QUANTIFYING LEAF LITTER PRODUCTION, DECOMPOSITION, AND NUTRIENT RELEASE IN CACAO PLANTATIONS IN SOUTHWEST NIGERIA

By

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ABSTRACT

Nutrient cycled from litterfall plays an important role in sustaining the fertility status in cacao (*Theobroma cacao* L.) plantations. However, the role of macrofauna in regulating the rate of decomposition and nutrient release remains uncertain despite its importance in litter decomposition and nutrient release. The study was carried out to assess the quantity and seasonal pattern of annual litterfall production in low-shade cacao plantations found in Southwestern Nigeria and also to determine the effects of macrofauna on the rate of cacao leaf litter decomposition and nutrients release.

Field studies were conducted in three locations for 13 months, and the study was a factorial experiment laid out in a randomized complete block design with two replicates; position, sampling dates, and incubation were used as factors. Litterbags and wooden frames were used to study the effects of macrofauna on the rate of decomposition and nutrient release.

The results of this study revealed that litterfall production in the study sites was subject to seasonal variation. Total annual litterfall production in the study sites ranged from 3.50 to 7.71 Mg DM ha^{-1} yr^{-1}. Macrofauna significantly ($p \leq 0.001$) increased the rate of decomposition and nutrient release, but the rate of decomposition was strongly regulated by litter quality. The rates of nutrients release were in the following order Nitrogen>Potassium>Phosphorus.

The nutrients release from cacao litter would play a significant role in the nutrition of the cacao tree.

Keywords: Cacao, Litter decomposition, Litterfall production, Litter bag, Nutrient release.

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CERTIFICATION

We certify that this study on "quantifying leaf litter production, decomposition and nutrients release" in cacao plantation was carried out by Oluwafemi OYEDELE of the Department of Soil Resources Management, University of Ibadan, Ibadan, Nigeria.

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DEDICATION

I dedicate this work to the Glory of God and my parents of blessed memory; late barrister J. O. Oyedele and late Mrs Grace Aina Oyedele.

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Abbreviations and Acronyms

DAI:	Days After Incubation
k:	Annual Decomposition Constant
Mg:	Megagram
DM:	Dry Matter
VPD:	Vapour Pressure Deficit
USDA:	United States Department of Agriculture
C/N:	Carbon/Nitrogen ratio
ha^{-1}:	Per hectare
ANOVA:	Analysis of Variance
LME:	Linear Mixed Effects Model
NLME:	Non-Linear Mixed Effects Model

Chapter 1

1.0 Introduction

1.1 Background to the study

Cocoa is an important cash crop that is mostly grown by small-holder farmers in West Africa, who depends on the crop for their livelihood (Fungenzi *et al*., 2021). Although, potential yield of cocoa exceed 6000 kg/ha, average farmers yield are often around 400 to 500 kg/ha (van Vliet *et al*., 2017). The major production constraints affecting small-holder farmers in West Africa is the depletion of soil nutrients due to continuous cropping with little or no nutrients replenishment (van Vliet *et al*., 2017). Most farmers maintain soil fertility under cacao plantations by recycling materials produced within the system (Dawoe *et al*., 2010 and van Vliet *et al*., 2017).

Litterfall and decomposition of litter, followed by nutrient release, are important biological processes for transporting materials from plants to soils (Dawoe *et al*., 2010). Litterfall is not only critical for maintaining nutrients cycling but also in sustaining soil fertility in cacao production system (Dawoe *et al*., 2010). Sreekala *et al*. (2001) reported that Litter serves as a nutrient input-output system, with decomposition serving as the primary means of returning organic matter and nutrients to the soil for re-absorption by plants. Litterfall is the primary source of nutrients to soils in the cacao production system, and the rates of decomposition and nutrients release depend on the chemical quality of the litter (Marco *et al*., 2013). Quimpang *et al*. (2017) argued that nutrients taken up by cacao trees during the growing season are returned to the soil through litterfall and are then progressively release during the decomposition process, which is then made available for uptake by plants or can be lost through leaching.

According to Bradford *et al*. (2016), decomposition is defined as the process by which carbon and nutrients are transferred from plant residues to decomposer organisms. Decomposition of litter is a critical biogeochemical process that regulates the transfer of carbon and nutrients to the soil (Sun *et al*., 2019). Decomposition is an important ecological process in the formation of soil organic matter, mineralization of organic

nutrients, and maintenance of carbon balance in forest ecosystems (Sanghaw *et al*. 2017).

Dawoe *et al*. (2010); Moretto and Pastur (2014), studied the decomposition rate of cacao litter, and they found out that the decomposition rate is not only regulated by the interactions between litter quality, climatic factors, and decomposer organisms, but also litter quality influence the mineralization and immobilization process.

1.2 Justification of the study

Decomposition rates of cacao litter are scarce in literatures. Some authors (Dawoe *et al*., 2010 and Fontes *et al*., 2014) confusingly used the "k" annotation for decomposition rate that differ from the (Olson's, 1963) decomposition constants (also annotated "k"). The method used in these studies involves simultaneous assessment of the litter production and the litter-stock at discrete time-points. The method is subject to large measurement errors related to the sampling strategy but only (Muoghalu and Odiwe, 2011) reported the "real" decomposition constants for cacao litter in West Africa. We need more studies to consolidate this knowledge which will not only improve the nutrition of the cacao tree but also enhance the carbon stock modelling.

1.3 The objectives of this study are:

1. To determine the quantity, and seasonal patterns of annual litter production in low-shade cacao plantations commonly found in Southwestern Nigeria.

2. To determine the effect of macrofauna and litter quality on the rate of cacao leaf litter decomposition and nutrients (N, P, and K) release

Chapter 2

Literature Review

2.1 Description of the Cacao Tree

Cacao (*Theobroma Cacao* L.) is a tropical crop that is small to medium-size in shape, 3-10 meters in height, and it belongs to the family Malvaceae; after 3 to 4 years of rapid growth, the cacao tree grows more steadily and flowers appears on the trunk and branches throughout the year as long as no extreme drought or seasonal fluctuations occur, it does not like too much sunlight and it grows in shade with banana, plantain, coconut, oil palm and orange (van Vliet *et al*., 2015). The pods develop within 5 to 6 months from the flowers and are pollinated by insects, mainly midges of the genus *Forcipomyia* species and *Lasiohelea* species and the fruit is egg-shaped, and its pod is red to brown and contains 30 to 50 seeds each of which is surrounded by a bitter-sweet white pulp. When the seeds are dried and fermented, they are brownish red, and known as cacao beans which is the principal ingredient of chocolate (van Vliet *et al*., 2015).

According to Almeida and Valle (2007), cacao flowers that is produced on the trunk and branches of the cacao tree which are not pollinated are abscised 24 to 36 days after opening and the percentage of flowers that set pods in cacao is usually about 0.5 to 5%. The young pods are called ‘cherelles’, and during the first 50 to 100 days after fruit set, the growth of cherelles can stop, and the cherelles then becomes yellow, shrivels, and blackens, this process is known as cherelