu g\ N\ m^{-2}$ stem h^{-1})	Annual stem N_2O fluxes (kg N $ha^{-1}\ yr^{-1}$)	Soil N_2O fluxes ($\mu g\ N\ m^{-2}$ soil h^{-1})	Annual soil N_2O fluxes (kg N $ha^{-1}\ yr^{-1}$)	Total (soil + stem) N_2O flux (kg N $ha^{-1}\ yr^{-1}$)	Contribution of stem to total N_2O flux (%)
Aloum						
Forest	$1.13 \pm 0.22^{a,A}$	0.13 ± 0.00	$13.7 \pm 2.2^{a,A}$	0.87 ± 0.14	1.00 ± 0.14	13.7 ± 1.8
CAF	$0.90 \pm 0.16^{a,A}$	0.09 ± 0.01 (0.02 ± 0.01)	$15.2 \pm 2.8^{a,A}$	1.06 ± 0.17	1.15 ± 0.17	7.8 ± 1.6
Biba Yezoum						
Forest	$2.38 \pm 0.48^{a,A}$	0.87 ± 0.05	$17.2 \pm 2.9^{a,A}$	1.46 ± 0.23	2.33 ± 0.24	38.2 ± 3.5
CAF	$1.11 \pm 0.21^{a,A}$	0.12 ± 0.01 (0.03 ± 0.01)	$10.6 \pm 2.1^{a,A}$	0.80 ± 0.20	0.92 ± 0.20	14.8 ± 3.0
Tomba						
Forest	$0.89 \pm 0.10^{a,A}$	0.14 ± 0.01	$15.0 \pm 1.7^{a,A}$	1.18 ± 0.18	1.31 ± 0.18	11.4 ± 2.2
CAF	$0.90 \pm 0.12^{a,A}$	0.12 ± 0.00 (0.05 ± 0.02)	$15.8 \pm 2.0^{a,A}$	1.25 ± 0.14	1.37 ± 0.14	8.9 ± 0.9

Note. Annual stem and soil N_2O fluxes were not statistically tested for differences among sites or between land-use types since these annual values are trapezoidal extrapolations (see section 2.3.3, pg. 32). Annual stem N_2O emissions in parentheses are from cacao trees only.

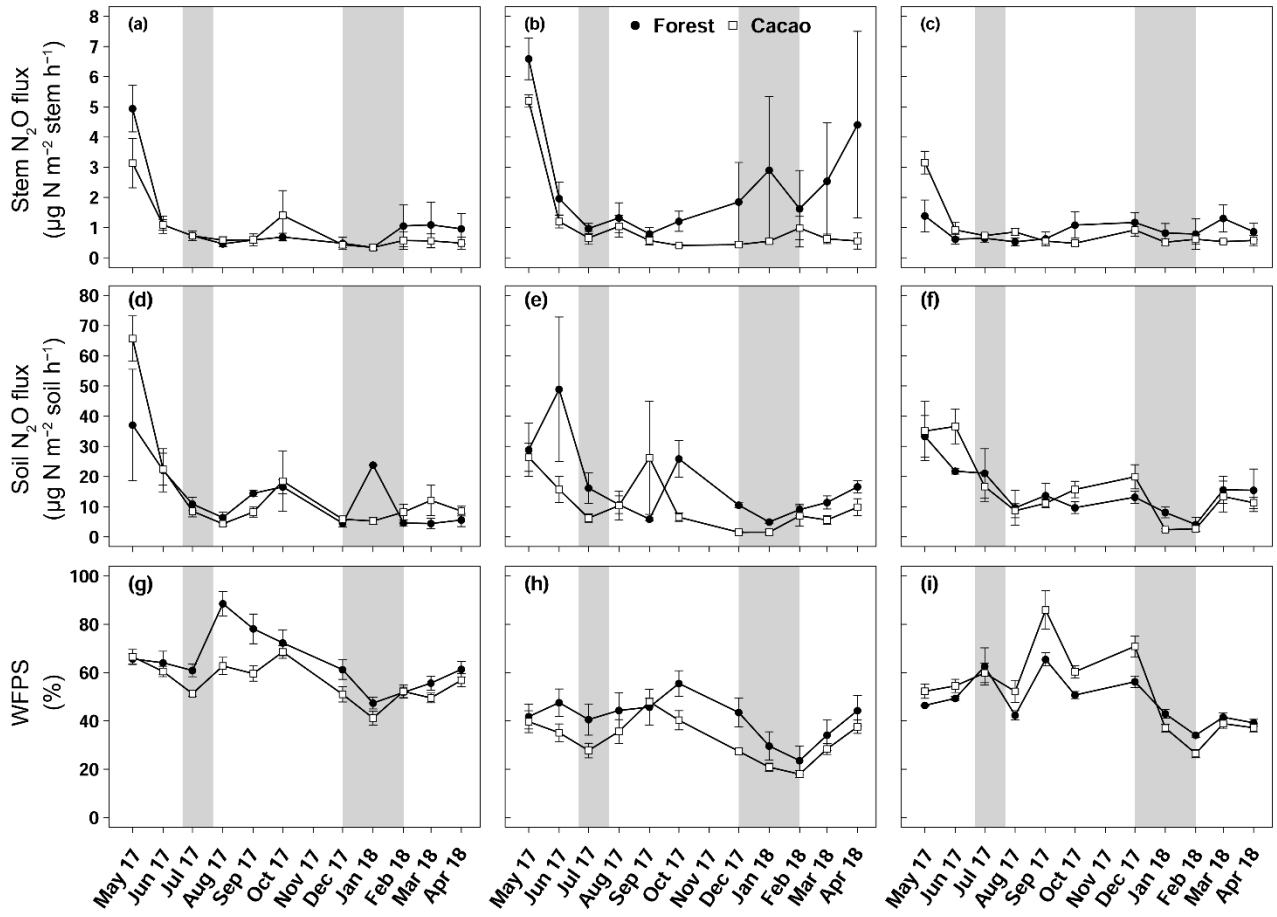

Figure 2.4. Mean (±SE, *n* = 4) stem N_2O fluxes (top panel), soil N_2O fluxes (middle panel) and water-filled pore space (bottom panel) in Aloum site (a, d and g), Biba Yezoum site (b, e and h) and Tomba site (c, f and i) in the Congo Basin, Cameroon, measured monthly from May 2017 to April 2018; grey shadings mark the dry season.

From the ^{15}N-tracing experiment, stem ^{15}N-N_2O emissions mirrored soil ^{15}N-N_2O emissions from both land uses (Fig.2.5). One day after ^{15}N addition to the soil, substantial ^{15}N-N_2O were emitted from the stem as well as from the soil. This diminished within two weeks as the added ^{15}N recycled within the soil-N-cycling processes, diluting the ^{15}N signatures; nevertheless, the ^{15}N signatures of stem- and soil-emitted N_2O remained elevated above the natural abundance level (Fig. 2.5).

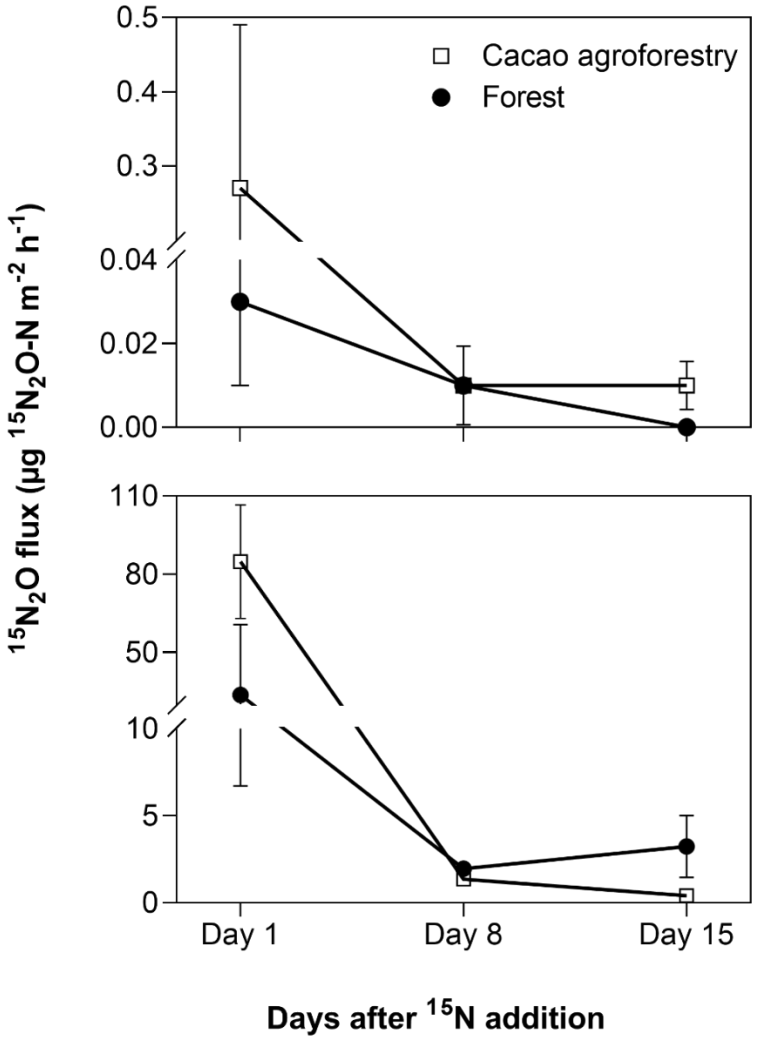

Figure 2.5. Mean (±SE, *n* = 3) $^{15}N_2O$ fluxes from stems (top panel, unit is per m^2 stem area) and soil (bottom panel, unit is per m^2 ground area) in the Congo Basin, Cameroon. In May 2018, 290 mg ^{15}N (in the form of $(^{15}NH_4)_2SO_4$ with 98% ^{15}N) was dissolved in 8 L distilled water and sprayed within 0.8-m^2 area around each tree (equal to 10 mm rain), which was only 20% of the extant mineral N in the top 10 cm soil and 49 ± 1% and 52 ± 2% water-filled pore space for the forest and CAF, respectively, comparable to the soil water content of the site (Fig. 2.4).

Across the study period, stem N_2O emissions from the forests were positively correlated with air temperature, soil-air N_2O concentrations and VPD (Table 2.3) and negatively correlated with WFPS and NH_4^+ contents (Table 2.3). The negative correlation of stem N_2O emissions

with WFPS was possibly spurious, as this correlation may have been driven by the autocorrelation between WFPS and air temperature (R = −0.59, p < 0.01, n = 33). In CAF, stem N_2O emissions were only positively correlated with soil N_2O emissions (Table 2.3).

Table 2.3. Spearman correlation coefficients of stem N_2O flux (μg N m^{-2} stem h^{-1}) and soil N_2O flux (μg N m^{-2} soil h^{-1}) with air temperature (°C), water-filled pore space (WFPS) (%, top 5-cm depth), extractable NH_4^+ (mg N kg^{-1}, top 5-cm depth), soil-air N_2O concentration (ppm N_2O at 50-cm depth), and vapour pressure deficit (VPD) (kPa), using the monthly means of the four replicate plots per land use across the three sites from May 2017 to April 2018 (n = 33).

Land use	Variable	Soil N_2O flux	Air temp.	WFPS	NH_4^+	Soil-air N_2O concentration	VPD
Forest	Stem N_2O flux	0.25	0.39[a]	−0.41[a]	−0.57[b]	0.41[a]	0.62[b]
	Soil N_2O flux		0.07	0.15	−0.43[a]	0.55[b]	−0.01
CAF	Stem N_2O flux	0.60[b]	−0.29	0.17	−0.26	0.21	0.21
	Soil N_2O flux		−0.34[a]	0.53[b]	−0.14	0.51[b]	0.10

[b] $p \leq 0.05$, [a] $p \leq 0.01$.

We detected no difference in WFPS between the forest and CAF (p = 0.15–0.28; Table 2.4) at any of the sites. For the CAF, we detected higher WFPS in the wet season compared to the dry season at two sites (p < 0.01; Fig. 2.4, Table S2.3) whereas there was no seasonal difference in WFPS for the forests at any sites (p = 0.31–0.92; Fig. 2.4, Table S2.2). At all the three sites, the dominant form of mineral N was NH_4^+ (Table 2.4). There was generally no difference in soil NH_4^+ and NO_3^- between the wet and dry seasons (p = 0.12–0.93), except for the forests at two sites with larger values in the dry than wet season (p < 0.01; Tables S2 and S3).

Table 2.4. Mean (±SE, n = 4) water-filled pore space (WFPS) and extractable mineral N in the top 5 cm of soil in forest and cacao agroforestry (CAF) within each site in Congo Basin, Cameroon, measured monthly from May 2017 to April 2018. Means followed by different lowercase letters indicate significant differences between land-use types within each site and different capital letters indicate significant differences among the three sites within a land-use type (linear mixed-effect models with Tukey's HSD at $p \leq 0.05$).

Site/ Land-use type	WFPS (%)	NH_4^+ (mg N kg^{-1})	NO_3^- (mg N kg^{-1})
Aloum			
Forest	$64.3 \pm 3.6^{a,A}$	$7.3 \pm 1.0^{a,A}$	$6.3 \pm 1.2^{a,A}$
CAF	$56.4 \pm 2.5^{a,A}$	$5.1 \pm 0.8^{a,B}$	$2.4 \pm 0.6^{b,A}$
Biba Yezoum			
Forest	$41.5 \pm 2.7^{a,B}$	$4.9 \pm 0.4^{b,B}$	$2.9 \pm 0.5^{a,B}$
CAF	$32.6 \pm 2.7^{a,B}$	$7.3 \pm 0.4^{a,A}$	$2.7 \pm 0.6^{a,A}$
Tomba			
Forest	$48.3 \pm 3.0^{a,B}$	$7.6 \pm 0.6^{a,A}$	$5.8 \pm 1.0^{a,A}$
CAF	$52.3 \pm 5.1^{a,A}$	$7.1 \pm 0.6^{a,A}$	$2.8 \pm 0.6^{b,A}$

2.4.2. Soil N_2O emissions

Soil N_2O emissions did not differ between forest and CAF at any site (p = 0.06–0.86; Table 2.2). Similarly, no differences in soil N_2O emissions were detected among sites for each land use (p = 0.26–0.44; Table 2.2). Soil N_2O emissions exhibited consistent seasonal patterns with larger fluxes in the wet than dry season for both land uses (all $p < 0.01$; Fig. 2.4, Tables S2 and S3).

Over the measurement period, soil N_2O emissions from the forests were positively correlated with soil-air N_2O concentrations and negatively correlated with NH_4^+ contents (Table 2.3). In the CAF, soil N_2O emissions were positively correlated with WFPS and soil-air N_2O

concentrations, and negatively correlated with air temperatures (Table 2.3). We did not detect any correlation between annual total N_2O fluxes and soil physical and biochemical characteristics. This was not surprising as the ranges of these soil characteristics were relatively small among sites, which reduce the likelihood that significant correlations will be detected.

2.4.3. Soil biochemical characteristics

Soil physical characteristics (clay content, bulk density) did not differ between forest and CAF at any of the sites (Table 2.1). Across sites, Biba Yezoum had lower clay content compared to the other sites for each land use ($p < 0.01$). Generally, the forest showed higher SOC and total N compared to the CAF ($p < 0.01$–0.05; Table 2.1). Soil ^{15}N natural abundance signatures, as an index of the long-term soil N availability, were generally similar between the forest and CAF except at Aloum site ($p < 0.01$; Table 2.1). Soil C/N ratio, another proxy for the long-term soil N status, was higher in the forest than in the CAF at all sites ($p < 0.01$–0.05). Soil pH and exchangeable bases were lower in the forest compared to the CAF at all sites and the converse was true for exchangeable Al ($p < 0.01$–0.05; Table 2.1). Soil ECEC did not differ between the land uses at two sites ($p < 0.01$; Table 2.1) and all were low congruent to Ferralsol soils.

2.5. Discussion

2.5.1. Stem and soil N_2O emissions from the forest

There has been no study on tree-stem N_2O emission from Africa, nor has any study on soil N_2O emission with year-long measurements and spatial replication been reported for the Congo Basin. Stems consistently emitted N_2O in both land uses (Fig. 2.1 and 2.4, Table 2.2), exemplifying that tropical trees on well-drained soils were important contributors of ecosystem N_2O emission. So far, there are only two tree species of tropical lowland forest reported with measurements of stem N_2O emissions (Welch *et al.*, 2019). Our present study included 23 tree species and their comparable stem N_2O emissions, at least from highly weathered Ferralsol

soils, across sites over a year of measurements provided support to our spatial extrapolation based on DBH of trees in the sites. Mean stem N_2O fluxes from our study were within the range of those reported for temperate forests (0.01–2.2 μg N m^{-2} stem h^{-1}; Díaz-Pinés *et al.,* 2016; Machacova *et al.,* 2016; Wen *et al.,* 2017), but substantially lower than the reported stem N_2O emissions of 51–759 μg N m^{-2} stem h^{-1} for a humid forest in Panama (Welch *et al.*, 2019). However, Welch *et al.* (2019) measured stem N_2O emissions at a lower stem height (0.3 m) compared to our study (1.3 m), which may partly explain their much larger N_2O emissions, as another study reported that larger N_2O emissions occur nearer to the stem base of trees (Barba *et al.*, 2019b). Moreover, the consistently higher stem than soil N_2O emissions found by Welch *et al.* (2019), which we did not observe in our study, may point to production of N_2O within the stem (e.g., Lenhart *et al.*, 2019). Nonetheless, such high stem N_2O emissions as reported by Welch *et al.* (2019) have not been observed anywhere else under field conditions.

Our annual soil N_2O emissions from forests (Table 2.2) were lower than the reported global average for humid tropical forests (2.81 kg N ha^{-1} yr^{-1}; summarised by Castaldi *et al.,* 2013). In contrast, the N_2O emissions from our forest soils were comparable to those reported for lowland forests on Ferralsol soils in Panama (0.35-1.07 kg N ha^{-1} yr^{-1}; Matson *et al.*, 2017), and lowland forests on Acrisol soils in Indonesia (0.9 & 1.0 kg N ha^{-1} yr^{-1}; Hassler *et al.*, 2017). These were possibly due to the generally similar soil N availability in our forest sites as these forest sites in Panama and Indonesia, indicated by their comparable soil mineral N contents and soil ^{15}N natural abundance signatures.

In comparison with studies from sub-Saharan Africa, annual soil N_2O emissions from our forests were lower than the annual N_2O emissions reported for the Mayombe forest in Congo (2.9 kg N ha^{-1} yr^{-1}; Serca *et al.,* 1994), Kakamega mountain rainforest in Kenya (2.6 kg N ha^{-1} yr^{-1}; Werner *et al.,* 2007), and Ankasa rainforest in Ghana (2.3 kg N ha^{-1} yr^{-1}; Castaldi

et al., 2013), but similar in magnitude as those reported for Mau Afromontane forest in Kenya (1.1 kg N ha^{-1} yr^{-1}; Wanyama *et al.,* 2018). Although these African sites have similar precipitation level and highly weathered acidic soils as our study sites, the Kakamega rainforest in Kenya had higher SOC (7.9–20%) and N contents (0.5–1.6%) in the topsoil layer compared to our forest sites (2.8–4.7% SOC, 0.2–0.4% total N), which may explain its correspondingly higher soil N_2O emissions. The study in Congo (Serca *et al.,* 1994), however, was conducted only in a short campaign (two rainy months and one dry month) with less sampling frequency and spatial replication, which may not be a good representation of the spatial and temporal dynamics of soil N_2O fluxes to achieve annual and large-scale estimate.

2.5.2. Source of tree-stem N_2O emissions and their contribution to total (soil + stem) N_2O emissions

Emitted N_2O from stems were found to originate predominantly from N_2O produced in the soil, as shown by the ^{15}N tracing experiment (Fig. 2.5). Additionally, the positive correlations of stem N_2O emissions with soil-air N_2O concentrations and soil N_2O emissions (Table 2.3) suggest that the seasonal variation in stem N_2O emissions (Fig. 2.4, Table S2.2) was likely driven by the temporal dynamics of produced N_2O in the soil, which partly supported our second hypothesis. While there has been suggestions of within-tree N_2O production (e.g., Lenhart et al., 2019), our finding from the ^{15}N tracing experiment, combined with the correlations of stem N_2O emissions with VPD and air temperature, pointed to a transport mechanism of dissolved N_2O in soil water by transpiration stream, which has been reported to be important for upland trees that do not have aerenchyma (Machacova *et al.,* 2016; Wen *et al.,* 2017; Welch *et al.,* 2019).

The contributions of up-scaled stem N_2O emissions from our studied forests to total (soil + stem) N_2O emissions (Table 2.2) were higher than those reported for temperate forests (1–

18%; Díaz-Pinés *et al.*, 2016; Machacova *et al.*, 2016; Wen *et al.*, 2017). Given the higher stem N_2O emissions in the wet than dry seasons (Table S2.2), coupled with the fact that we consistently measured positive fluxes or net stem N_2O emissions throughout our measurement period (Fig. 2.4), we conclude that tree stems in these well-drained Ferralsol soils were efficient conduits for releasing N_2O from the soil. This has significant implications especially during the rainy season as this pathway bypasses the chance for complete denitrification (N_2O to N_2 reduction) in the soil.

2.5.3. Factors controlling temporal variability of stem and soil N_2O fluxes

The positive correlation of stem N_2O emissions with VPD and air temperature in the forest suggests for transport of N_2O via sap flow, for which the latter had been shown to be stimulated with increasing VPD and air temperature (O'Brien *et al.*, 2004; McJannet *et al.*, 2007). Soil water containing dissolved N_2O is transported through the xylem via the transpiration stream and eventually emitted from the stem surface to the atmosphere (Díaz-Pinés *et al.*, 2016; Welch et al., 2019; Wen *et al.*, 2017).

Soil moisture has been shown to affect strongly the seasonal variation of soil N_2O emissions from tropical ecosystems, with increases in soil N_2O emissions by predominantly denitrification process at high WFPS (Werner *et al.*, 2006; Koehler *et al.*, 2009b; Corre *et al.*, 2014; Matson *et al.*, 2017). The larger stem N_2O emissions from the forest and soil N_2O emissions from both land uses in the wet than the dry seasons (Tables S2.2 and S2.3) signified the favourable soil N_2O production during the wet season, which suggests that denitrification was the dominant N_2O-producing process. However, the moderate WFPS across the year (Table 2.4) suggests that nitrification may also have contributed to N_2O emissions, especially at Biba Yezoum (with lower rainfall and clay contents; Tables 2.1 and S21) where the low WFPS (Table 2.4) likely favoured nitrification (Corre *et al.*, 2014). For the forest, the negative

correlation of the stem and soil N_2O emissions with soil NH_4^+ (Table 2.3) may be indicative of a conservative soil N cycle in our forest sites, as supported by the dominance of soil NH_4^+ over NO_3^- (Table 2.4) and by the lower soil N_2O emissions at our sites compared to NO_3^--dominated systems (Davidson *et al.*, 2000a). Although the soil mineral N content alone does not indicate the N-supplying capacity of the soil, the relative contents of NH_4^+ over NO_3^- can be a good indicator of whether the soil-N cycling is conservative with low N_2O losses or increasingly leaky (Corre *et al.*, 2010, 2014).

2.5.4. Land-use change effects on soil N_2O emissions

The annual soil N_2O emissions from CAF (Table 2.2) were comparable with those reported for rubber agroforestry in Indonesia (0.6–1.2 kg N ha^{-1} yr^{-1}; Hassler *et al.*, 2017) and from multistrata agroforestry systems in Peru (0.6 kg N ha^{-1} yr^{-1}; Palm *et al.*, 2002). However, our soil N_2O emissions from CAF were higher than those from an extensively managed homegarden in Tanzania (0.35 kg N ha^{-1} yr^{-1}; Gütlein *et al.*, 2018). In a review, Kim *et al.* (2016b) reported mean annual N_2O emission from tropical agroforestry systems to be 7.7 kg N ha^{-1} yr^{-1}. Most of the data used in their review were from intensively managed agroforestry systems with varied fertilizer inputs, which were absent in our extensively managed CAF systems. In line with this, our measured soil N_2O emissions from the CAF were also lower than the emissions reported for 10-23-year old CAF in Indonesia (3.1 kg N ha^{-1} yr^{-1}; Veldkamp *et al.*, 2008). Our measured N_2O emissions provide the first estimates for traditional CAF systems in Africa, as these production systems were not represented in extrapolation of GHG budgets despite their extensive coverage in Africa.

Soil N_2O emissions did not differ between forest and CAF systems, which supported our first hypothesis. This is possibly due to the presence of leguminous trees in both systems (Table S2.1), which can compensate for N export from harvest and other losses (Erickson *et*

al., 2002; Veldkamp *et al.*, 2008). Although studies have hinted on increased N_2O emissions from managed systems that utilize leguminous trees as cover crops (Veldkamp et al., 2008), the similar abundance of leguminous trees between forest and CAF at our sites may have offset this effect (Table S1). Previous studies have indeed reported similar soil N_2O fluxes between reference forests and unfertilized agroforestry systems (Van Lent *et al.*, 2015). Despite the general absence of heavy soil physical disturbance, cultivation and fertilization in these traditional CAF systems, some soil biochemical characteristics have decreased (Table 2.1); however, these did not translate into detectable differences in soil N_2O emissions with those from forest.

2.6. Implications

The biophysical conditions of our forest sites were representative of approximately two-thirds of the rainforest area in the Congo Basin (1.137×10^6 km^2; Fig. 3), considering the same Ferralsol soils, similar elevation ($\leq$ 1000 m asl), and annual rainfall between 1,500 and 2,100 mm yr^{-1}. Using the total (soil + stem) N_2O emission from our forest sites (1.55 ± 0.20 N_2O-N kg ha^{-1} yr^{-1}; Table 2.2), our extrapolated emission for the two-thirds of the Congo Basin was 0.18 ± 0.05 Tg N_2O-N yr^{-1} (error estimate is the 95% confidence interval). This accounted 52% of the earlier estimate of soil N_2O emissions from tropical rainforests in Africa (0.34 Tg N_2O-N yr^{-1}; Werner *et al.*, 2007), or 27% based on the more recent estimate (0.65 Tg N_2O-N yr^{-1}; Valentini *et al.*, 2014). We acknowledge, however, that there are uncertainties in our extrapolation (as is the case of these cited estimates) because our up-scaling approach from plot to regional level did not account for the spatial variability of large-scale drivers of soil N_2O emissions, such as soil texture, landforms and vegetation characteristics (e.g. Corre *et al.*, 1999). These limitations of our estimate of N_2O source strength for the Congo Basin rainforests call for further investigations in Africa to address the geographic bias of studies in the tropical region (e.g. Powers *et al.*, 2011).

Our year-round measurements of stem and soil N_2O fluxes were the first detailed study carried out in the Congo Basin, with key implications on improved estimates of N_2O budget for Africa. Our results revealed that trees on well-drained, highly weathered soils served as an important N_2O emission pathway, with the potential to overlook up to 38% of N_2O emissions if trees are not considered in the ecosystem N_2O budget. Additionally, forest conversion to traditional, mature (> 20 years old) CAF systems had no effect on stem and soil N_2O emissions, because of similarities in soil moisture and soil texture, absence of fertilizer application, and comparable abundance of leguminous trees in both land uses, which can compensate for N export from harvest or other losses. Further multi-temporal and spatially replicated studies are needed to provide additional insights on the effect of forest conversion to other land uses on GHG fluxes from the African continent in order to improve GHG budget estimations for the region.

2.7. Acknowledgements

This study was funded by the German Research Foundation (DFG, VE 219/14-1, STR 1375/1-1). We gratefully acknowledge our counterparts in Cameroon, the International Institute for Tropical Agriculture (IITA) and Dr. Rachid Hanna, for granting us access and use of their storage facilities. We are especially grateful to our Cameroonian field assistant Leonel Boris Gadjui Youatou for assisting in the coordination of the project. Many thanks to Narcis Lekeng, Yannick Eyenga Alfred, Denis Djiyo and all the field workers for their great support with field measurements, as well as Raphael Manu for helping with the GIS work and Rodine Tchiofo Lontsi for many discussions on soil processes and Cameroonian settings. We also thank the village leaders and local plot owners for granting us access to their forest and cacao farms. We thank Andrea Bauer, Kerstin Langs, Martina Knaust and Lars Szwec for their assistance with laboratory analyses.

2.8. References

Abada Mbolo, M. M., Zekeng, J. C., Mala, W. A., Fobane, J. L., Djomo Chimi, C., Tangboulou Ngavounsia, … Tamanjong, Y. V. (2016). The role of cocoa agroforestry systems in conserving forest tree diversity in the Central region of Cameroon. *Agroforestry Systems*, *90*(4), 577–590. https://doi.org/10.1007/s10457-016-9945-8

Allen, R. G., Pereira, L. S., Raes, D., & Smith, M. (1998). Determination of ET_0, crop evapotranspiration. *Guidelines for Computing Crop Water Requirements-FAO Irrigation and Drainage Paper 56*, 309. Retrieved from http://www.hidmet.gov.rs/podaci/agro/table of contens_files.pdf

Barba, J., Poyatos, R., & Vargas, R. (2019). Automated measurements of greenhouse gases fluxes from tree stems and soils: magnitudes, patterns and drivers. *Scientific Reports*, *9*(1), 1–13. https://doi.org/10.1038/s41598-019-39663-8

Bouwman, A. F., Van Der Hoek, K. W., & Olivier, J. G. J. (1995). Uncertainties in the global source distribution of nitrous oxide. *Journal of Geophysical Research*, *100*(D2), 2785–2800. https://doi.org/10.1029/94JD02946

Breuer, L., Papen, H., & Butterbach-Bahl, K. (2000). N_2O emission from tropical forest soils of Australia. *Journal of Geophysical Research Atmospheres*, *105*(D21), 26353–26367. https://doi.org/10.1029/2000JD900424

Castaldi, S., Bertolini, T., Valente, A., Chiti, T., & Valentini, R. (2013). Nitrous oxide emissions from soil of an African rain forest in Ghana. *Biogeosciences*, *10*(6), 4179–4187. https://doi.org/10.5194/bg-10-4179-2013

Climate-Data.org. (2019). Cameroon climate. Retrieved May 21, 2019, from https://en.climate-data.org/africa/cameroon-142/

Corre, M. D., Dechert, G., & Veldkamp, E. (2006). Soil nitrogen cycling following montane forest conversion in Central Sulawesi, Indonesia. *Soil Science Society of America Journal*, *70*(2), 359–366. https://doi.org/10.2136/sssaj2005.0061

Corre, M. D., Pennock, D. J., Van Kessel, C., & Elliott, D. K. (1999). Estimation of annual nitrous oxide emissions from a transitional grassland-forest region in Saskatchewan, Canada. *Biogeochemistry*, *44*(1), 29–49. https://doi.org/10.1023/A:1006025907180

Corre, M. D., Sueta, J. P., & Veldkamp, E. (2014). Nitrogen-oxide emissions from tropical forest soils exposed to elevated nitrogen input strongly interact with rainfall quantity and seasonality. *Biogeochemistry*, *118*(1–3), 103–120. https://doi.org/10.1007/s10533-013-9908-3

Corre, M. D., Veldkamp, E., Arnold, J., & Joseph Wright, S. (2010). Impact of elevated N input on soil N cycling and losses in old-growth lowland and montane forests in Panama. *Ecology*, *91*(6), 1715–1729. https://doi.org/10.1890/09-0274.1

Crawley, M. J. (2009). *The R Book*. Chichester, UK: John Wiley & Sons Ltd.

Curtis, J. T., & McIntosh, R. P. (1951). An Upland Forest Continuum in the Prairie-Forest Border Region of Wisconsin. *Ecology*, *32*(3), 476–496. https://doi.org/10.2307/1931725

Davidson, E. A., & Kanter, D. (2014). Inventories and scenarios of nitrous oxide emissions. *Environmental Research Letters*, *9*(10). https://doi.org/10.1088/1748-9326/9/10/105012

Davidson, E. A., Keller, M., Erickson, H. E., Verchot, L. V., & Veldkamp, E. (2000). Testing a Conceptual Model of Soil Emissions of Nitrous and Nitric Oxides. *BioScience*, *50*(8), 667. https://doi.org/10.1641/0006-3568(2000)050[0667:tacmos]2.0.co;2

Davidson, E. A., & Verchot, L. V. (2000). Testing the hole-in-the-pipe model of nitric and nitrous oxide emissions from soils using the TRAGNET database. *Global Biogeochemical Cycles*, *14*(4), 1035–1043. https://doi.org/10.1029/1999GB001223

Denman, K. L., Brasseur, G., Chidthaisong, A., Ciais, P., Cox, P. M., Dickinson, R. E., … Zhang, X. (2007). Couplings Between Changes in the Climate System and Biogeochemistry. In *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA. Retrieved from https://www.ipcc.ch/site/assets/uploads/2018/02/ar4-wg1-chapter7-1.pdf

Díaz-Pinés, E., Heras, P., Gasche, R., Rubio, A., Rennenberg, H., Butterbach-Bahl, K., & Kiese, R. (2016). Nitrous oxide emissions from stems of ash (*Fraxinus angustifolia Vahl*) and European beech (*Fagus sylvatica L.*). *Plant and Soil*, *398*(1–2), 35–45. https://doi.org/https://doi.org/10.1007/s11104-015-2629-8

Dkamela, G. P. (2010). The context of REDD+ in Cameroon: Drivers, agents and institutions (Occasional). CIFOR, Bogor, Indonesia.

Erickson, H. E., Davidson, E. A., & Keller, M. (2002). Former land-use and tree species affect nitrogen oxide emissions from a tropical dry forest. *Oecologia*, *130*(2), 297–308. https://doi.org/10.1007/s004420100801

FAO/IIASA/ISRIC/ISS-CAS/JRC. (2012). Harmonized World Soil Database (version 1.2). FAO, Rome, Italy and IIASA, Laxenburg, Austria. Retrieved September 13, 2019, from http://webarchive.iiasa.ac.at/Research/LUC/External-World-soil-database/HTML/

Gütlein, A., Gerschlauer, F., Kikoti, I., & Kiese, R. (2018). Impacts of climate and land use on N_2O and CH_4 fluxes from tropical ecosystems in the Mt. Kilimanjaro region, Tanzania. *Global Change Biology*, *24*, 1239–1255. https://doi.org/10.1111/gcb.13944

Gwanfogbe, M., Meligui, A., Moukam, J., & Nguoghia, J. (1983). *Geography of Cameroon.* Macmillan Education Ltd, Hong Kong.

Hassler, E., Corre, M. D., Kurniawan, S., & Veldkamp, E. (2017). Soil nitrogen oxide fluxes from lowland forests converted to smallholder rubber and oil palm plantations in Sumatra, Indonesia. *Biogeosciences*, *14*(11), 2781–2798. https://doi.org/https://doi.org/10.5194/bg-14-2781-2017

Hassler, E., Corre, M. D., Tjoa, A., Damris, M., Utami, S. R., & Veldkamp, E. (2015). Soil fertility controls soil-atmosphere carbon dioxide and methane fluxes in a tropical landscape converted from lowland forest to rubber and oil palm plantations. *Biogeosciences Discussions*, *12*(12), 9163–9207. https://doi.org/10.5194/bgd-12-9163-2015

Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G., & Jarvis, A. (2005). Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, *25*(15), 1965–1978. https://doi.org/10.1002/joc.1276

Huang, J., Golombeck, A., Prinn, R. G., Weiss, R. F., Fraser, P., Simmonds, P., … Porter, L. (2008). Estimation of regional emissions of nitrous oxide from 1997 to 2005 using multinetwork measurements, a chemical transport model, and an inverse method. *Journal of Geophysical Research Atmospheres*, *113*(17), 1–19. https://doi.org/10.1029/2007JD009381

Ishizuka, S., Tsuruta, H., & Murdiyarso, D. (2002). An intensive field study on CO_2, CH_4, and N_2O emissions from soils at four land-use types in Sumatra, Indonesia. *Global Biogeochemical Cycles*, *16*(3), 1049. https://doi.org/10.1029/2001gb001614

IUSS Working Group WRB. (2015). World Reference Base for Soil Resources 2014, update 2015 International soil classification system for naming soils and creating legends for soil maps. *World Soil Resources Reports No. 106.* FAO, Rome.

Jarvis, A., Reuter, H. I., Nelson, A., & Guevara, E. (2008). Hole-filled SRTM for the globe, Version 4. CGIAR-CSI SRTM 90m Database. *International Center for Tropical Agriculture, Cali, Columbia. Http://Srtm.Csi.Cgiar.Org*, (September 2017).

Kiese, R., Hewett, B., Graham, A., & Butterbach-Bahl, K. (2003). Seasonal variability of N_2O emissions and CH_4 uptake by tropical rainforest soils of Queensland, Australia. *Global Biogeochemical Cycles*, *17*(2), 1043. https://doi.org/10.1029/2002gb002014

Kim, D. G., Thomas, A. D., Pelster, D. E., Rosenstock, T. S., & Sanz-Cobena, A. (2016). Greenhouse gas emissions from natural ecosystems and agricultural lands in sub-Saharan Africa: Synthesis of available data and suggestions for further research. *Biogeosciences*, *13*(16), 4789–4809. https://doi.org/10.5194/bg-13-4789-2016

Koehler, B., Corre, M. D., Veldkamp, E., Wullaert, H., & Wright, S. J. (2009). Immediate and long-term nitrogen oxide emissions from tropical forest soils exposed to elevated nitrogen input. *Global Change Biology*, *15*(8), 2049–2066. https://doi.org/10.1111/j.1365-2486.2008.01826.x

Kotto, J. S., Moukam, A., Njomgang, R., Tiki-Manga, T., Tonye, J., Diaw, C., … Tondoh, J. (2002). Alternatives to slash-and-burn in Indonesia: summary report & synthesis of phase II in Cameroon. *ASB-Indonesia report*. Nairobi. Kenya.

Kreuzwieser, J., Buchholz, J., & Rennenberg, H. (2003). Emission of Methane and Nitrous Oxide by Australian Mangrove Ecosystems. *Plant Biology*, *5*(4), 423–431. https://doi.org/10.1055/s-2003-42712

Kroetsch, D., & Wang, C. (2008). Particle size distribution. In *Soil Sampling and Methods of Analysis, Second Edition* (pp. 713–725).

Lenhart, K., Behrendt, T., Greiner, S., Steinkamp, J., Well, R., Giesemann, A., & Keppler, F. (2019). Nitrous oxide effluxes from plants as a potentially important source to the atmosphere. *New Phytologist*, *221*(3), 1398–1408. https://doi.org/10.1111/nph.15455

Machacova, K., Bäck, J., Vanhatalo, A., Halmeenmäki, E., Kolari, P., Mammarella, I., …

Pihlatie, M. (2016). *Pinus sylvestris* as a missing source of nitrous oxide and methane in boreal forest. *Scientific Reports*, *6*(March), 1–8. https://doi.org/10.1038/srep23410

Machacova, K., Maier, M., Svobodova, K., Lang, F., & Urban, O. (2017). Cryptogamic stem covers may contribute to nitrous oxide consumption by mature beech trees. *Scientific Reports*, *7*(1), 1–7. https://doi.org/10.1038/s41598-017-13781-7

Machacova, K., Papen, H., Kreuzwieser, J., & Rennenberg, H. (2013). Inundation strongly stimulates nitrous oxide emissions from stems of the upland tree *Fagus sylvatica* and the riparian tree *Alnus glutinosa*. *Plant and Soil*, *364*(1–2), 287–301. https://doi.org/10.1007/s11104-012-1359-4

Matson, A. L., Corre, M. D., Langs, K., & Veldkamp, E. (2017). Soil trace gas fluxes along orthogonal precipitation and soil fertility gradients in tropical lowland forests of Panama. *Biogeosciences*, *14*(14), 3509–3524. https://doi.org/10.5194/bg-14-3509-2017

McJannet, D., Fitch, P., Disher, M., & Wallace, J. (2007). Measurements of transpiration in four tropical rainforest types of north Queensland, Australia. *HYDROLOGICAL PROCESSES*, *21*, 3549–3564. https://doi.org/https://doi.org/10.1002/hyp.6576

Müller, A. K., Matson, A. L., Corre, M. D., & Veldkamp, E. (2015). Soil N_2O fluxes along an elevation gradient of tropical montane forests under experimental nitrogen and phosphorus addition. *Frontiers in Earth Science*, *3*(October), 1–12. https://doi.org/10.3389/feart.2015.00066

Neill, C., Steudler, P. A., Garcia-Montiel, D. C., Melillo, J. M., Feigl, B. J., Piccolo, M. C., & Cerri, C. C. (2005). Rates and controls of nitrous oxide and nitric oxide emissions following conversion of forest to pasture in Rondônia. *Nutrient Cycling in Agroecosystems*, *71*(1), 1–15. https://doi.org/10.1007/s10705-004-0378-9

O'Brien, J. J., Oberbauer, S. F., & Clark, D. B. (2004). Whole tree xylem sap flow responses to multiple environmental variables in a wet tropical forest. *Plant, Cell and Environment*, *27*(5), 551–567. https://doi.org/10.1111/j.1365-3040.2003.01160.x

Palm, C. A., Alegre, J. C., Arevalo, L., Mutuo, P. K., Mosier, A. R., & Coe, R. (2002). Nitrous oxide and methane fluxes in six different land use systems in the Peruvian Amazon. *Global Biogeochemical Cycles*, *16*(4), 1073. https://doi.org/10.1029/2001gb001855

Powers, J. S., Corre, M. D., Twine, T. E., & Veldkamp, E. (2011). Geographic bias of field observations of soil carbon stocks with tropical land-use changes precludes spatial extrapolation. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(15), 6318–6322. https://doi.org/10.1073/pnas.1016774108

Purbopuspito, J., Veldkamp, E., Brumme, R., & Murdiyarso, D. (2006). Trace gas fluxes and nitrogen cycling along an elevation sequence of tropical montane forests in Central Sulawesi, Indonesia. *Global Biogeochemical Cycles*, *20*(3), 1–11. https://doi.org/10.1029/2005GB002516

Ravishankara, A. R., Daniel, J. S., & Portmann, R. W. (2009). Nitrous oxide (N_2O): The dominant ozone-depleting substance emitted in the 21st century. *Science*, *326*(5949), 123–125. https://doi.org/10.1126/science.1176985

Rusch, H., & Rennenberg, H. (1998). Black alder (*Alnus glutinosa (L.) Gaertn.*) trees mediate methane and nitrous oxide emission from the soil to the atmosphere. *Plant and Soil*, *201*(1), 1–7. https://doi.org/10.1023/A:1004331521059

Saj, S., Jagoret, P., & Todem Ngogue, H. (2013). Carbon storage and density dynamics of associated trees in three contrasting *Theobroma cacao* agroforests of Central Cameroon. *Agroforestry Systems*, *87*(6), 1309–1320. https://doi.org/10.1007/s10457-013-9639-4

Serca, D., Delmas, R., Jambert, C., & Labroue, L. (1994). Emissions of nitrogen oxides from equatorial rain forest in central Africa: *Tellus B: Chemical and Physical Meteorology*, *46*(4), 243–254. https://doi.org/10.3402/tellusb.v46i4.15795

Sonwa, D. J., Nkongmeneck, B. A., Weise, S. F., Tchatat, M., Adesina, A. A., & Janssens, M. J. J. (2007). Diversity of plants in cocoa agroforests in the humid forest zone of Southern Cameroon. *Biodiversity and Conservation*, *16*(8), 2385–2400. https://doi.org/10.1007/s10531-007-9187-1

Tchiofo Lontsi, R., Corre, M. D., van Straaten, O., & Veldkamp, E. (2019). Changes in soil organic carbon and nutrient stocks in conventional selective logging versus reduced-impact logging in rainforests on highly weathered soils in Southern Cameroon. *Forest Ecology and Management*, *451*(August), 117522. https://doi.org/10.1016/j.foreco.2019.117522

Thompson, R. L., Chevallier, F., Crotwell, A. M., Dutton, G., Langenfelds, R. L., Prinn, R. G., … Aoki, S. (2014). Nitrous oxide emissions 1999 to 2009 from a global atmospheric inversion. *Atmospheric Chemistry and Physics*, *14*(4), 1801–1817. https://doi.org/10.5194/acp-14-1801-2014

Valentini, R., Arneth, A., Bombelli, A., Castaldi, S., Cazzolla Gatti, R., Chevallier, F., … Scholes, R. J. (2014). A full greenhouse gases budget of africa: Synthesis, uncertainties, and vulnerabilities. *Biogeosciences*, *11*(2), 381–407. https://doi.org/10.5194/bg-11-381-2014

Van Lent, J., Hergoualc'H, K., & Verchot, L. V. (2015). Reviews and syntheses: Soil N_2O and NO emissions from land use and land-use change in the tropics and subtropics: A meta-analysis. *Biogeosciences*, *12*(23), 7299–7313. https://doi.org/10.5194/bg-12-7299-2015

van Straaten, O., Corre, M. D., Wolf, K., Tchienkoua, M., Cuellar, E., Matthews, R. B., & Veldkamp, E. (2015). Conversion of lowland tropical forests to tree cash crop plantations loses up to one-half of stored soil organic carbon. *Proceedings of the National Academy of Sciences*, *112*(32), 9956–9960. https://doi.org/10.1073/pnas.1504628112

Veldkamp, E. (1994). Organic Carbon Turnover in Three Tropical Soils under Pasture after Deforestation. *Soil Science Society of America Journal*, *58*(1), 175–180. https://doi.org/10.2136/sssaj1994.03615995005800010025x

Veldkamp, E., Koehler, B., & Corre, M. D. (2013). Indications of nitrogen-limited methane uptake in tropical forest soils. *Biogeosciences*, *10*(8), 5367–5379. https://doi.org/10.5194/bg-10-5367-2013

Veldkamp, E., Purbopuspito, J., Corre, M. D., Brumme, R., & Murdiyarso, D. (2008). Land use change effects on trace gas fluxes in the forest margins of Central Sulawesi, Indonesia. *Journal of Geophysical Research: Biogeosciences*, *113*(2), 1–11. https://doi.org/10.1029/2007JG000522

Verchot, L. V., Hutabarat, L., Hairiah, K., & van Noordwijk, M. (2006). Nitrogen availability and soil N_2O emissions following conversion of forests to coffee in southern Sumatra. *Global Biogeochemical Cycles*, *20*(4), 1–12. https://doi.org/10.1029/2005GB002469

Wanyama, I., Pelster, D. E., Arias-Navarro, C., Butterbach-Bahl, K., Verchot, L. V., & Rufino, M. C. (2018). Management intensity controls soil N_2O fluxes in an Afromontane ecosystem. *Science of the Total Environment*, *624*(December), 769–780. https://doi.org/10.1016/j.scitotenv.2017.12.081

Welch, B., Gauci, V., & Sayer, E. J. (2019). Tree stem bases are sources of CH_4 and N_2O in a tropical forest on upland soil during the dry to wet season transition. *Global Change Biology*, *25*(1), 361–372. https://doi.org/10.1111/gcb.14498

Wen, Y., Corre, M. D., Rachow, C., Chen, L., & Veldkamp, E. (2017). Nitrous oxide emissions from stems of alder, beech and spruce in a temperate forest. *Plant Soil*. https://doi.org/10.1007/s11104-017-3416-5

Werner, C., Butterbach-Bahl, K., Haas, E., Hickler, T., & Kiese, R. (2007a). A global inventory of N_2O emissions from tropical rainforest soils using a detailed biogeochemical model. *Global Biogeochemical Cycles*, *21*(3). https://doi.org/10.1029/2006GB002909

Werner, C., Kiese, R., & Butterbach-Bahl, K. (2007b). Soil-atmosphere exchange of N_2O, CH_4, and CO_2 and controlling environmental factors for tropical rain forest sites in western Kenya. *Journal of Geophysical Research*, *112*(3), D03308. https://doi.org/10.1029/2006JD007388

Werner, C., Zheng, X., Tang, J., Xie, B., Liu, C., Kiese, R., & Butterbach-Bahl, K. (2006). N_2O, CH_4 and CO_2 emissions from seasonal tropical rainforests and a rubber plantation in Southwest China. *Plant and Soil*, *289*(1–2), 335–353. https://doi.org/10.1007/s11104-006-9143-y

Wolf, K., Veldkamp, E., Homeier, J., & Martinson, G. O. (2011). Nitrogen availability links forest productivity, soil nitrous oxide and nitric oxide fluxes of a tropical montane forest in southern Ecuador. *Global Biogeochemical Cycles*, *25*(4), GB4009. https://doi.org/10.1029/2010GB003876

Zapfack, L., Engwald, S., Sonke, B., Achoundong, G., & Madong, B. A. (2002). The impact of land conversion on plant biodiversity in the forest zone of Cameroon. *Biodiversity and Conservation*, *11*(11), 2047–2061. https://doi.org/https://doi.org/10.1023/A:1020861925294

2.9. Appendix

Table S2.1. Vegetation and site characteristics of the study sites on highly weathered soils in the Congo Basin, Cameroon.

Site	Aloum		Biba Yezoum		Tomba	
Land use	Forest	Cacao agroforestry[a]	Forest	Cacao agroforestry[a]	Forest	Cacao agroforestry[a]
Tree density (n ha^{-1})	594 ± 29	403 ± 60 (140 ± 37)	619 ± 16	267 ± 24 (96 ± 16)	453 ± 34	430 ± 51 (292 ± 79)
Total basal area (m^2 ha^{-1})	35 ± 1.4	27 ± 2.5 (1.5 ± 0.5)	33 ± 2.9	27 ± 2.0 (0.9 ± 0.2)	34 ± 2.3	30 ± 3.2 (3.8 ± 1.3)
Legume abundance (% of the number of trees)	7.7 ± 1.7	5.9 ± 1.4	9.3 ± 1.9	6.5 ± 2.3	7.4 ± 1.6	4.8 ± 1.4
Tree height (m)	18.6 ± 0.5	15.1 ± 0.9 (6.8 ± 0.1)	20.6 ± 0.5	16.1 ± 0.4 (6.2 ± 0.3)	19.5 ± 0.4	11.7 ± 1.7 (6.1 ± 0.3)
Diameter at breast height (cm)	23.2 ± 0.6	23.3 ± 1.6 (11.4 ± 0.2)	22.6 ± 0.8	27.2 ± 0.2 (10.8 ± 0.2)	24.8 ± 1.0	23.5 ± 2.7 (12.3 ± 0.6)
Three most abundant tree species in the forest plots at each site[b]	*Cleistopholis patens* *Coelocaryon preussi* *Pycnanthus angolensis*		*Celtis sp.* *Diospyros sp* *Petersianthus macrocarpus*		*Celtis sp.* *Carapa procera* *Funtumia elastica*	
Elevation (m above sea level)	651		674		752	
Precipitation (mm yr^{-1}; from 1982 to 2012)[c]	2064		1639		1577	

Note. All vegetation characteristics were determined from trees with ≥ 10 cm diameter at breast height in both forest and cacao agroforestry.

[a] For cacao agroforestry, the first values are for both cacao and remnant shade trees, and the second values in parentheses are for cacao trees only.

[b] Determined using Importance Value Index (IVI = relative density + relative frequency + relative dominance (Curtis and McIntosh, 1951)).

[c] Climate-Data.org, 2019

Table S2.2. Seasonal mean (±SE, n = 4) water-filled pore space (WFPS), extractable mineral N (measured in the top 5 cm of soil) and nitrous oxide (N_2O) fluxes in forests on highly weathered soils in the Congo Basin, Cameroon. Means followed by different lowercase letters indicate significant differences between seasons for each site (linear mixed-effect models with Tukey's HSD at $p \leq 0.05$).

Site/ season	Stem N_2O flux (µg N m^{-2} stem h^{-1})	Soil N_2O flux (µg N m^{-2} h^{-1})	WFPS (%)	Soil NH_4^+ (mg N kg^{-1})	Soil NO_3^- (mg N kg^{-1})
Wet seasson					
Aloum	1.56 ± 0.36[a]	16.7 ± 3.7[a]	66.2 ± 2.2[a]	6.0 ± 0.6[a]	6.0 ± 0.8[a]
Biba Yezoum	2.92 ± 0.73[a]	22.9 ± 4.9[a]	44.8 ± 2.6[a]	4.4 ± 0.3[a]	2.2 ± 0.2[b]
Tomba	1.01 ± 0.13[a]	18.6 ± 2.2[a]	49.4 ± 1.8[a]	6.9 ± 0.5[b]	5.4 ± 0.8[a]
Dry season					
Aloum	0.61 ± 0.14[b]	10.0 ± 1.8[b]	62.0 ± 3.6[a]	8.7 ± 1.3[a]	6.6 ± 1.0[a]
Biba Yezoum	1.73 ± 0.57[b]	10.3 ± 1.4[b]	36.3 ± 3.2[a]	5.5 ± 0.4[a]	3.6 ± 0.5[a]
Tomba	0.69 ± 0.15[b]	8.9 ± 1.9[b]	46.2 ± 3.1[a]	8.7 ± 0.8[a]	6.5 ± 1.1[a]

Table S2.3. Seasonal mean (±SE, n = 4) water-filled pore space (WFPS), extractable mineral N (measured in the top 5 cm of soil) and nitrous oxide (N_2O) fluxes in cacao agroforestry sites located on highly weathered soils in the Congo Basin, Cameroon. Means followed by different lowercase letters indicate significant differences between seasons for each site (linear mixed-effect models with Tukey's HSD at $p \leq 0.05$).

Site/ season	Stem N_2O flux (µg N m^{-2} stem h^{-1})	Soil N_2O flux (µg N m^{-2} h^{-1})	WFPS (%)	Soil NH_4^+ (mg N kg^{-1})	Soil NO_3^- (mg N kg^{-1})
Wet season					
Aloum	1.21 ± 0.27[a]	22.6 ± 4.7[a]	60.3 ± 1.6[a]	4.3 ± 0.4[a]	2.1 ± 0.4[a]
Biba Yezoum	1.43 ± 0.36[a]	15.0 ± 3.5[a]	38.2 ± 1.7[a]	7.0 ± 0.6[a]	2.2 ± 0.4[a]
Tomba	1.05 ± 0.18[a]	21.2 ± 2.6[a]	53.4 ± 2.4[a]	7.3 ± 0.8[a]	2.5 ± 0.3[a]
Dry season					
Aloum	0.53 ± 0.07[b]	6.4 ± 0.7[b]	51.7 ± 1.9[b]	6.0 ± 1.0[a]	2.7 ± 0.6[a]
Biba Yezoum	0.74 ± 0.12[a]	5.3 ± 1.3[b]	25.9 ± 1.8[b]	7.5 ± 0.6[a]	3.2 ± 0.7[a]
Tomba	0.63 ± 0.06[a]	6.2 ± 1.2[b]	50.4 ± 6.2[a]	6.9 ± 0.9[a]	3.4 ± 0.7[a]

CHAPTER 3

TREE STEM AND SOIL METHANE AND CARBON DIOXIDE FLUXES FROM RAINFOREST AND CACAO AGROFOREST ON HIGHLY WEATHERED SOILS IN THE CONGO BASIN

Najeeb A. Iddris[1], Marife D. Corre[1], Oliver van Straaten[1,2], Rodine Tchiofo Lontsi[1], Edzo Veldkamp[1]

[1]Soil Science of Tropical and Subtropical Ecosystems, University of Goettingen, Goettingen, Germany

[2] Now at: Northwest German Forest Research Institute, Goettingen, Germany

3.1. Abstract

Despite increasing evidence from the last decade pointing to significant tree-stem CH_4 emissions, estimates of CH_4 budget from terrestrial ecosystems are still restricted to net fluxes from the soil surface only. The vast majority of tree greenhouse gas (GHG) emission studies have been conducted in tropical wetland forests, but it remains unknown whether trees in tropical lowland forests on heavily weathered soils, are substantial contributors to CH_4 emissions. Additionally, despite the availability of data on trace soil GHG fluxes from other parts of the world, very little is known about the effect of land-use change on trace GHG fluxes in natural African ecosystems. Here, we measured stem and soil CH_4 fluxes and soil CO_2 fluxes with forest conversion to cacao agroforestry in central and southern Cameroon. Stem and soil trace gas fluxes were measured monthly from May 2017 to April 2018. All the studied trees emitted measureable CH_4 at some point during the measurement period. The annual stem CH_4 emissions were 0.33 ± 0.06 kg C ha^{-1} yr^{-1} from the forest and 0.20 ± 0.03 kg C ha^{-1} yr^{-1} from cacao agroforestry, whereas the annual soil CH_4 uptake was -2.95 ± 0.40 kg C ha^{-1} yr^{-1} for the forest and -3.42 ± 0.44 kg C ha^{-1} yr^{-1} for the cacao agroforestry. Thus, the balance between the soil and stem CH_4 fluxes indicated that there was a net CH_4 sink in both land uses. Our upscaling suggested that tree emissions offset 5–18% and 3–14% of the soil CH_4 sink in the forest and cacao agroforestry, respectively. The annual soil CO_2 emissions were 10.1 ± 0.27 Mg C ha^{-1} yr^{-1} for the forest, and 10.3 ± 0.42 Mg C ha^{-1} yr^{-1} for the cacao agroforestry. Forest conversion to traditional, mature cacao agroforestry had no effect on stem and soil trace gas fluxes (p = 0.12 – 0.95). Overall, our results demonstrate that tropical trees on well drained, highly weathered soils represent potential CH_4 emission pathways that have largely been ignored, with stem CH_4 emissions constituting a considerable offset of the soil CH_4 sink.

Keywords: Africa, cacao agroforest, carbon dioxide, Congo Basin, land-use change, methane, soil respiration, trace greenhouse gases, tree stem emissions, tropical rainforest

3.2. Introduction

Carbon dioxide (CO_2) and methane (CH_4) constitute two of the most important trace greenhouse gases (GHG), with CH_4 particularly having 32–45 times the global warming potential of CO_2 by mass over a century (Neubauer & Megonigal, 2015). Forest CO_2 dynamics feature prominently in global carbon cycle studies, but the role of forests in the CH_4 cycle are relatively poorly understood. Although a considerable number of research have been undertaken to constrain the net balance of CH_4, the global CH_4 budget is still characterised by high uncertainty (Saunois *et al.*, 2016), especially for the tropics (Valentini *et al.*, 2014). The widely differing CH_4 estimates between bottom-up models and top-down approaches highlights the considerable uncertainty regarding the relative contributions of individual sources and sinks of CH_4 (IPCC, 2013). There is increasing evidence from the last decade pointing to significant tree stem CH_4 emissions from wetland and upland (well-drained) forests (Barba *et al.*, 2019a; Covey & Megonigal, 2019). Yet, estimates of CH_4 budget from terrestrial ecosystems are still restricted to net fluxes from soils only (Kirschke *et al.*, 2013; Saunois *et al.*, 2016).

The variation and magnitude of stem CH_4 emissions may depend on the tree species, age, site characteristics and environmental conditions (Barba *et al.*, 2019a; Covey & Megonigal, 2019). Stem emitted CH_4 could be produced within the heartwood of trees by methanogenic archaea populations (Covey *et al.*, 2012; Wang *et al.*, 2016, 2017; Pitz & Megonigal, 2017; Yip *et al.*, 2019), or could originate from soil-produced CH_4 under anoxic conditions by methanogens (Pitz & Megonigal, 2017; Barba *et al.*, 2019b; Welch *et al.*, 2019). The vast majority of tree GHG emission studies have been conducted in tropical wetland forests, where trees have adapted by developing specialist tissues such as lenticels and aerenchyma tissue to facilitate the transport of atmospheric oxygen to anoxic soil layers (Pangala *et al.*, 2014). These aerenchyma tissue have also been related to the transport of dissolved CH_4 from the soil through the tree, followed by diffusion through the stem surface to

the atmosphere largely through the lenticels (Pangala *et al.*, 2013, 2017). However, recent studies have observed tree stem CH_4 emissions in well-drained forests where soils predominantly acts as CH_4 sinks (Warner *et al.*, 2017; Pitz *et al.*, 2018; Barba *et al.*, 2019b; Welch *et al.*, 2019). Here, trees typically lack aerenchyma tissue, and hence, stem CH_4 emissions may originate from root uptake of dissolved CH_4 produced in deep anoxic soil layers or methanogenic microsites (von Fischer & Hedin, 2007; Brewer *et al.*, 2018).

Plant-mediated CH_4 fluxes have been found to be significant at the ecosystem level; tree stem fluxes accounted for 62–87% of the total ecosystem CH_4 flux in a tropical forested peatland in Panama (Pangala *et al.*, 2013), and accounts for half of the CH_4 emission in the Amazonian floodplain (Pangala *et al.*, 2017). Presently, most tropical forests grow on well-drained soils that tend to act as significant sinks for atmospheric CH_4 (Kiese *et al.*, 2003). Dutaur & Verchot (2007) estimated that about 28% of the global CH_4 sink occur in tropical soils. However, it is possible that even minor CH_4 emissions from tree stems could extrapolate to large fluxes globally, which may be significant enough to alter the sink strength of tropical forests, consequently influencing global CH_4 budgets. Indeed, previous studies have shown that emissions from tree stems could reduce the CH_4 sink of well-drained forests (Pitz & Megonigal, 2017). This emphasizes the need for further research on the sources, temporal and spatial patterns and magnitudes of tree stem CH_4 fluxes in tropical forests. To date, only one study has been published on tree stem CH_4 emissions from tropical forests on well-drained soils, and this was conducted in Panama. All tree stem bases in this study emitted significant CH_4, in contrast to soil CH_4 uptake and emissions in the dry and wet season, respectively. However, until now, the relative contribution of tree to total (soil + stem) CH_4 fluxes from lowland tropical forests on well-drained soils remains unknown.

Tropical soils are the largest natural source of atmospheric CO_2, contributing *ca.* 64% of the estimated global mean soil respiration of about 91 Pg C yr^{-1} (Hashimoto *et al.*, 2015).

Soil CO_2 efflux at the soil surface results from the combined activity of autotrophic (root) and heterotrophic (soil fauna and microbial communities) respiration processes (Luo & Zhou, 2006). Soil CO_2 fluxes are temporarily influenced by soil moisture and temperature (Werner *et al.*, 2007; Wanyama *et al.*, 2019). Spatial differences in soil CO_2 fluxes are driven by changes in soil physical and chemical properties (Raich & Schlesinger, 1998; Luo & Zhou, 2006), following land-use change most notably the texture of the soil due its strong effect on gas diffusivity within soils (Sotta *et al.*, 2006). Tropical forests also play a significant role in atmospheric CH_4 production and uptake (Keller & Matson, 1994), with well-drained soils constituting the largest biogenic sink of atmospheric CH_4 (Dutaur & Verchot, 2007). Soil CH_4 fluxes results from the simultaneous activities of methanogens (CH_4 producers) under anaerobic conditions and methanotrophs (CH_4 consumers) in aerobic soil conditions. For well-drained soils such as in our sites, CH_4 oxidation by methanotrophic bacteria exceeds CH_4 production, resulting in net CH_4 sink. Soil CH_4 fluxes are primarily controlled by soil moisture and soil texture, with the most important distal regulators been soil fertility and microbial activity (Veldkamp *et al.*, 2013; Hassler *et al.*, 2015). Presently, Africa remains the continent with the lowest numbers of published field studies on soil trace GHG fluxes from the tropical forest biome. Consequently, field studies covering sufficiently large spatial and temporal scales remains key to improving the sink-source estimates of these GHG for this important region (Valentini *et al.*, 2014; Kim *et al.*, 2016b).

Relatively well preserved, the high level of species endemism makes the Congo Basin an important repository of biodiversity and other ecosystem services, supporting the livelihood of 60 million people (de Wasseige *et al.*, 2014). However, conversion of natural forests to agricultural lands such as traditional cacao agroforestry is widespread in the Congo Basin countries, most notably in Cameroon (Sonwa *et al.*, 2007). These cacao farms are typically hand-planted under the shade of forests' remnant trees, and are extensively managed by

mechanical weeding and no fertilizer inputs. Nevertheless, changes in land use have been found to affect soil CO_2 and CH_4 fluxes due to changes in soil texture, soil bulk density, soil water content and management practices such as N-fertilization of agricultural lands (Veldkamp *et al.*, 2008, 2013; Hassler *et al.*, 2015). Despite the availability of data on trace soil GHG fluxes from other parts of the world, very little is known about the effect of land-use change on CH_4 and CO_2 fluxes in African ecosystems.

Given these knowledge gaps, our study provides the first spatially replicated quantification with a full year of measurements of stem and soil CH_4 fluxes and soil CO_2 fluxes in the Congo Basin, and contributes to the much-needed information on GHG budget from these important ecosystems. Our objectives were to (i) assess whether trees in tropical rainforests and CAF are important conduits of CH_4, (ii) quantify changes in soil-atmosphere fluxes of CH_4 and CO_2 fluxes with land-use change, and (iii) determine the temporal and spatial controls of stem and soil CH_4 and CO_2 fluxes. Stem and soil GHG fluxes were measured in the reference forest and the converted CAF at monthly intervals from May 2017 to April 2018. We hypothesized that (i) stem and soil CH_4 and CO_2 fluxes from extensively managed CAF systems (unfertilized and manual harvest) will be comparable to the natural forests, and (ii) trees in tropical forests and cacao trees in cacao agroforestry will emit CH_4 from stems.

3.3. Materials and methods

3.3.1. Study area and experimental design

Stem and soil GHG fluxes were measured at three study sites (Tomba, Biba Yezoum and Aloum; Fig. 1.1) located in south and central regions of Cameroon, where conversion of forest to smallholder cacao agroforests is widespread (Sonwa *et al.*, 2007). The study sites have a mean annual temperature of 23.5 °C (Table S1.1). Rainfall is bimodal, with rainy seasons from March to June and from September to November (Fig. 1.2). The geological substrate of the

study sites are underlain by Precambrian basement rocks made up of metamorphic schists, phyllites, quartzites and gneiss (Gwanfogbe *et al.*, 1983). The soils are heavily weathered and classified as Ferralsols (IUSS Working Group WRB, 2015).

We investigated two land-use types in each site: the reference forest and the converted CAF system. The cacao farms typically occurred in continuous clusters surrounded by mosaics of secondary forest and cash crop farms, and were hand-planted under the shade of a few remnant forest trees that were selectively retained by farmers during forest clearing. On average, each cacao agroforest had a size of about 0.95 ha (with a range of 0.3–2.5 ha). Interviews with farmers revealed that localised weeding was done using hand tools, and none of the studied cacao farms have been fertilised. A more detailed description of the study sites and experimental design is reported in Chapter 2.3.

For each of the two land-use types per site, we selected four replicate plots; each replicate plot was 50 m × 50 m with a minimum distance of 100 m between plots (Fig. 1.1). Within each plot, we identified and measured the diameter at breast height (DBH) and height of all stems including cacao trees with a DBH ≥ 10 cm. Stem and soil trace gas fluxes and all associated measurements were then conducted within a 40 m × 40 m core zone in each plot in order to minimise edge effects.

We assessed the effects of land-use change on stem and soil trace GHG fluxes by first testing the implicit assumption that the initial conditions between the forest and cacao agroforests were similar prior to forest conversion. To do this, we compared the clay contents in 30-50 cm depth between the forest and cacao agroforests within each site. There was no difference in clay contents between the two land uses in each site (Table 2.1), which suggest that both land uses in each site had comparable initial soil conditions prior to conversion. Therefore, any measured differences in GHG fluxes can be attributed solely to land-use change.

Such as described in Chapter 2.3 above, we measured stem CH_4 fluxes by selecting six cacao trees per replicate plot in the cacao agroforests, and six trees of the most dominant species within each replicate plot in the forest, based on their importance value index (IVI) (Table S1.1). For each of the six selected trees per plot, we measured stem CH_4 fluxes at 1.3 m height above the ground at monthly interval from May 2017 to April 2018. We also sampled stem fluxes at different heights along the stems of 16 individual trees per land use type in May 2018, in order to assess the influence of tree height on stem CH_4 fluxes. Stem chambers were installed at three different heights (1.3 m, 2.6 m and 3.9 m above the ground) per tree in the forest, and at two different heights (1.3 m and 2.6 m above the ground) per tree in the cacao agroforests due to the limited height of the cacao trees.

Within each plot, we also installed four permanent chamber bases, which were randomly distributed within the 40 x 40 m core zone to measure CH_4 and CO_2 fluxes from the soil surface. Additionally, we permanently installed a stainless steel soil gas sampler (1-mm internal diameter) located ~1 m from the measured trees in each replicate plot to measure soil-air CH_4 concentrations at 50 cm below the soil surface. Stem and soil CH_4 fluxes and soil CO_2 fluxes were measured monthly from May 2017 to April 2018, together with meteorological and soil variables.

3.3.2. Measurement of stem and soil CH_4 fluxes and soil CO_2 fluxes

Stem CH_4 fluxes were measured using stem chambers made of polyethylene-terephthalate foil (same method as described in Chapter 2.3.2). One month prior to the measurement, we prepared trees for stem chamber installation by applying permanent strips of silicone (Otto Seal ® S110, Hermann Otto GmbH, Fridolfing, Germany) around the stem of each tree, 20 cm apart, with the center of the two strips at a height between 1.2 m and 1.4 m from the ground. The installation of these stem chambers is quick, hence, new chambers were installed to the stems on every

measurement date rather than installing them permanently. The permanent silicone strips were used as a mark to ensure that the same 20-cm length stem section was sampled. A Luer-lock sampling port was fixed onto the foil, which was then wrapped around the tree stem on top of the silicone strips, and the vertical ends of the foil taped together to form a chamber around the stem. The foil was then shrunk to fit closely onto the silicone strips using a heat gun, after which we attached polyethylene foam over the foil above and below the silicone strips. The foams were tightly adjusted over the foil and silicone with lashing straps using ratchet tensioners to ensure an airtight fitting. Using a syringe fitted with a Luer-lock one-way check valve, we completely evacuated the headspace inside the stem chamber, and replaced it with a known volume of ambient air using a manual bicycle pump, to allow for stem CH_4 flux calculations. Immediately after stem chamber refilling and closure, a 25-mL gas sample was taken with syringe and stored with overpressure in pre-evacuated 12-mL exetainers (Labco Limited, Lampeter, UK) with rubber septa, and then gain after 20, 40 and 60 minutes.

Soil CH_4 and CO_2 fluxes were measured simultaneously with stem CH_4 fluxes using vented, static chambers (made of polyvinyl chloride pipe with 0.04 m^2-area and ~0.02-m insertion into the soil) that were permanently installed in the soil one month prior to the start of trace gas measurements. The chamber bases were covered with vented, static polyethylene hoods (resulting in 11 L total headspace volume) on each sampling day. From each chamber, samples of the enclosed headspace were taken four times over a 32-minute closure period: t_0 at 2 minutes after closure and three samples thereafter at 10-minute interval. The gas samples were taken using a 25-mL syringe and immediately stored with overpressure in pre-evacuated 12-mL exetainers.

On the same day, soil-air CH_4 concentrations were sampled from the permanently installed stainless steel sampling probes by fitting the probes with Luer-lock sampling ports. Using a syringe, we first removed and discarded the top 5-mL of air to clear the probes of

"dead" air volume, after which we took 25 mL gas samples and stored them in 12 mL pre-evacuated exetainers. The exetainers we used have been confirmed to be leak-proof in our previous studies (e.g. Hassler *et al.*, 2015; Matson *et al.*, 2017; van Straaten *et al.*, 2019). All the gas samples were brought to the University of Goettingen, Germany for analysis.

3.3.3. Trace greenhouse gas analysis and flux rate calculation

Gas samples were analysed for CH_4 and CO_2 concentrations using a gas chromatograph (SRI 8610C, SRI Instruments Europe GmbH, Bad Honnef, Germany), equipped with a flame ionization detector (FID), an electron capture detector (ECD) and an autosampler (AS-210, SRI Instruments). Before analysing the gas samples, the gas chromatograph was calibrated with three calibration gases (Deuste Steininger GmbH, Mühlhausen, Germany), taking into the consideration the concentration ranges of our field samples. Stem and soil CH_4 and soil CO_2 fluxes were calculated from the linear change in headspace concentrations over time of chamber closure, and corrected with air temperature and atmospheric pressure measured in each replicate plot at the time of sampling. Individual chamber measurements were quality checked using the linear increase in CO_2 concentration with time. In a small number of cases where CO_2 concentration curved at a particular point, we excluded the data point and calculated the fluxes based on the remaining three sampling data points that showed a linearity of increase in CO_2 concentrations with time ($R^2 > 0.9$). Nonetheless, our data analyses included zero and negative fluxes in order to avoid overestimation of stem and soil fluxes.

We calculated annual stem and soil CH_4 and CO_2 fluxes following the same extrapolation method described in Chapter 2.3.3 (pg. 32) above. The annual CH_4 fluxes from each replicate plot, expressed on a hectare basis, were represented by the sum of the stem and soil CH_4 fluxes.

3.3.4. Soil and climatic variables

Concurrent with stem and soil trace gas flux measurements, we determined soil and air temperature, moisture and mineral N concentrations from the top 0.05 m of soil near the chamber bases on each sampling day. Soil and air temperature were measured close to the stem and soil chambers using a digital thermometer (GTH 175, Greisinger electronic GmbH, Regenstauf, Germany). Soil moisture and mineral N concentrations were determined from a composite sample that were pooled from four sampling locations ~1 m away from the chamber bases per replicate plot on each sampling day. Some of the soil subsample were oven-dried at 105 °C for 24 h to determine the gravimetric moisture content, and then converted to soil water-filled pore space (WFPS) using the mineral soil particle density of 2.65 g cm^{-3} and the average soil bulk densities from each plot (Table 2.1). Soil mineral N concentrations were extracted in-situ in the field by adding some of the composite soil samples to already prepared bottles filled with 150 mL solution of 0.5 M K_2SO_4 and shaken thoroughly. The mineral N samples were shaken for an hour and filtered through K_2SO_4 pre-washed filter papers upon arrival at the field station. The filtered extracts were then stored in 20 mL scintillation vials and immediately frozen for transport by air to the University of Goettingen, Germany, where they were analysed for NH_4^+ and NO_3^- concentrations using continuous flow injection colorimetry (SEAL Analytical AA3, SEAL Analytical GmbH, Norderstedt, Germany).

We also recorded the relative humidity, air temperature and solar irradiance of each plot over the course of each sampling day using a portable weather station. From this data, we calculated vapour pressure deficit (VPD) using measured air temperature and relative humidity (Allen *et al.*, 1998).

In April 2017, we conducted a one-time soil sampling at three depth intervals down to 50 cm to determine the soil biochemical characteristics. To capture the spatial variability in

each replicate plot, soil samples were collected from ten randomly selected sampling points per plot from the top 0-0.1 m depth, and five samples each from 0.1–0.3 and 0.3–0.5 m depths. In total, 480 soil samples were collected from the 24 plots (three sites × two land uses × four replicate plots × 20 soil samples per plot). The soil samples were air-dried, 2-mm sieved, transported by air to Germany and dried again at 40 °C prior to analysis in the laboratory. Soil pH was analysed from a 1:2.5 soil-to-distilled water ratio. We determined the soil texture of each replicate plot using the pipette method after iron oxide and organic matter removal (Kroetsch & Wang, 2008). The effective cation exchange capacity (ECEC) was determined from the soil samples by percolating with unbuffered 1M NH4Cl and measuring the exchangeable element concentrations (Al, Ca, Fe, K, Mg, Mn, and Na) in the percolates using an inductively coupled plasma-atomic emission spectrometer (ICP-AES; iCAP 6300 Duo VIEW ICP Spectrometer, Thermo Fischer Scientific GmbH, Dreieich, Germany). Base and Al saturations were calculated, respectively, as the percent exchangeable bases (Mg, Ca, K and Na) and Al of the ECEC. Grounded soil and litter samples were used to analyse for total N, total organic C (using a CN analyser; Vario EL Cube; Elementar Analysis Systems GmbH, Hanau, Germany) and ^{15}N natural abundance signatures (using isotope ratio mass spectrometry; Delta Plus; Finnigan MAT, Bremen, Germany). Soil organic C and total N of the cacao agroforestry were calculated using the bulk densities of the forest, to avoid overestimation of stocks resulting from increases in bulk densities due to land-use change.

3.3.5. Statistical analysis

Statistical tests of stem and soil CH_4 and CO_2 gas fluxes were based on the average of the six trees and of the four chambers that represent each replicate plot on a given sampling day. We first checked the data for normality using Shapiro-Wilk's test, and those exhibiting non-normal distributions were log- or square root-transformed. Linear mixed-effect models (LMEs) were used to assess the differences in stem and soil CH_4 and CO_2 fluxes and accompanying soil

factors (soil temperature, WFPS and mineral N concentrations) between the two land-use types for each site, and among the three sites for each land-use type. When applying LMEs, land-use (when comparing land-use types within each site) and site (when comparing sites for each land-use type) were used as fixed effects in the model, and replicate plots and sampling days as random effects. Differences between the land uses per site or among sites per land-use type were assessed using analysis of variance (ANOVA) with Fisher's least significant difference (LSD) test.

Additionally, stem CH_4 emissions were tested for differences among tree species across the four forest plots at each site as well as across the three sites for the forest land use using LME. Here, tree species were used as fixed effect in the model, and the random effects were trees belonging to each species and sampling days.

We used one-way ANOVA with Fisher's LSD test to test for differences in soil biochemical and litter characteristics between the two land uses at each site, and among sites for each land-use type, when the parameter exhibits a normal distribution and homogenous variance. Kruskal-Wallis ANOVA with multiple comparison extension test was applied when assumptions of normality and variance homogeneity were not met.

Using the means of the four replicate plots for each land-use type per site, we determined the temporal controls of stem and soil trace gas fluxes by testing their correlations with the soil controlling factors using the Spearman correlation test. The correlations were conducted separately for each land-use type across the three sites and sampling days (n = 33 (3 sites × 11 monthly measurements)). Spatial controls of stem and soil annual trace gas fluxes were determined by assessing their relationship with soil biochemical characteristics, conducted across land uses and sites (n = 24 (3 sites × 2 land uses × 4 replicate plots)). The statistical

significance for all the tests were set at $p \leq 0.05$. Data analysis were performed using the R (version 3.5.2) open source software (R Core Team, 2018).

3.4. Results

3.4.1. Stem CH_4 fluxes

All the studied trees emitted measureable CH_4 at some point during the measurement period (Fig. 3.1). Stem CH_4 fluxes neither differed between the forest and cacao agroforestry at each site (p = 0.12–0.71; Table 3.1), nor among the three sites for each land use type (p = 0.24–0.43; Table 3.1).

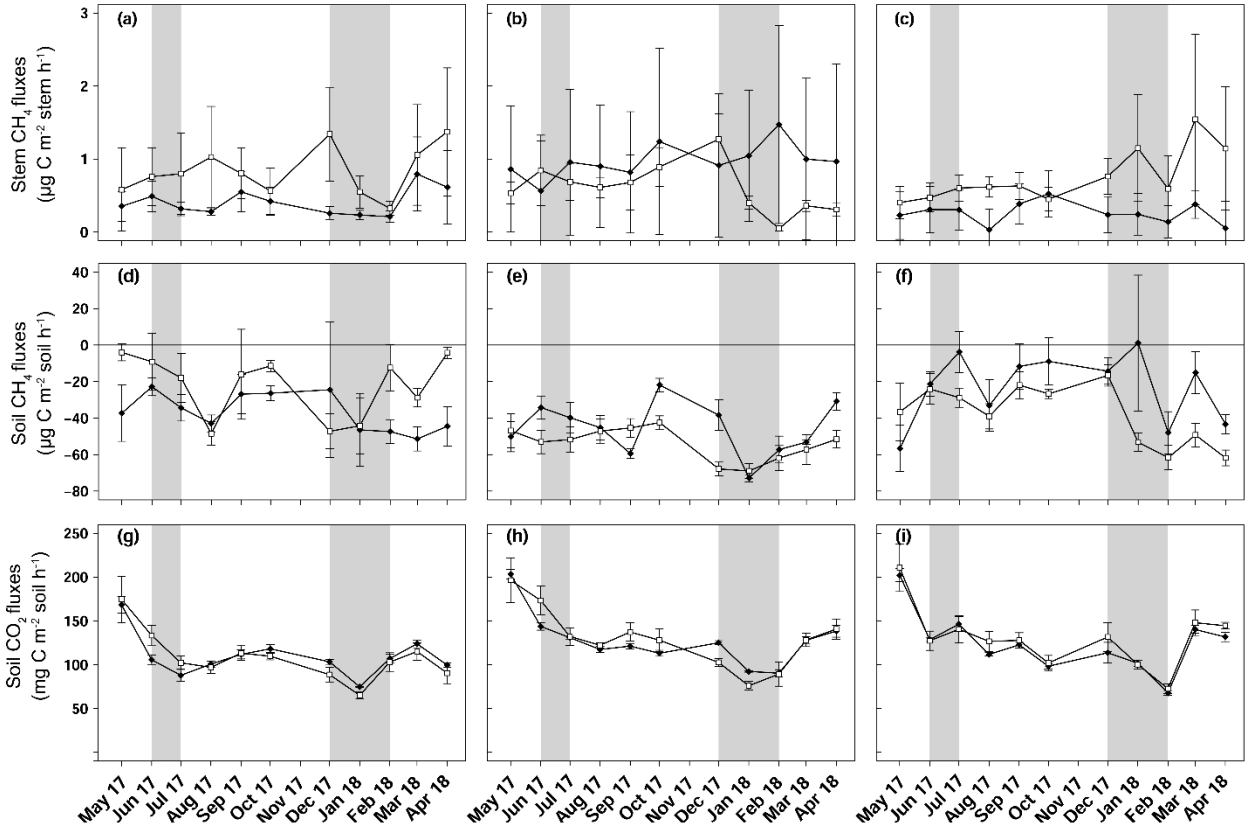

Figure 3.1. Mean (±SE, n = 4) stem CH_4 fluxes (top panel), soil CH_4 fluxes (middle panel) and soil CO_2 fluxes in Aloum site (a, d and g), Biba Yezoum site (b, e and h) and Tomba site (c, f and i) on highly weathered soils in the Congo Basin, Cameroon, measured monthly from May 2017 to April 2018. Forest (♦) and cacao agroforestry (□); grey shadings mark the dry season.

We did not also detect any differences in stem CH_4 fluxes between the wet season and the dry season for both land uses (p = 0.55–0.80; Fig. 3.1, Tables S3.1 and S3.2). Additionally, stem CH_4 emissions did not vary among tree species in forest plots at each site as well as across the three sites (p = 0.13–0.83; Fig. 3.2), nor among tree diameter sizes (p = 0.38–0.51).

Table 3.1. Mean (±SE, n = 4) stem CH_4 emission, soil CH_4 uptake and soil CO_2 emissions from forest and cacao agroforestry system within each site in the Congo Basin, Cameroon. Means followed by different lowercase letters indicate significant differences between land-use types within each site and different capital letters indicate significant differences among the three sites within a land-use type (linear mixed-effect models with Tukey's HSD at $p \leq 0.05$).

Site/Land-use type	Stem CH_4 fluxes (µg C m^{-2} stem h^{-1})	Soil CH_4 fluxes (µg C m^{-2} h^{-1})	Soil CO_2 fluxes (mg C m^{-2} h^{-1})
Aloum			
Forest	$0.41 \pm 0.07^{a,A}$	$-36.8 \pm 4.2^{a,A}$	$109.1 \pm 4.1^{a,A}$
Cacao	$0.83 \pm 0.15^{a,A}$	$-22.2 \pm 4.1^{a,B}$	$108.3 \pm 5.1^{a,A}$
Biba Yezoum			
Forest	$0.98 \pm 0.28^{a,A}$	$-45.8 \pm 2.7^{a,A}$	$127.5 \pm 4.7^{a,A}$
Cacao	$0.60 \pm 0.08^{a,A}$	$-54.0 \pm 2.1^{a,A}$	$129.6 \pm 6.1^{a,A}$
Tomba			
Forest	$0.26 \pm 0.08^{a,A}$	$-23.2 \pm 4.9^{a,A}$	$124.0 \pm 5.6^{a,A}$
Cacao	$0.76 \pm 0.15^{a,A}$	$-38.2 \pm 3.1^{a,AB}$	$130.1 \pm 6.2^{a,A}$

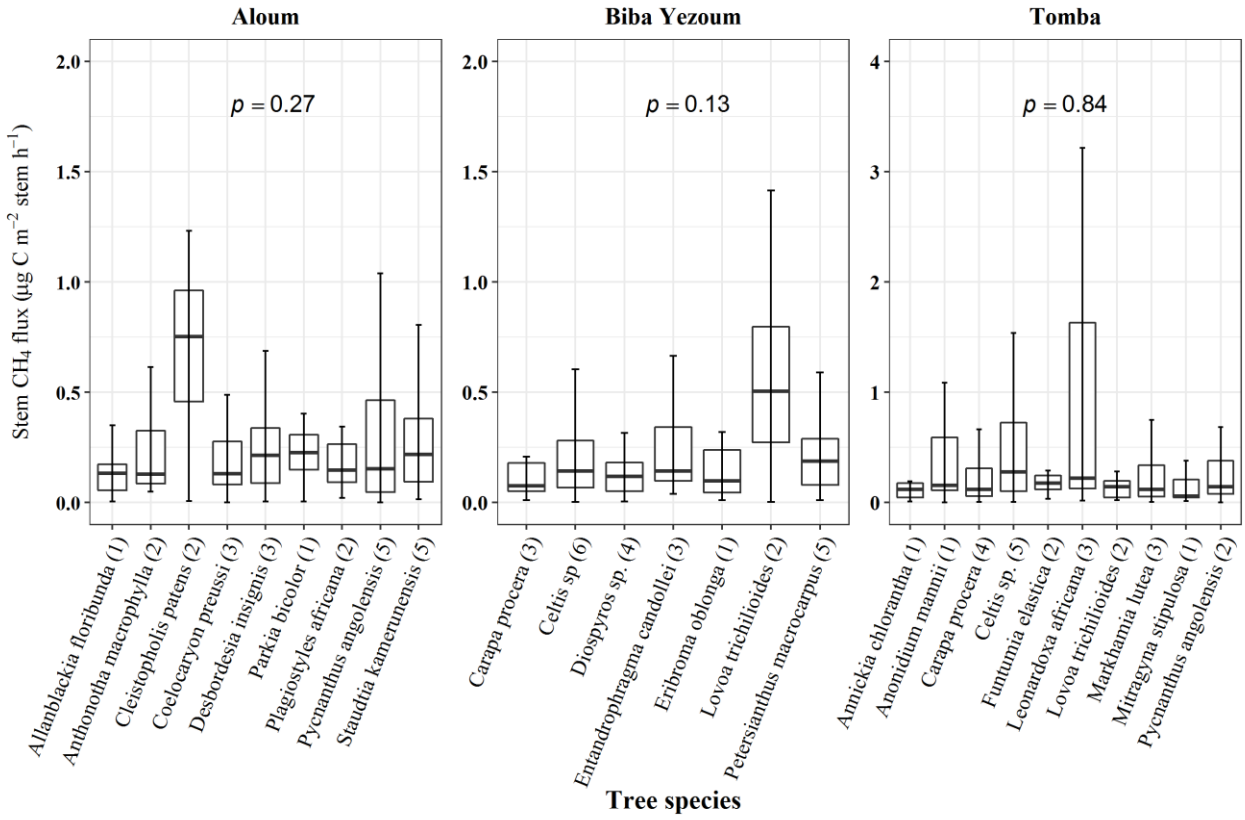

Figure 3.2. Stem CH_4 fluxes from 22 tree species at three forest sites (Aloum, Biba Yezoum and Tomba) across central and south Cameroon in the Congo Basin. Boxes (25th, median and 75th percentile) and whiskers (1.5 × interquartile range) are based on CH_4 fluxes measured monthly from May 2017 to April 2018 for each tree species, and the values in parentheses represent the number of trees measured per species. There were no differences in CH_4 fluxes among species (linear mixed-effect models with Tukey's HSD at $p > 0.13$).

Stem CH_4 emissions decreased with increasing stem height in both land uses (Fig. 3.3). Using the upscaling method described in the Materials and Methods section (see Chapter 2.3.3 above), the mean annual stem CH_4 fluxes were 0.33 ± 0.06 kg C ha^{-1} yr^{-1} for the forest and 0.20 ± 0.03 kg C ha^{-1} yr^{-1} for the cacao agroforestry, when including the shade trees in the cacao plots. This was equivalent to *ca.* 5–18% and 3–14% of the amount of CH_4 consumed by the soils in the forest and cacao agroforestry, respectively.

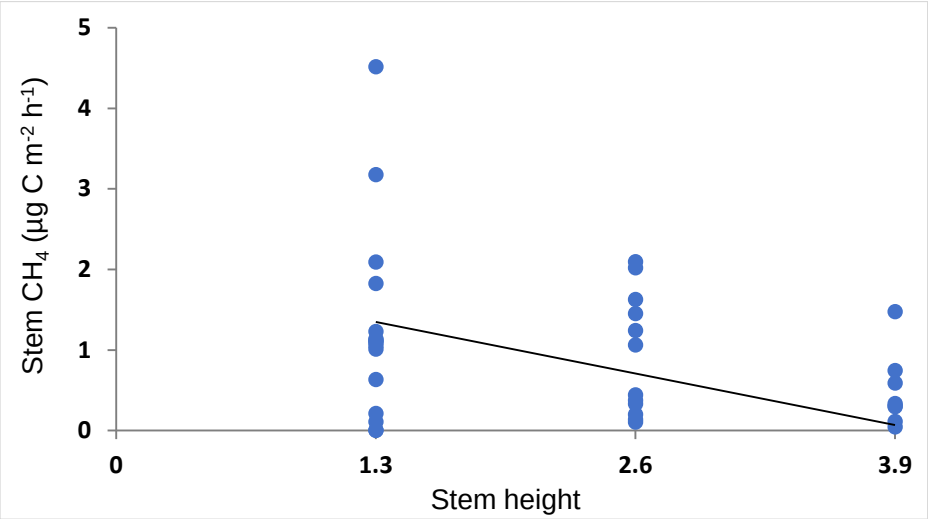

Figure 3.3. Mean (*n* = 16) stem CH_4 emissions at three different heights along the tree stem on highly weathered soils in the Congo Basin, Cameroon.

Table 3.2. Annual trace gas fluxes (mean ± SE, *n* = 4) from lowland rainforest and cacao agroforestry system within each site on highly weathered soils in the Congo Basin, Cameroon. Annual fluxes were not statistically tested for differences among sites or between land-use types since these annual values are trapezoidal extrapolations.

Site/ Land-use type	Annual stem CH_4 fluxes (Kg C ha^{-1} yr^{-1})	Annual soil CH_4 fluxes (Kg C ha^{-1} yr^{-1})	Total (soil + stem) CH_4 flu (Kg C ha^{-1} yr^{-1})	Annual soil CO_2 fluxes (Mg C ha^{-1} yr^{-1})
Aloum				
Forest	0.56 ± 0.02	−3.16 ± 0.52	−2.60 ± 0.59	10.24 ± 0.45
Cacao	0.31 ± 0.05 (0.04 ± 0.01)	−2.16 ± 0.72	−1.85 ± 0.80	10.78 ± 0.65
Biba Yezoum				
Forest	0.35 ± 0.02	−3.98 ± 0.27	−3.62 ± 0.33	10.71 ± 0.16
Cacao	0.17 ± 0.02 (0.02 ± 0.00)	−4.80 ± 0.34	−4.62 ± 0.40	10.87 ± 0.56
Tomba				
Forest	0.08 ± 0.00	−1.72 ± 0.55	−1.64 ± 0.64	9.30 ± 0.42
Cacao	0.10 ± 0.01 (0.06 ± 0.02)	−3.31 ± 0.33	−3.20 ± 0.39	9.13 ± 0.58

Note. Annual stem and soil CH_4 and CO_2 fluxes were not statistically tested for differences among sites or between land-use types since these annual values are trapezoidal extrapolations (see section 2.3.3). Annual stem CH_4 emissions in parentheses are from cacao trees only.

Three individual trees from the forest and one tree from the cacao agroforestry consistently emitted high CH_4 throughout the study period, with stem CH_4 fluxes ranging from 4.9 to 154.8 µg C m^{-2} h^{-1} in the forest and 6.1 to 68.6 µg C m^{-2} h^{-1} in the cacao agroforestry (Fig. 3.4). When including these trees in the annual flux calculations, the balance between the soil and stem CH_4 fluxes indicated that the replicate plots containing these high emitting trees could be net CH_4 sources.

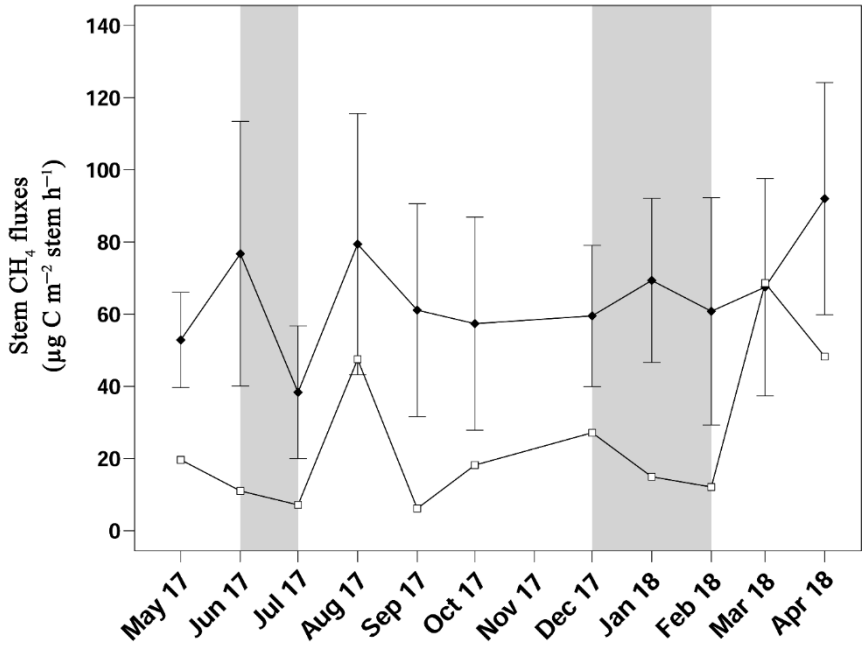

Figure 3.4. Mean stem CH_4 emissions on highly weathered soils in the Congo Basin, Cameroon, measured monthly from May 2017 to April 2018. Stem values are average of three trees for the forest (♦) and one tree for the cacao agroforestry (□); grey shadings mark the dry season.

Across the study period, average stem CH_4 emissions from the forest were positively correlated with average WFPS (Spearman ρ = 0.37, $p < 0.05$, n = 33), while in the cacao agroforestry, we found positive correlations of stem CH_4 emissions with soil-air CH_4 concentration across sampling dates (ρ = 0.35, $p < 0.05$, n = 33).

3.4.2. Soil CH_4 fluxes

Soil CH_4 fluxes were comparable between the forest and cacao agroforestry at each site (p = 0.20–0.89; Table 3.1). In the cacao agroforestry, soil CH_4 uptake was higher at Biba Yezoum than at the Aloum site ($p < 0.01$; Table 3.1), but, in the forest, Soil CH_4 fluxes did not differ across sites ($p = 0.32$; Table 3.1). When compared between seasons, soil CH_4 uptake was higher in the dry season than in the wet season at Aloum for the cacao agroforestry system ($p = 0.05$; Fig. 3.1, Table S2). However, we did not detect any seasonal differences among the study sites in the forest (p = 0.14–0.92; Fig. 3.1, Table S3). The mean annual soil CH_4 uptake was -2.95 ± 0.38 kg C ha^{-1} yr^{-1} for the forest and -3.42 ± 0.42 kg C ha^{-1} yr^{-1} for the cacao agroforestry. Thus, the balance between the soil and stem CH_4 fluxes indicated that there was a net CH_4 sink in both land uses (Table 3.2).

Over the measurement period, moisture was the dominant controlling factor of soil CH_4 fluxes, with average monthly soil CH_4 fluxes correlating positively with WFPS in both land uses (ρ = 0.45–0.86, $p < 0.01$, $n = 33$). Additionally, soil CH_4 fluxes from the cacao agroforestry were positively correlated with average soil-air CH_4 concentrations ($\rho = 0.36$, $p < 0.05$, $n = 33$), and negatively correlated with soil NH_4^+ content ($\rho = 0.42$, $p < 0.05$, $n = 33$).

Of the soil physical and biochemical characteristics measured once, annual soil CH_4 fluxes were correlated positively with clay contents (Spearman $\rho = 0.50$, $p < 0.05$, $n = 24$) and aluminium saturation ($\rho = 0.45$, $p < 0.05$, $n = 24$).

3.4.3. Soil CO_2 fluxes

We did not detect any differences in soil CO_2 emissions between the forest and cacao agroforestry at each site (p = 0.60–0.95; Table 3.1), nor among the three study sites for each land use type (p = 0.14–0.19; Table 3.1). In both land uses, average soil CO_2 emissions were highest at the beginning of our measurement period, which coincided with the end of the rainy

season (Fig. 3.1). This was followed by a gradual decline in soil CO_2 efflux as the soil moisture levels decreased during the dry season. The beginning of the wet season in August stimulated soil CO_2 emissions again, but further reductions in soil moisture during the second dry season resulted in decreasing soil CO_2 emissions (Fig. 3.1). Accordingly, average soil CO_2 emissions showed clear seasonal variability with larger fluxes in the wet season than the dry season for both land uses ($p < 0.05$; Fig. 3.1, Tables S3.1 and S3.2). The mean annual soil CO_2 emissions were 10.1 ± 0.27 Mg C ha^{-1} yr^{-1} for the forest, and 10.3 ± 0.42 Mg C ha^{-1} yr^{-1} for the cacao agroforestry.

While soil CO_2 fluxes did not correlate with soil WFPS, we did find a parabolic relationship between soil CO_2 emissions and soil moisture in both land uses. Across sites and land uses, the only significant correlation between annual soil CO_2 emissions and soil physical and biochemical characteristics was with sand content ($\rho = 0.45$, $p < 0.05$, $n = 24$).

3.5. Discussion

3.5.1. Stem CH_4 emissions and their contribution to total (soil + stem) CH_4 emissions

To our knowledge, this study provides the first year-round simultaneous measurements of stem and soil CH_4 fluxes from tropical Africa. The mean stem CH_4 emissions we measured from our sites (Table 3.1) were in the lower range of those reported for temperate and boreal upland (well-drained) forests (0.004–22.6 µg C m^{-2} $h^{-1;}$ (Machacova *et al.*, 2016; Wang *et al.*, 2016; Warner *et al.*, 2017; Maier *et al.*, 2018; Pitz *et al.*, 2018; Barba *et al.*, 2019b; Welch *et al.*, 2019). Our stem CH_4 emissions were also significantly lower than those reported for wetland and floodplain ecosystems (42.6–427.0 µg C m^{-2} h^{-1}; Gauci *et al.*, 2010; Pangala *et al.*, 2013, 2015; Terazawa *et al.*, 2015; Pitz *et al.*, 2018). The high stem CH_4 emissions in wetlands and floodplains may be characteristic of the equally high soil CH_4 concentrations resulting from the dominance of methanogenic activity in the soils of these ecosystems (Terazawa *et al.*, 2007),

which have been found to be predominantly emitted via plants (Terazawa *et al.*, 2007; Pangala *et al.*, 2013). Additionally, our mean stem emission values were 11-fold lower than the mean stem CH_4 emissions reported for *Heisteria concinna* (75.9 µg C m^{-2} h^{-1}) and *Simarouba amara* (65.9 µg C m^{-2} h^{-1}) tree species in a moist tropical forest in Panama (Welch *et al.*, 2019). It is more likely that the results of Welch *et al.* (2019) may have calculation errors, especially because they were not able to measure any significant stem CH_4 emissions during the dry season. Additionally, they consistently measured higher tree-stem than soil fluxes, which has not been shown in any upland study elsewhere. Nevertheless, the wide range of stem emissions reported in the literature signifies the substantial spatial and temporal variability in stem CH_4 fluxes, and highlights the complexity in accounting for stem emissions in global GHG budgets.

More recent evidence suggest that differences in tree diameter sizes, age or species (Pangala *et al.*, 2015; Wang *et al.*, 2016; Warner *et al.*, 2017; Pitz *et al.*, 2018; Welch *et al.*, 2019) can significantly influence tree stem emissions, although we could not corroborate these findings in our study (Fig. 3.2). This may possibly be due to the small diameter range of our measured trees (10–18 cm DBH for cacao trees and 10–30 cm DBH for the forest trees). Indeed, Pitz *et al.* (2018) found a positive correlation between stem CH_4 emissions and tree diameter (tree DBH range from 16 to 93 cm). Compared to young, small trees, older and bigger trees are suggested to emit higher CH_4 owing to their large, deep tap root system which can tap deep into anoxic soil layers (Pierret *et al.*, 2016; Barba *et al.*, 2019a) or groundwater, which are both potential CH_4 sources. It is possible that the different tree species in our study sites utilise similar CH_4 transport mechanisms, which may have accounted for the lack of differences in stem emissions among species (Fig. 3.2) and between land uses, supporting our first hypothesis (Table 1). Recent literature reviews (Barba *et al.*, 2019a; Covey & Megonigal, 2019) show that tree physiology and traits of wood anatomy can influence species-level CH_4 emissions; however, these have mostly been demonstrated for wetland species. For example, wood specific

density and lenticel density have been shown to affect wetland tree stem CH_4 fluxes (Pangala *et al.*, 2013, 2014). Evapotranspiration rate and wood density were possibly the reason for the higher stem CH_4 emissions of the fast-growing *Simarouba* compared to the shade-tolerant *Heisteria* species in an upland tropical forest in Panama (Welch *et al.*, 2019). However, there is still limited knowledge about how tree species traits contribute to stem flux differences in tropical trees. New studies that measures several trees of different species, diameter classes and ages could further our understanding of the spatial variability of stem emissions from ecosystem to regional and global levels.

Our results demonstrate that tropical trees on well drained soils represent potential CH_4 emission pathways that have largely been ignored, with stem CH_4 emissions constituting a considerable offset of the soil CH_4 sink in both forest and cacao agroforestry, supporting our second hypothesis (Table 2). This finding is particularly important considering that trees occupy less than 10% ground area in the study plots. Tree stem emissions were found to offset 5–18% of the soil sink in our forest sites, which brackets the range of estimates reported for two upland forests in America (16%; Pitz & Megonigal, 2017; Warner *et al.*, 2017). These estimates are lower than those reported for wetland forests, where tree-mediated CH_4 emissions were found to account for 20–87% of the total (soil + stem) CH_4 efflux (Gauci *et al.*, 2010; Pangala *et al.*, 2013), but higher than the estimates from two upland forests where tree stem emissions equated to less than 1% of the soil sink (Machacova *et al.*, 2016; Plain *et al.*, 2019). The degree to which tree-mediated emissions may offset soil CH_4 sinks, especially in well-drained tropical soils, remains highly uncertain, as evidenced by the wide range of stem emission estimates in the literature. Nevertheless, the consistent measurement of positive net stem CH_4 emissions in our study sites suggests that stem CH_4 emissions could be widespread in lowland tropical forests, and illustrates the need for further investigation.

Our study provides evidence that tropical trees on well-drained soils can also emit CH_4 (Fig. 3.2). However, the origin of stem emitted CH_4 is a subject of ongoing debate, with studies suggesting microbial production of CH_4 in the heartwood (Covey *et al.*, 2012; Wang *et al.*, 2016; Yip *et al.*, 2019), soil-derived CH_4 from low depths (Machacova *et al.*, 2016; Pitz & Megonigal, 2017; Barba *et al.*, 2019b), and to a lesser extent from cryptogamic covers on stem bark (Lenhart *et al.*, 2015). Nonetheless, our findings of decreasing stem CH_4 emissions with height suggest a potential belowground (soil) origin, which concurs with the findings of other studies (Pangala *et al.*, 2013; Pitz & Megonigal, 2017; Barba *et al.*, 2019b). Despite the soils acting as net CH_4 sinks, it is possible that soil processes occurring in deeper soil depth could regulate the source of stem emitted CH_4. Indeed, studies have found soils to produce CH_4 at depth while acting as net sinks at the surface level (Maier *et al.*, 2018). We found positive correlations of stem CH_4 emissions with soil moisture, and with soil-air CH_4 concentrations at 50 cm depth, which is consistent with root uptake of soil water containing dissolved CH_4 produced in deep anoxic layers or methanogenic microsites (von Fischer & Hedin, 2007; Brewer *et al.*, 2018). This active transport of dissolved CH_4 is likely driven by sap flow via transpiration streams of the trees, and then emitted to the atmosphere through the stem surfaces, bypassing the soil methanotrophic layers (Megonigal & Guenther, 2008).

Our findings of decreasing stem emissions with height (Fig. 3.3) are consistent with previous results found in wetland and upland forests (Pangala *et al.*, 2013, 2017; Wang *et al.*, 2016; Pitz & Megonigal, 2017; Barba *et al.*, 2019b). As many of the studied trees had buttresses (e.g. Fig. 2.2), we measured stem emissions at trunk heights of 1.3 m above the ground, leaving an open question about lower stem CH_4 emission rates. Mean CH_4 emission of trees growing in a floodplain forest were found to decrease from 132 to 73 μg C m^{-2} h^{-1} when measuring at trunk heights of 15 and 70 cm, respectively (Terazawa *et al.*, 2007). Similarly, CH_4 emission of temperate upland trees were 19.9 μg C m^{-2} h^{-1} at a trunk height of 75 cm, reducing to 12.1

µg C m^{-2} h^{-1} at an upper stem height of 150 cm (Barba *et al.*, 2019b). The pattern is similar in tropical wetland forests, where stem emissions were found to range from 139 to 13 µg C m^{-2} h^{-1} at stem heights of 20–50, 60–90 and 100–130 cm (Pangala *et al.*, 2013). These findings, coupled with our measurements of decreasing stem emissions with height suggest that there may be high emissions occurring at lower tree height, and possibly an underestimation of tree stem emissions from this important tropical region. Further research efforts are necessary to provide additional insights into mechanisms of stem CH_4 production and magnitudes, in order to improve regional and global CH_4 budget estimations.

3.5.2. Factors controlling temporal and spatial variability of soil fluxes

Studies have shown that soil moisture is the dominant factor controlling the seasonal variation in soil CH_4 and CO_2 fluxes in tropical systems (Verchot *et al.*, 2000; Veldkamp *et al.*, 2013; Matson *et al.*, 2017; Wanyama *et al.*, 2019). Indeed, we found a positive correlation between soil CH_4 fluxes and WFPS in both land uses, which is consistent with diffusional limitation of atmospheric CH_4 into the soil at high soil moisture conditions (Keller & Reiners, 1994). Such inhibited diffusion of CH_4 from the atmosphere into the soil affects methanotrophic CH_4 oxidation, and/or creates conditions for anaerobic decomposition by methanogenic archaea, thereby producing CH_4. However, the effect of soil moisture changes on soil CH_4 uptake was less pronounced in our study, as indicated by the similar CH_4 uptake rates between the wet and dry season in both land uses (Tables S3.1 and S3.2). We also found strong indications of potential N limitation on CH_4 uptake in the cacao agroforestry, as shown by the negative correlation of soil CH_4 fluxes with soil NH_4^+ content, and the positive correlation of CH_4 fluxes with aluminium saturation (see Sect. 3.4). CH_4 and NH_4^+ oxidizers compete for the methane monooxygenase enzyme responsible for both the oxidation of CH_4 to CO_2 and NH_4^+ to NO_2^- (Bedard & Knowles, 1989). As such, the activities of methanotrophs can be inhibited by increasing NH_4^+ availability in the soil. Additionally, the intermediate and end products of

methanotrophic NH_4^+ oxidation have been found to be toxic to soil methanotrophic bacteria (Schnell & King, 1994), which may also inhibit CH_4 consumption. Indications of the inhibitory effect of soil NH_4^+ content on methanotrophic activity have been reported for tropical forests in Ecuador (Wolf *et al.*, 2012), Panama (Veldkamp *et al.*, 2013; Matson *et al.*, 2017), Australia (Kiese *et al.*, 2003) and Kenya (Wanyama *et al.*, 2019), while both increasing $NH4^+$ availability and exchangeable Al in the soil have been shown to be toxic for both plants and methanotrophs in a tropical forest in Indonesia (Hassler *et al.*, 2015).

Soil texture has been shown to largely control atmospheric CH_4 uptake by soils, due to its direct effect on gas diffusivity into the soil (Veldkamp *et al.*, 2013). In their review of studies conducted in (sub)tropical forests, Veldkamp *et al.* (2013) found annual soil CH_4 fluxes to positively correlate with clay contents, which is consistent with the findings of this study. A high clay content reduces the diffusivity of atmospheric CH_4 into the soil, thereby limiting aerobic CH_4 oxidation and consumption, while increasing anaerobic CH_4 production (Keller *et al.*, 1993; Veldkamp *et al.*, 2008). For such clayey soils such as in our sites, CH_4 uptake also decreases at high WFPS due to inhibited diffusion of atmospheric CH_4 into the soil because of the high soil water content. Indeed, for our cacao agroforestry sites, Biba Yezoum had a lower clay content (Table 2.1) and a lower WFPS (Table 2.4) compared to the Aloum, and hence had correspondingly higher soil CH_4 uptake (Tables 3.1).

Soil CO_2 fluxes at our sites showed clear seasonal variability (Tables S3.1 and S3.2), controlled by soil water content. We measured the highest soil CO_2 fluxes at the beginning of our measurement period (Fig. 3.1), which coincided with the end of the rainy season when soil mineralization activity was still high. The lower soil CO_2 fluxes in the dry season may reflect water limitation of plant root and soil microbial activity as well as limited litter decomposition due to low soil moisture content (Yavitt *et al.*, 2004). The relationship between soil CO_2 fluxes

and WFPS reflected the parabolic relationship typically found in tropical forest studies, with the highest soil CO_2 fluxes measured at field capacity (WFPS between 50 and 55%), after which increasing soil moisture content inhibited soil CO_2 production in the soil, and/or slowed the diffusion of soil CO_2 from the soil (Schwendenmann *et al.*, 2003; Sotta *et al.*, 2006; Koehler *et al.*, 2009a; van Straaten *et al.*, 2011; Hassler *et al.*, 2015). The positive correlation of soil CO_2 emissions with sand content across our sites and land uses was similar to the findings of other studies conducted in tropical forests (Silver *et al.*, 2000; Sotta *et al.*, 2006). Sandy soils tend to have higher root biomass, and consequently, higher autotrophic root respiration, which has been shown to contribute up to 35% of soil respiration (Silver *et al.*, 2000; van Straaten *et al.*, 2011).

3.5.3. Effects of land-use change on soil CO_2 and CH_4 fluxes

Our mean soil CO_2 emissions from the forests (Table 3.1) were within the range of values (93–228 mg C m^{-2} h^{-1}) reported for tropical rainforests on Ferralsol soils in Central and South America (Davidson *et al.*, 2000b, 2004; Schwendenmann *et al.*, 2003; Chambers *et al.*, 2004; Keller *et al.*, 2005; Sotta *et al.*, 2006; Matson *et al.*, 2017). Compared to the few studies conducted in Africa, mean soil CO_2 emissions from our forests were higher than those reported for tropical montane forests in Kenya (71.8–95.2 mg C m^{-2} h^{-1}; Wanyama *et al.*, 2019; Werner *et al.*, 2007). For Werner *et al.* (2007), their short measuring campaign (3 months), which included two dry months, may have resulted in lower soil CO_2 emissions compared to our study. The forest sites in Wanyama *et al.* (2019) study reportedly had low tree density, which could have affected autotrophic root respiration, leading to lower CO_2 emissions.

Soil CO_2 emissions did not differ between the forest and cacao agroforestry in this study (Table 3.1), in support of our first hypothesis. In tropical regions marked by periods of wet and dry conditions such as in our sites, soil CO_2 fluxes are primarily controlled by soil moisture (Hassler *et al.*, 2015; Matson *et al.*, 2017; van Straaten *et al.*, 2019), especially when there is

little fluctuation in temperature (Schwendenmann *et al.*, 2003). The lack of differences in soil water content between the two land uses (Table 2.4) may therefore have resulted in the similar soil CO_2 fluxes. Moreover, forest conversion to cacao agroforestry in our study regions generally lacked heavy soil physical disturbance and preparation activities, which may have resulted in the similar soil texture and soil bulk density the two land uses (Table 2.1), and consequently, comparable CO_2 emissions. Indeed, differences in soil characteristics such as soil texture (Sotta *et al.*, 2006; da Costa *et al.*, 2018), bulk density (Zhong *et al.*, 2016), SOC and total N (Schwendenmann *et al.*, 2003) have been shown to control spatial and temporal variation in soil CO_2 fluxes. While we measured differences in SOC between the land uses in two of our sites (Table S1), it is possible that the microbial communities involved in heterotrophic respiration may have adapted to any differences in the quantity of substrate between the forest and cacao agroforest, resulting in their similar soil CO_2 fluxes. Additionally, the high density of shade trees in the cacao agroforestry and their comparable basal area with the forests (Table S2.1) may have partly offset any differences in autotrophic root respiration between the two land uses. da Costa *et al.* (2018) also presented similar results where soil CO_2 emissions did not differ between cacao agroforestry and a reference forest in Brazil. Similarly, Hassler *et al.* (2015) found no differences in soil CO_2 emissions between jungle rubber agroforestry and forests in Indonesia. Our mean soil CO_2 fluxes from the cacao agroforestry (Table 3.1) were comparable to those reported for cacao agroforestry systems in Brazil and Indonesia (125–137 mg C m^{-2} h^{-1}; da Costa *et al.*, 2018; van Straaten *et al.*, 2010).

Our forest sites acted as sinks of atmospheric CH_4 (Fig. 3.1), similarly to what has been found in previous studies conducted on well-drained soils (e.g., Werner *et al.*, 2007; Veldkamp *et al.*, 2013; Wanyama *et al.*, 2019). The mean soil CH_4 uptake from our forest sites (Table 3.1) was within the range of other reported values for (sub)tropical lowland forests (−6.28 to −55.9 µg C m^{-2} h^{-1}; summarized by Veldkamp *et al.*, 2013), but higher than those found for three

lowland forests on Ferralsol soils in Panama (−10.7 to −22.6 µg C m^{-2} h^{-1}; Matson *et al.*, 2017). The latter study has higher clay contents compared to our study sites, which explains their correspondingly lower CH_4 uptake rate. Compared to measurements conducted in sub-Saharan Africa, our lowland forests had comparable soil CH_4 uptake rates as those reported for tropical montane forests in Kenya (−35.4 to −66.2 µg C m^{-2} h^{-1}; Wanyama *et al.*, 2019) and in Tanzania (−31.0 to −44.6 µg C m^{-2} h^{-1}; Gütlein *et al.*, 2018). Conversely, our mean soil CH_4 uptake was lower than reported for tropical montane forests in Kenya (−56.4 µg C m^{-2} h^{-1}; Werner *et al.*, 2007), which had a comparably sandy texture compared to our sites.

In line with our first hypothesis, soil CH_4 fluxes did not differ between the forest and cacao agroforestry at each site (Table 3.1). All our cacao sites were unfertilized and the soils were minimally disturbed, as reflected in the comparable soil texture (i.e. clay contents; Table 2.1) and soil moisture content (Table 2.4) between the two land uses. Since atmospheric CH_4 diffusion into the soil has been suggested as the main limitation of soil CH_4 oxidation by methanotrophic bacteria in the soil (Palm *et al.*, 2002; Veldkamp *et al.*, 2013), the comparable soil texture and soil moisture content between the forest and cacao agroforestry may be the primary reason for the similar CH_4 rates.

Cacao agroforestry in our study (Table 3.1) had slightly higher soil CH_4 uptake than a managed homegarden in Tanzania (−32.6 µg C m^{-2} h^{-1}; Gütlein *et al.*, 2018) and an agroforestry system in Peru (−24.2 µg C m^{-2} h^{-1}; (Palm *et al.*, 2002), and was also higher than a jungle rubber agroforestry in Indonesia (−20.8 to −26.9 µg C m^{-2} h^{-1}; Hassler *et al.*, 2015). Our mean soil CH_4 uptake in the cacao agroforestry were also higher than the reported average for agroforestry systems worldwide (−18.3 µg C m^{-2} h^{-1}; Kim *et al.*, 2016a). These trends in soil CH_4 fluxes may be explained by differences in gas diffusivity resulting from compaction and soil fertility; the agroforestry systems in Gütlein *et al.* (2018) study sites had comparably higher

soil bulk densities than our cacao sites. Moreover, the higher soil CH_4 uptake in our cacao agroforestry compared to the jungle rubber agroforestry (Hassler *et al.*, 2015) may be the result of our comparably higher soil NO_3^- content, which have been found to simulate CH_4 consumption and/or reduce its production in the soil (Veldkamp *et al.*, 2013; Matson *et al.*, 2017). This could also explain the lower CH_4 uptake rate reported by Kim *et al.* (2016a) for the reviewed cacao agroforestry sites, in which ammonium-N fertilizers have been applied. As discussed above, increasing NH_4^+ concentrations in the soil owing to nitrogenous fertilizer applications can inhibit CH_4 oxidation rates (Veldkamp *et al.*, 2001; Bodelier & Laanbroek, 2004).

3.6. Conclusions

Our study provides evidence that tropical trees on well-drained, highly weathered soils represent potential CH_4 emission pathways. Stem contribution to total CH_4 fluxes suggests that tropical soils may be a weaker sink of atmospheric CH_4 than previously estimated. Positive correlations of stem CH_4 emissions with WFPS and soil-air CH_4 concentrations points to a belowground origin of stem CH_4 emissions. However, the consistently high CH_4 emissions from a few of our sampled trees suggests there may be other contributing mechanisms. These findings highlight the need for additional studies to constrain the magnitude and mechanisms of stem CH_4 fluxes in tropical well-drained forests, so that this important CH_4 source can be accounted for in GHG budget estimations. In contrast to other studies, stem CH_4 emissions did not differ among tree species in our study. Overall, we did not observe any effects of land-use change on stem and soil CH_4 and CO_2 fluxes, due to similarities in soil texture and soil moisture content between the forest and cacao agroforestry.

3.7. Acknowledgement

This study was funded by the German Research Foundation (DFG, VE 219/14-1, STR 1375/1-1). We gratefully acknowledge our counterparts in Cameroon, the International Institute for Tropical Agriculture (IITA) or granting us access and use of their storage facilities. We are grateful to our Cameroonian field assistants: Leonel Boris Gadjui Youatou, Yannick Eyenga Alfred, Denis Djiyo, and all the field workers for their great support with field measurements. We also thank the village leaders and local plot owners for granting us access to their forest and cacao farms. We thank Andrea Bauer, Kerstin Langs and Martina Knaust for their assistance with laboratory analyses.

3.8. References

Allen, R. G., Pereira, L. S., Raes, D., & Smith, M. (1998). Determination of ET_0, crop evapotranspiration. *Guidelines for Computing Crop Water Requirements-FAO Irrigation and Drainage Paper 56*, 309. Retrieved from http://www.hidmet.gov.rs/podaci/agro/table of contens_files.pdf

Barba, J., Bradford, M. A., Brewer, P. E., Bruhn, D., Covey, K. R., van Haren, J., et al. (2019a). *Methane emissions from tree stems: a new frontier in the global carbon cycle*. *New Phytologist* (Vol. 222). https://doi.org/10.1111/nph.15582

Barba, J., Poyatos, R., & Vargas, R. (2019b). Automated measurements of greenhouse gases fluxes from tree stems and soils: magnitudes, patterns and drivers. *Scientific Reports*, *9*(1), 1–13. https://doi.org/10.1038/s41598-019-39663-8

Bedard, C., & Knowles, R. (1989). CO Oxidation by Methanotrophs and Nitrifiers. *Microbiology*, *53*(1), 68–84. Retrieved from http://apps.isiknowledge.com/full_record.do?product=UA&search_mode=GeneralSearch&qid=11&SID=X16536DGgEAkOn8a882&page=1&doc=1&colname=WOS

Bodelier, P. L. E., & Laanbroek, H. J. (2004). Nitrogen as a regulatory factor of methane oxidation in soils and sediments. *FEMS Microbiology Ecology*, *47*(3), 265–277. https://doi.org/10.1016/S0168-6496(03)00304-0

Brewer, P. E., Calderón, F., Vigil, M., & von Fischer, J. C. (2018). Impacts of moisture, soil respiration, and agricultural practices on methanogenesis in upland soils as measured with stable isotope pool dilution. *Soil Biology and Biochemistry*, *127*, 239–251. https://doi.org/10.1016/j.soilbio.2018.09.014

Chambers, J. Q., Tribuzy, E. S., Toledo, L. C., Crispim, B. F., Higuchi, N., Dos Santos, J., et al. (2004). Respiration from a tropical forest ecosystem: Partitioning of sources and low carbon use efficiency. *Ecological Applications*, *14*(4 SUPPL.), 72–88. https://doi.org/10.1890/01-6012

da Costa, E. N. D., de Souza, M. F. L., Lima Marrocos, P. C., Lobão, D., & da Silva, D. M. L. (2018). Soil organic matter and CO2 fluxes in small tropical watersheds under forest and cacao agroforestry. *PLoS ONE*, *13*(7), 1–22. https://doi.org/10.1371/journal.pone.0200550

Covey, K. R., & Megonigal, J. P. (2019). Methane production and emissions in trees and forests. *New Phytologist*, *222*(1), 35–51. https://doi.org/10.1111/nph.15624

Covey, K. R., Wood, S. A., Warren, R. J., Lee, X., & Bradford, M. A. (2012). Elevated methane concentrations in trees of an upland forest. *Geophysical Research Letters*, *39*(15), 1–6. https://doi.org/10.1029/2012GL052361

Davidson, E. A., Verchot, L. V., Henrique Cattânio, J., Ackerman, I. L., & Carvalho, J. E. M. (2000). Effects of soil water content on soil respiration in forests and cattle pastures of eastern Amazonia. *Biogeochemistry*, *48*(1), 53–69. https://doi.org/10.1023/A:1006204113917

Davidson, E. A., Ishida, F. Y., & Nepstad, D. C. (2004). Agricultural & Applied Economics Budgets. *Global Change Biology*, *10*(5), 718–730. https://doi.org/10.1111/j.1529-8817.2003.00762.x

Dutaur, L., & Verchot, L. V. (2007). A global inventory of the soil CH4 sink. *Global Biogeochemical Cycles*, *21*(4), 1–9. https://doi.org/10.1029/2006GB002734

von Fischer, J. C., & Hedin, L. O. (2007). Controls on soil methane fluxes: Tests of biophysical mechanisms using table isotope tracers. *Global Biogeochemical Cycles*, *21*(2), 1–9. https://doi.org/10.1029/2006GB002687

Gauci, V., Gowing, D. J. G., Hornibrook, E. R. C., Davis, J. M., & Dise, N. B. (2010). Woody stem methane emission in mature wetland alder trees. *Atmospheric Environment*. https://doi.org/10.1016/j.atmosenv.2010.02.034

Gütlein, A., Gerschlauer, F., Kikoti, I., & Kiese, R. (2018). Impacts of climate and land use on N2O and CH4 fluxes from tropical ecosystems in the Mt. Kilimanjaro region, Tanzania. *Global Change Biology*, *24*, 1239–1255. https://doi.org/10.1111/gcb.13944

Gwanfogbe, M., Meligui, A., Moukam, J., & Nguoghia, J. (1983). *Geography of Cameroon.* Macmillan Education Ltd, Hong Kong.

Hashimoto, S., Carvalhais, N., Ito, A., Migliavacca, M., Nishina, K., & Reichstein, M. (2015). Global spatiotemporal distribution of soil respiration modeled using a global database. *Biogeosciences*, *12*(13), 4121–4132. https://doi.org/10.5194/bg-12-4121-2015

Hassler, E., Corre, M. D., Tjoa, A., Damris, M., Utami, S. R., & Veldkamp, E. (2015). Soil fertility controls soil-atmosphere carbon dioxide and methane fluxes in a tropical landscape converted from lowland forest to rubber and oil palm plantations. *Biogeosciences Discussions*, *12*(12), 9163–9207. https://doi.org/10.5194/bgd-12-9163-2015

IPCC. (2013). *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia,*. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

IUSS Working Group WRB. (2015). *World Reference Base for Soil Resources 2014, update 2015 International soil classification system for naming soils and creating legends for soil maps. World Soil Resources Reports No. 106.* FAO, Rome.

Keller, M., & Matson, P. A. (1994). Biosphere-Atmosphere Exchange of Trace Gases in the Tropics: Evaluating the Effects of Land Use Changes BT - Global Atmospheric-Biospheric Chemistry. In R. G. Prinn (Ed.) (pp. 103–117). Boston, MA: Springer US. https://doi.org/10.1007/978-1-4615-2524-0_7

Keller, M., & Reiners, W. A. (1994). Soil-atmosphere exchange of nitrous oxide, nitric oxide, and methane under secondary succession of pasture to forest in the Atlantic lowlands of Costa Rica. *Global Biogeochemical Cycles*, *8*(4), 399–409. https://doi.org/10.1029/94GB01660

Keller, M., Veldkamp, E., Weitz, A. M., & Reiners, W. A. (1993). Effect of pasture age on soil trace-gas emissions from a deforested area of Costa Rica. *Nature*, *365*(6443), 244–246. https://doi.org/10.1038/365244a0

Keller, M., Varner, R., Dias, J. D., Silva, H., Crill, P., De Oliveira, R. C., & Asner, G. P. (2005). Soil-atmosphere exchange of nitrous oxide, nitric oxide, methane, and carbon dioxide in logged and undisturbed forest in the Tapajos National Forest, Brazil. *Earth Interactions*, *9*(23), 1–28. https://doi.org/10.1175/EI125.1

Kiese, R., Hewett, B., Graham, A., & Butterbach-Bahl, K. (2003). Seasonal variability of N2O emissions and CH4 uptake by tropical rainforest soils of Queensland, Australia. *Global Biogeochemical Cycles*, *17*(2), 1043. https://doi.org/10.1029/2002gb002014

Kim, D. G., Kirschbaum, M. U. F., & Beedy, T. L. (2016a). Carbon sequestration and net emissions of CH4 and N2O under agroforestry: Synthesizing available data and suggestions for future studies. *Agriculture, Ecosystems and Environment*, *226*, 65–78. https://doi.org/10.1016/j.agee.2016.04.011

Kim, D. G., Thomas, A. D., Pelster, D. E., Rosenstock, T. S., & Sanz-Cobena, A. (2016b). Greenhouse gas emissions from natural ecosystems and agricultural lands in sub-Saharan Africa: Synthesis of available data and suggestions for further research. *Biogeosciences*, *13*(16), 4789–4809. https://doi.org/10.5194/bg-13-4789-2016

Kirschke, S., Bousquet, P., Ciais, P., Saunois, M., Canadell, J. G., Dlugokencky, E. J., et al. (2013). Three decades of global methane sources and sinks. *Nature Geoscience*, *6*(10), 813–823. https://doi.org/10.1038/ngeo1955

Koehler, B., Corre, M. D., Veldkamp, E., & Sueta, J. P. (2009). Chronic nitrogen addition causes a reduction in soil carbon dioxide efflux during the high stem-growth period in a tropical montane forest but no response from a tropical lowland forest in decadal scale. *Biogeosciences Discussions*, *6*(5), 8633–8660. https://doi.org/10.5194/bgd-6-8633-2009

Kroetsch, D., & Wang, C. (2008). Particle size distribution. In *Soil Sampling and Methods of Analysis, Second Edition* (pp. 713–725).

Lenhart, K., Weber, B., Elbert, W., Steinkamp, J., Clough, T., Crutzen, P., et al. (2015). Nitrous oxide and methane emissions from cryptogamic covers. *Global Change Biology*, *21*(10), 3889–3900. https://doi.org/10.1111/gcb.12995

Luo, Y., & Zhou, X. (2006). *Soil Respiration and the Environment*. Amsterdam, Netherlands, Netherlands: Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-088782-

8.X5000-1

Machacova, K., Bäck, J., Vanhatalo, A., Halmeenmäki, E., Kolari, P., Mammarella, I., et al. (2016). Pinus sylvestris as a missing source of nitrous oxide and methane in boreal forest. *Scientific Reports*, *6*(March), 1–8. https://doi.org/10.1038/srep23410

Maier, M., Machacova, K., Lang, F., Svobodova, K., & Urban, O. (2018). Combining soil and tree-stem flux measurements and soil gas profiles to understand CH4 pathways in Fagus sylvatica forests. *Journal of Plant Nutrition and Soil Science*, *181*(1), 31–35. https://doi.org/10.1002/jpln.201600405

Matson, A. L., Corre, M. D., Langs, K., & Veldkamp, E. (2017). Soil trace gas fluxes along orthogonal precipitation and soil fertility gradients in tropical lowland forests of Panama. *Biogeosciences*, *14*(14), 3509–3524. https://doi.org/10.5194/bg-14-3509-2017

Megonigal, J. P., & Guenther, A. B. (2008). Methane emissions from upland forest soils and vegetation. *Tree Physiology*, *28*(4), 491–498. https://doi.org/10.1093/treephys/28.4.491

Neubauer, S. C., & Megonigal, J. P. (2015). Moving Beyond Global Warming Potentials to Quantify the Climatic Role of Ecosystems. *Ecosystems*, *18*(6), 1000–1013. https://doi.org/10.1007/s10021-015-9879-4

Palm, C. A., Alegre, J. C., Arevalo, L., Mutuo, P. K., Mosier, A. R., & Coe, R. (2002). Nitrous oxide and methane fluxes in six different land use systems in the Peruvian Amazon. *Global Biogeochemical Cycles*, *16*(4), 1073. https://doi.org/10.1029/2001gb001855

Pangala, S. R., Moore, S., Hornibrook, E. R. C., & Gauci, V. (2013). Trees are major conduits for methane egress from tropical forested wetlands. *New Phytologist*, *197*(2), 524–531. https://doi.org/10.1111/nph.12031

Pangala, S. R., Gowing, D. J. G., Hornibrook, E. R. C., & Gauci, V. (2014). Controls on methane emissions from Alnus glutinosa saplings. *New Phytologist*, *201*(3), 887–896. https://doi.org/10.1111/nph.12561

Pangala, S. R., Hornibrook, E. R. C., Gowing, D. J. G., & Gauci, V. (2015). The contribution of trees to ecosystem methane emissions in a temperate forested wetland. *Global Change Biology*, *21*(7), 2642–2654. https://doi.org/10.1111/gcb.12891

Pangala, S. R., Enrich-Prast, A., Basso, L. S., Peixoto, R. B., Bastviken, D., Hornibrook, E. R. C., et al. (2017). Large emissions from floodplain trees close the Amazon methane budget. *Nature*, *552*(7684), 230–234. https://doi.org/10.1038/nature24639

Pierret, A., Maeght, J. L., Clément, C., Montoroi, J. P., Hartmann, C., & Gonkhamdee, S. (2016). Understanding deep roots and their functions in ecosystems: An advocacy for more unconventional research. *Annals of Botany*, *118*(4), 621–635. https://doi.org/10.1093/aob/mcw130

Pitz, S. L., & Megonigal, J. P. (2017). Temperate forest methane sink diminished by tree emissions. *New Phytologist*, *214*(4), 1432–1439. https://doi.org/10.1111/nph.14559

Pitz, S. L., Megonigal, J. P., Chang, C. H., & Szlavecz, K. (2018). Methane fluxes from tree stems and soils along a habitat gradient. *Biogeochemistry*, *137*(3), 307–320. https://doi.org/10.1007/s10533-017-0400-3

Plain, C., Ndiaye, F. K., Bonnaud, P., Ranger, J., & Epron, D. (2019). Impact of vegetation on

the methane budget of a temperate forest. *New Phytologist*, *221*(3), 1447–1456. https://doi.org/10.1111/nph.15452

R Core Team (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Raich, J. W., & Schlesinger, W. H. (1998). The global carbon dioxide flux in soil respiration and its relationship to vegetation and climate. *Tellus B*, *44B*, 81–99.

Saunois, M., Bousquet, P., Poulter, B., Peregon, A., Ciais, P., Canadell, J. G., et al. (2016). The global methane budget 2000-2012. *Earth System Science Data*, *8*(2), 697–751. https://doi.org/10.5194/essd-8-697-2016

Schnell, S., & King, G. M. (1994). Mechanistic analysis of ammonium inhibition of atmospheric methane consumption in forest soils. *Applied and Environmental Microbiology*, *60*(10), 3514–3521. https://doi.org/10.1128/aem.60.10.3514-3521.1994

Schwendenmann, L., Veldkamp, E., Brenes, T., O'Brien, J. J., & Mackensen, J. (2003). Spatial and temporal variation in soil CO2 efflux in an old-growth neotropical rain forest, La Selva, Costa Rica. *Biogeochemistry*, *64*(1), 111–128. https://doi.org/10.1023/A:1024941614919

Silver, W. L., Neff, J., McGroddy, M., Veldkamp, E., Keller, M., & Cosme, R. (2000). Effects of Soil Texture on Belowground Carbon and Nutrient Storage in a Lowland Amazonian Forest Ecosystem. *Ecosystems*, *3*(2), 193–209. https://doi.org/10.1007/s100210000019

Sonwa, D. J., Nkongmeneck, B. A., Weise, S. F., Tchatat, M., Adesina, A. A., & Janssens, M. J. J. (2007). Diversity of plants in cocoa agroforests in the humid forest zone of Southern Cameroon. *Biodiversity and Conservation*, *16*(8), 2385–2400. https://doi.org/10.1007/s10531-007-9187-1

Sotta, E. D., Veldkamp, E., Guimarães, B. R., Paixão, R. K., Ruivo, M. L. P., & Almeida, S. S. (2006). Landscape and climatic controls on spatial and temporal variation in soil CO2 efflux in an Eastern Amazonian Rainforest, Caxiuanã, Brazil. *Forest Ecology and Management*, *237*(1–3), 57–64. https://doi.org/10.1016/j.foreco.2006.09.027

van Straaten, O., Veldkamp, E., Köhler, M., & Anas, I. (2010). Spatial and temporal effects of drought on soil CO2 efflux in a cacao agroforestry system in Sulawesi, Indonesia. *Biogeosciences*, *7*(4), 1223–1235. https://doi.org/10.5194/bg-7-1223-2010

van Straaten, O., Veldkamp, E., & Corre, M. D. (2011). Simulated drought reduces soil CO 2 efflux and production in a tropical forest in Sulawesi, Indonesia . *Ecosphere*, *2*(10), art119. https://doi.org/10.1890/es11-00079.1

van Straaten, O., Doamba, S. W. M. F., Corre, M. D., & Veldkamp, E. (2019). Impacts of burning on soil trace gas fluxes in two wooded savanna sites in Burkina Faso. *Journal of Arid Environments*, *165*(February), 132–140. https://doi.org/10.1016/j.jaridenv.2019.02.013

Terazawa, K., Ishizuka, S., Sakata, T., Yamada, K., & Takahashi, M. (2007). Methane emissions from stems of Fraxinus mandshurica var. japonica trees in a floodplain forest. *Soil Biology and Biochemistry*, *39*(10), 2689–2692.

Terazawa, K., Yamada, K., Ohno, Y., Sakata, T., & Ishizuka, S. (2015). Spatial and temporal

variability in methane emissions from tree stems of Fraxinus mandshurica in a cool-temperate floodplain forest. *Biogeochemistry*, *123*(3), 349–362. https://doi.org/10.1007/s10533-015-0070-y

Valentini, R., Arneth, A., Bombelli, A., Castaldi, S., Cazzolla Gatti, R., Chevallier, F., et al. (2014). A full greenhouse gases budget of africa: Synthesis, uncertainties, and vulnerabilities. *Biogeosciences*, *11*(2), 381–407. https://doi.org/10.5194/bg-11-381-2014

Veldkamp, E., Weitz, A. M., & Keller, M. (2001). Management effects on methane fluxes in humid tropical pasture soils. *Soil Biology and Biochemistry*, *33*(11), 1493–1499. https://doi.org/10.1016/S0038-0717(01)00060-8

Veldkamp, E., Purbopuspito, J., Corre, M. D., Brumme, R., & Murdiyarso, D. (2008). Land use change effects on trace gas fluxes in the forest margins of Central Sulawesi, Indonesia. *Journal of Geophysical Research: Biogeosciences*, *113*(2), 1–11. https://doi.org/10.1029/2007JG000522

Veldkamp, E., Koehler, B., & Corre, M. D. (2013). Indications of nitrogen-limited methane uptake in tropical forest soils. *Biogeosciences*, *10*(8), 5367–5379. https://doi.org/10.5194/bg-10-5367-2013

Verchot, L. V., Davidson, E. A., Cattânio, J. H., & Ackerman, I. L. (2000). Land-use change and biogeochemical controls of methane fluxes in soils of eastern Amazonia. *Ecosystems*, *3*(1), 41–56. https://doi.org/10.1007/s100210000009

Wang, Z. P., Gu, Q., Deng, F. D., Huang, J. H., Megonigal, J. P., Yu, Q., et al. (2016). Methane emissions from the trunks of living trees on upland soils. *The New Phytologist*, *211*(2), 429–439. https://doi.org/10.1111/nph.13909

Wang, Z. P., Han, S. J., Li, H. L., Deng, F. D., Zheng, Y. H., Liu, H. F., & Han, X. G. (2017). Methane Production Explained Largely by Water Content in the Heartwood of Living Trees in Upland Forests. *Journal of Geophysical Research: Biogeosciences*, *122*(10), 2479–2489. https://doi.org/10.1002/2017JG003991

Wanyama, I., Pelster, D. E., Butterbach-Bahl, K., Verchot, L. V., Martius, C., & Rufino, M. C. (2019). Soil carbon dioxide and methane fluxes from forests and other land use types in an African tropical montane region. *Biogeochemistry*, *143*(2), 171–190. https://doi.org/10.1007/s10533-019-00555-8

Warner, D. L., Villarreal, S., McWilliams, K., Inamdar, S., & Vargas, R. (2017). Carbon Dioxide and Methane Fluxes From Tree Stems, Coarse Woody Debris, and Soils in an Upland Temperate Forest. *Ecosystems*, *20*(6), 1205–1216. https://doi.org/10.1007/s10021-016-0106-8

Welch, B., Gauci, V., & Sayer, E. J. (2019). Tree stem bases are sources of CH4 and N2O in a tropical forest on upland soil during the dry to wet season transition. *Global Change Biology*, *25*(1), 361–372. https://doi.org/10.1111/gcb.14498

Werner, C., Kiese, R., & Butterbach-Bahl, K. (2007). Soil-atmosphere exchange of N2O, CH4, and CO2 and controlling environmental factors for tropical rain forest sites in western Kenya. *Journal of Geophysical Research*, *112*(3), D03308. https://doi.org/10.1029/2006JD007388

Wolf, K., Flessa, H., & Veldkamp, E. (2012). Atmospheric methane uptake by tropical montane forest soils and the contribution of organic layers. *Biogeochemistry*, *111*(1–3),

469–483. https://doi.org/10.1007/s10533-011-9681-0

Yavitt, J. B., Wright, S. J., & Wieder, R. K. (2004). Seasonal drought and dry-season irrigation influence leaf-litter nutrients and soil enzymes in a moist, lowland forest in Panama. *Austral Ecology*, *29*(2), 177–188. https://doi.org/10.1111/j.1442-9993.2004.01334.x

Yip, D. Z., Veach, A. M., Yang, Z. K., Cregger, M. A., & Schadt, C. W. (2019). Methanogenic Archaea dominate mature heartwood habitats of Eastern Cottonwood (Populus deltoides). *New Phytologist*, *222*(1), 115–121. https://doi.org/10.1111/nph.15346

Zhong, Y., Yan, W., & Shangguan, Z. (2016). The effects of nitrogen enrichment on soil CO2 fluxes depending on temperature and soil properties. *Global Ecology and Biogeography*, *25*(4), 475–488. https://doi.org/10.1111/geb.12430

3.9. Appendix

Table S3.1. Seasonal mean (±SE, n = 4) stem CH_4 flux, soil CH_4 flux and soil CO_2 flux in forests on highly weathered soils in the Congo Basin, Cameroon. Means followed by different lowercase letters indicate significant differences between seasons for each site (linear mixed-effect models with Tukey's HSD at $P \leq 0.05$).

Site/season	Stem CH_4 flux ($\mu g\ N\ m^{-2}$ stem h^{-1})	Soil CH_4 flux ($\mu g\ N\ m^{-2}$ soil h^{-1})	Soil CO_2 flux ($mg\ C\ m^{-2}$ soil h^{-1})
Wet seasson			
Aloum	5.70 ± 0.88^{a}	-34.9 ± 4.7^{a}	121.1 ± 10.0^{a}
Biba Yezoum	4.18 ± 0.40^{a}	-25.4 ± 12.8^{a}	141.2 ± 13.2^{a}
Tomba	0.31 ± 0.07^{a}	-26.2 ± 7.9^{a}	137.2 ± 14.2^{a}
Dry season			
Aloum	4.97 ± 0.82^{a}	-39.1 ± 4.3^{a}	94.7 ± 6.0^{b}
Biba Yezoum	4.01 ± 0.16^{a}	-44.1 ± 11.1^{a}	111.1 ± 8.4^{b}
Tomba	0.19 ± 0.05^{a}	-19.7 ± 9.2^{a}	108.2 ± 12.6^{b}

Table S3.2. Seasonal mean (±SE, $n = 4$) stem CH_4 flux, soil CH_4 flux and soil CO_2 flux in cacao agroforestry sites located on highly weathered soils in the Congo Basin, Cameroon. Means followed by different lowercase letters indicate significant differences between seasons for each site (linear mixed-effect models with Tukey's HSD at $P \leq 0.05$).

Site/season	Stem CH_4 flux ($\mu g\ N\ m^{-2}$ stem h^{-1})	Soil CH_4 flux ($\mu g\ N\ m^{-2}$ soil h^{-1})	Soil CO_2 flux ($mg\ C\ m^{-2}$ soil h^{-1})
Wet seasson			
Aloum	0.86 ± 0.13^{a}	-12.3 ± 3.8^{a}	122.7 ± 11.8^{a}
Biba Yezoum	0.60 ± 0.10^{a}	-49.5 ± 2.3^{a}	150.6 ± 11.4^{a}
Tomba	1.97 ± 0.60^{a}	-36.8 ± 6.5^{a}	143.3 ± 15.0^{a}
Dry season			
Aloum	0.81 ± 0.18^{a}	-34.1 ± 7.8^{b}	91.1 ± 7.01^{b}
Biba Yezoum	0.60 ± 0.20^{a}	-59.5 ± 4.4^{a}	104.4 ± 10.3^{b}
Tomba	1.64 ± 0.30^{a}	-39.9 ± 8.1^{a}	114.3 ± 12.4^{b}

CHAPTER 4

SYNTHESIS

4.1. Key findings of this thesis

Chapter 2: Stem and soil nitrous oxide fluxes from rainforest and cacao agroforest

The conversion of forests to extensively managed cacao agroforestry systems had no effect on stem and soil nitrous oxide (N_2O) emissions, due to similarities in soil water content, soil texture and leguminous tree density in both land uses. All the studied trees emitted measureable N_2O at some point during the measurement period. In contrast to findings from other studies (Wen *et al.*, 2017; Welch *et al.*, 2019), stem N_2O emissions did not differ among the different tree species in our study, which supported our spatial extrapolation based on diameter at breast height (DBH) of trees in our sites. The up-scaled N_2O fluxes suggest that trees could be important to consider in N_2O budgets, with the potential to overlook up to 38% of N_2O emissions in the forests and 15% of N_2O emissions in cacao agroforests if tree stems are not considered in the ecosystem N_2O budget. These estimates of tree contributions to total stem+soil fluxes are the highest reported for any upland forests, and the first estimates for tropical Africa. ^{15}N-isotope tracing from soil mineral nitrogen (N) to stem-emitted $^{15}N_2O$ together with the relationships between stem and soil N_2O emissions as well as their controlling factors suggest that tree stem N_2O emissions originate mainly from produced N_2O in the soil.

Chapter 3: Stem and soil methane and soil carbon dioxide fluxes from rainforest and cacao agroforest

Forest conversion to cacao agroforestry had no effect on stem and soil methane (CH_4) fluxes. Similarly, soil carbon dioxide (CO_2) fluxes did not differ between the two land uses. The lack of differences in soil CH_4 and CO_2 fluxes was due to the comparable soil texture and soil moisture content between the two land uses, which influences gas diffusivity into and out of the soil. Tree stems were a net source of CH_4, and our results points to a possible soil origin driven by transpiration. Our upscaling suggests that tree stem emissions offset 3–18% of the annual soil CH_4 sink in both land uses. Our results demonstrate that tropical trees on well-

drained soils represent potential CH_4 emission pathways that have largely been ignored, thus highlighting the urgent need for additional studies to further constrain regional and global CH_4 budgets.

4.2. Revising the African greenhouse gas budget

As discussed in previous chapters, trace gas budgets for the African continent is difficult to constrain due to limited number of *in situ* flux measurements, leaving considerable uncertainties in recent estimates. The most current estimates of the magnitude of trace gas fluxes from the African continent are from Valentini *et al.* (2014), who estimated the mean annual N_2O emissions of natural ecosystems to be 1.13 Tg N_2O yr^{-1}, with a standard deviation of 0.9 Tg N_2O yr^{-1}, signifying the high variability of the limited dataset used in the estimation. The range of uncertainty is similar for soil CH_4 fluxes, with tropical humid forests estimated to emit 0.27 ± 0.16 Tg CH_4 yr^{-1}, which is almost balanced by the net sink of seasonally dry forests (−0.21 ± 0.42 Tg CH_4 yr^{-1}; Valentini *et al.*, 2014). Given that we did not find land-use change effects on trace gas fluxes in our study, this sub-chapter aims to recalculate the source strength and sink of tropical forests in Africa, taken into consideration emissions from tree stems, which was not included in previous estimates. We only provide estimates for N_2O and CH_4, and not for CO_2 because what we measured from our sites is not the net ecosystem CO_2 fluxes. Additionally, we do not provide an estimate for agroforestry systems due to lack of studies on trace gas fluxes from unfertilized agroforestry systems in Africa.

Using the "measure and multiply" method commonly employed in bottom-up approaches (Schimel & Potter, 1995; Corre *et al.*, 1999), we estimated the N_2O and CH_4 fluxes for tropical forests in African by synthesizing currently available data on trace gas fluxes from *in situ* field measurements (Table 4.1). The total flux was calculated by multiplying the annual trace gas flux with the areal coverage of the land use (in this case, the area of tropical forest in

Africa) as estimated from land cover maps using geographic information system (GIS) technology (Table 4.1). Trace gas fluxes were converted to CO_2 equivalent (CO_2 eq.) assuming global warming potentials (GWP) of 263 kg CO_2 eq. for N_2O and 32 kg CO_2 eq. for CH_4, over a 100-year time scale (Neubauer & Megonigal, 2015). Tree stem contribution to total trace gas fluxes were emphasized by categorising the estimates into fluxes from soils only, and from soils + stems (Table 4.1).

Table 4.1. Mean (± SE) N_2O (n = 16) and CH_4 (n = 10) fluxes from tropical rainforest in Africa

	Area[1] (Mha)	N_2O emission (kg N ha^{-1} yr^{-1})	N_2O source strength (Tg N yr^{-1})	GWP[2] (Tg CO_2 eq. yr^{-1})
Soil fluxes	305.5	1.6 ± 0.2	0.50 ± 0.10	132 ± 26
Soil + stem fluxes	305.5	1.7 ± 0.2	0.52 ± 0.11	137 ± 29
	Area (Mha)	CH_4 emission (kg C ha^{-1} yr^{-1})	CH_4 source strength (Tg C yr^{-1})	GWP (Tg CO_2 eq. yr^{-1})
Soil fluxes	305.5	−3.4 ± 0.4	−1.04 ± 0.11	−33 ± 3
Soil + stem fluxes	305.5	−2.9 ± 0.5	−0.89 ± 0.14	−29 ± 5

[1] Estimate from GlobCover 2009 (http://due.esrin.esa.int/page_globcover.php)

[2] GWP, global warming potential

Our estimated total N_2O source strength for the African tropical forests (Table 4.1) were higher than the 0.34 ± 0.08 Tg N_2O-N yr^{-1} estimated by (Werner *et al.*, 2007). Their estimate was based on N_2O simulations from a data-calibrated mechanistic model (ForestDNDC-tropica) coupled with a global GIS. As pointed out by the authors, site measured N_2O fluxes do not always match with simulated N_2O fluxes due to differences in soil properties and vegetation. Valentini et al. (2014) modelled the relationship between field-measured N_2O emissions and annual precipitation, and estimated the N_2O source strength of tropical forests in Africa to be

0.65 Tg N_2O-N yr^{-1}. Similarly, the estimated net CH_4 sink strength of tropical forest (−0.21 Tg N_2O-N yr^{-1}) in Valentini *et al.* (2014) study was significantly lower than estimated in our study (Table 4.1). It is difficult to relate their estimated CH_4 flux to our value, since they do not provide details on their upscaling method. Nevertheless, it is noteworthy that our estimated N_2O source strength of 0.52 Tg N_2O-N yr^{-1}, together with the uncertainty (0.11 Tg N_2O-N yr^{-1}), is within the range of previously reported values from bottom-up approaches, and shows that tropical forests in Africa are indeed important sources of global N_2O.

We also estimated the N_2O source strength for the entire African continent using our measured fluxes and synthesized data from the literature (Table 4.2). When including emissions from tree stems, our estimated N_2O source strength was higher than the other estimates, and is equivalent to 23% of global N_2O emissions (Thompson *et al.*, 2014). For all the estimation approaches considered, our estimated total N_2O emission in Africa was in agreement with the previous estimates from Valentini *et al.* (2014) when excluding tree stem emissions (Table 4.2). The estimate from Thompson *et al.* (2014) were based on modelling of atmospheric observations and an inversion method, and included fluxes from coastal and ocean surfaces. It is therefore likely that their estimated N_2O source strength for Africa will be smaller than our estimate when excluding oceanic fluxes. Similarly, our estimate was higher than the value reported by Huang *et al.* (2008) from atmospheric measurements and inversion models. The limited data from atmospheric inversions for the African continent makes it difficult to constrain trace gas emissions using top-down approaches (Tian *et al.*, 2016), which may explain their comparably lower estimate. Our estimated N_2O source strength was also higher than estimated by Tian *et al.* (2016) using a bottom-up approach; however, their estimate did not include emissions from N deposition and leaching, which is large enough to explain the discrepancy between the two estimates (Table 4.2).

Table 4.2. Estimated annual N_2O (± SE) emissions from the African continent

Approach	N_2O-emitting pathways	N_2O fluxes (Tg N_2O-N yr^{-1})	Study
Bottom-up	Trees	0.3 ± 0.1	This study
	Natural soils	1.1 ± 0.2	This study
	Agriculture	1.4 ± 0.7	Kim *et al.* 2016
	Biomass burning	0.3 ± 0.0	Tian *et al.* 2016
	Nitrogen depositions & nitrogen leaching	0.68	Valentini *et al.* 2014
	Other natural sources (wetlands, termite mounds, savannah & grasslands)	0.2 ± 0.0	Kim *et al.* 2016
Total emissions (including trees)		**3.9 ± 0.7**	This study
Total emissions (no trees)		**3.6 ± 0.9**	This study
Bottom-up (no trees)		**3.3 ± 1.3**	Valentini *et al.* 2014
Bottom-up (no trees)		**2.9 ± 0.3**	Tian *et al.* 2016
Top-down		**3.5**	Thompson *et al.* 2014
Top-down		**2.9**	Huang *et al.* 2008

We acknowledge, however, that our simple upscaling of trace gas fluxes from small scales to regional scales based on a few studies have a high degree of uncertainty, especially because we did not account for spatial and temporal differences in soil properties, vegetation and climate. Moreover, a central assumption of the "measure and multiply" approach is that the trace gas flux data used in the upscaling are representative for the investigated land use throughout the study region. Given the relatively few number of studies and the lack of data for some countries (Fig. 4.1), it is likely that the degree of uncertainty for the upscaled fluxes is higher than indicated in Tables 4.1 and 4.2. These limitations of our estimates for N_2O and CH_4

fluxes highlights the need for further investigations in Africa to provide additional insights on the natural sources and sinks of these important trace gases, and to improve estimations from this important region.

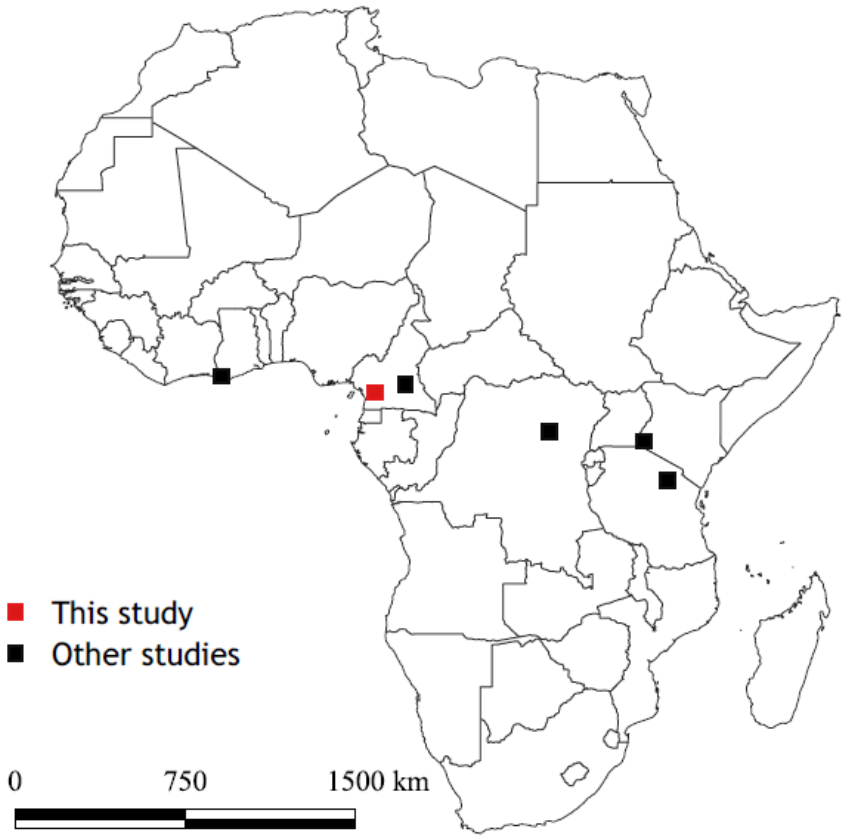

Figure 4.1. Map showing study sites of soil trace gas studies from moist natural forests in Africa.

4.3. Tree stem emissions and implications for global greenhouse gas budgets

This study provide the first attempt, albeit uncertain, to estimate the role of tree stem emissions in N_2O and CH_4 budgets in Africa. Tree stems accounted for 8% of the total N_2O emissions in Africa (Table 4.2), and reduced the net CH_4 sink of African rainforests by 15% (Table 4.1). The GWP of the forests was also higher when including tree stem emissions (Table 4.1). These findings have important implications for global greenhouse gas budgets, considering that well-drained soils such as found in our study sites constitute the largest terrestrial CH_4 sink (Saunois

et al., 2016). It is possible that even small stem CH_4 emissions might change a forest from a net sink to a net source (Shoemaker *et al.*, 2014; Pitz & Megonigal, 2017). This was partly the case in our study; when including the consistently high emitting trees (Fig. 3.4) in our upscaling method, the balance between soil and stem CH_4 fluxes indicated that those sites could be net CH_4 sources. At the global scale, plant-based CH_4 emissions has been estimated to contribute up to 22% of the total global flux (Carmichael *et al.*, 2014), but the role of trees in global N_2O budgets remains unknown. Currently, tree density at the global scale is estimated at *ca.* 3.04 trillion (Crowther *et al.*, 2015), hence, it is plausible to assume that even if we measure low stem emissions at the site-scale, these could possibly upscale to a larger flux at the global scale.

4.4. Outlook

Our study highlights the increasing importance of including tree-mediated fluxes in trace gas budgets, with implications for refining ecosystem-scale estimates, as well as global greenhouse gas budgets. The results show for the first time that N_2O and CH_4 emissions from tree stems on well-drained soils are apparently widespread and detectable in many tropical trees in Africa. These findings emphasize the need for additional studies on tree stem fluxes in order to better quantify stem flux magnitudes and their mechanisms. Efforts should be concentrated on measuring fluxes at different stem heights (especially near the base of the tree wherever possible), among several diameter sizes, and for long periods (such as employed in this study). The primacy of the African continent in global climate dynamics is unquestionable, yet, our understanding of the contribution of the continent to global greenhouse gas budgets is still characterised by high uncertainty. Concurrent measurements of soil and stem greenhouse gas fluxes from different regions, soil types and land uses as well as their controlling factors is crucial for a rigorous understanding of Africa's greenhouse gas balance. Presently, close to half of the available studies on trace gas fluxes in Africa are from laboratory incubations, but these often do not capture the high spatial and temporal variability that drive *in situ* fluxes. Most of

the few remaining studies that were conducted in the field are also limited by their short measuring campaigns and lack of replications with independent plots. Such as employed in this study, further approaches should additionally focus on collecting data over longer periods and over sufficient spatial replications.

4.5. References

Carmichael, M. J., Bernhardt, E. S., Bräuer, S. L., & Smith, W. K. (2014). The role of vegetation in methane flux to the atmosphere: should vegetation be included as a distinct category in the global methane budget? *Biogeochemistry*, *119*(1), 1–24. https://doi.org/10.1007/s10533-014-9974-1

Corre, M. D., Pennock, D. J., Van Kessel, C., & Elliott, D. K. (1999). Estimation of annual nitrous oxide emissions from a transitional grassland-forest region in Saskatchewan, Canada. *Biogeochemistry*, *44*(1), 29–49. https://doi.org/10.1023/A:1006025907180

Crowther, T. W., Glick, H. B., Covey, K. R., Bettigole, C., Maynard, D. S., Thomas, S. M., et al. (2015). Mapping tree density at a global scale. *Nature*, *525*(7568), 201–205. https://doi.org/10.1038/nature14967

Huang, J., Golombeck, A., Prinn, R. G., Weiss, R. F., Fraser, P. J., Simmonds, P., et al. (2008). Estimation of regional emissions of nitrous oxide from 1997 to 2005 using multinetwork measurements, a chemical transport model, and an inverse method. *Journal of Geophysical Research Atmospheres*, *113*(17), 1–19. https://doi.org/10.1029/2007JD009381

Kim, D. G., Thomas, A. D., Pelster, D. E., Rosenstock, T. S., & Sanz-Cobena, A. (2016). Greenhouse gas emissions from natural ecosystems and agricultural lands in sub-Saharan Africa: Synthesis of available data and suggestions for further research. *Biogeosciences*, *13*(16), 4789–4809. https://doi.org/10.5194/bg-13-4789-2016

Pitz, S. L., & Megonigal, J. P. (2017). Temperate forest methane sink diminished by tree emissions. *New Phytologist*, *214*(4), 1432–1439. https://doi.org/10.1111/nph.14559

Saunois, M., Bousquet, P., Poulter, B., Peregon, A., Ciais, P., Canadell, J. G., et al. (2016). The global methane budget 2000-2012. *Earth System Science Data*, *8*(2), 697–751. https://doi.org/10.5194/essd-8-697-2016

Schimel, D. S., & Potter, C. S. (1995). Process modelling and spatial extrapolation. In P. A. Matson & R. C. Harriss (Eds.), *Biogenic Trace Gases: Measuring Emissions from Soil and Water* (pp. 358–383). Cambridge, Massachusetts: Blackwell Sci Publication.

Shoemaker, J. K., Keenan, T. F., Hollinger, D. Y., & Richardson, A. D. (2014). Forest ecosystem changes from annual methane source to sink depending on late summer water balance. *Geophysical Research Letters*, *41*(2), 673–679. https://doi.org/10.1002/2013GL058691

Thompson, R. L., Chevallier, F., Crotwell, A. M., Dutton, G., Langenfelds, R. L., Prinn, R. G., et al. (2014). Nitrous oxide emissions 1999 to 2009 from a global atmospheric inversion. *Atmospheric Chemistry and Physics*, *14*(4), 1801–1817. https://doi.org/10.5194/acp-14-1801-2014

Tian, H., Lu, C., Ciais, P., Michalak, A. M., Canadell, J. G., Saikawa, E., et al. (2016). The terrestrial biosphere as a net source of greenhouse gases to the atmosphere. *Nature*, *531*(7593), 225–228. https://doi.org/10.1038/nature16946

Valentini, R., Arneth, A., Bombelli, A., Castaldi, S., Cazzolla Gatti, R., Chevallier, F., et al. (2014). A full greenhouse gases budget of africa: Synthesis, uncertainties, and vulnerabilities. *Biogeosciences*, *11*(2), 381–407. https://doi.org/10.5194/bg-11-381-2014

Welch, B., Gauci, V., & Sayer, E. J. (2019). Tree stem bases are sources of CH4 and N2O in a tropical forest on upland soil during the dry to wet season transition. *Global Change Biology*, *25*(1), 361–372. https://doi.org/10.1111/gcb.14498

Wen, Y., Corre, M. D., Rachow, C., Chen, L., & Veldkamp, E. (2017). Nitrous oxide emissions from stems of alder, beech and spruce in a temperate forest. *Plant Soil*. https://doi.org/10.1007/s11104-017-3416-5

Werner, C., Butterbach-Bahl, K., Haas, E., Hickler, T., & Kiese, R. (2007). A global inventory of N2O emissions from tropical rainforest soils using a detailed biogeochemical model. *Global Biogeochemical Cycles*, *21*(3). https://doi.org/10.1029/2006GB002909

ACKNOWLEDGEMENT

Alhamdulillah!

I am very grateful to the German Research Foundation who provided the funding for my PhD research.

We are only as sturdy as the shoulders we stand upon, and I was fortunate enough to stand atop two brilliant and supportive ones. I am forever indebted to my PhD supervisor, Professor Dr. Edzo Veldkamp for accepting me into his working group, from the masters' level all through my PhD, and for his continuous confidence in me. Ed, your encouraging attitude and emails for a job well done made me feel more confident in myself and in my work. Your door was always open with a warm smile when I had a problem or question, and you created an intellectually stimulating yet informal work culture that made me feel so much at ease to express myself as a scientist. Thank you for been supportive and encouraging on my strides and setbacks.

To Dr. Marife Corre, my co-supervisor: I am grateful to God that you took a chance on me during our bioclimatology class seminar, and I value every opportunity to learn from you. Your enthusiasm and passion for knowledge sharing and good research is both contagious and motivational for me. You have been my primary resource in learning and building scientific expertise. It was your critical feedbacks, several hours of engaged discussions and teaching, selfless acts of kindness, understanding and encouragement that fuelled my stride to the finish line. You remain my role model for a scientist and a mentor, and I hope to emulate your sharp intellect.

I am sincerely grateful to my thesis committee members: Professor Dr. Alexander Knohl for making time to come to my presentations, for the numerous discussions and for his insightful comments and feedbacks; and to Dr. Guntars Martinson for his constructive feedback during my thesis committee meetings. Special thanks to Professor Dr. Klaus Dittert for joining my examination committee, especially considering the circumstances we are in, and for engaging with my PhD viva and making the process a happy experience.

My fieldwork would not have been possible without the continuous effort of Dr. Oliver van Straaten. Among many other things, he coordinated the safe transport of research equipment to Cameroon, assisted with site selections, and was always available to assist me when I had questions in the field. His expertise, supervision and friendship during my difficult moments contributed immensely to the successful completion of my PhD.

Sharing an office with Rodine Tchiofo and Raphael Manu has been one of the highlights of my PhD studies. We had many laughs, regular and constructive discussions, and I benefitted tremendously from their expertise. I am thankful to Rodine, who showed me what an ideal doctoral student looks like with her inspiring work ethic. To Raphael and Belinda, for their lovely home, hospitality and good food that has been my source of comfort in Germany, during good times and difficult ones.

The Department of Soil Science of Tropical and Subtropical Ecosystems has been a source of friendships, support and collaboration. Special thanks to Joost and Greta, for their immense help when my mama was ill, for the many discussions we had, and for all the good laughs; to Xenia, for translating my abstract and sharing the office with me on weekends; to Marcus, for all his R tutorials; to Andrea, for been so kind to me; to Kerstin, Martina, Andrea, Dirk, and Lars for their assistance with laboratory analyses; and to Cecille, Jie, Guodong, Guan-tao, Dan, Lukas and Leonie, for the friendships. I am sincerely grateful to the department for their support during my time of need. Many thanks.

I gratefully acknowledge our counterparts in Cameroon, the International Institute for Tropical Agriculture (IITA) for granting us access and use of their storage facilities, and for other logistical support.

I am especially grateful to my field assistant and friend Leonel Boris Gadjui Youatou, for assisting in the coordination of the project. He was hardworking, trustworthy, and extremely dedicated to practicing good science. Many thanks to Narcis Lekeng, Yannick Eyenga Alfred, Denis Djiyo and all the other field workers for their tireless effort in the field, often under very difficult conditions. I also thank Walther Pohl for helping to transport research equipment to Cameroon and for assisting with site selections.

My heartfelt gratitude goes to my friends: Dr. Issaka Abdulai and his wonderful family for their support and kindness; Grace Cudjoe for her constant support; Divine Torkutsah, Abdul Latif Moudassirrou, Osei-Wusu Emmanuel and Teddy Freduah Agyemang for simply been there for me; Sheikh Bawa Aransa Awuze for all his advice and prayers; and Aastha Tyagi for the good laughs and company. Parisa Mazinanian deserves special thanks for her immense support and encouragement, I could not have done this without her; Hakuna Matata Parpar ☺

Lastly, I would like to thank my family for all their unwavering support and love. I am especially grateful to my Parents, Iddris Issah and Rabiatu Umar for putting aside their dreams so I can achieve my mine, JazākumAllāhu Khayran. You are the best there ever was.

THESIS DECLARATION

I, Najeeb Al-Amin Iddris, hereby declare that I have composed the present thesis independently using no other sources and resources than those stated. In particular, I have completed all parts of the thesis myself; I have neither, nor will I, accept unauthorised outside assistance either free of charge or subject to a fee.

I furthermore declare that this work has not been submitted elsewhere in any form as part of another thesis procedure.

Göttingen, March 2020 _____

(Najeeb Al-Amin Iddris)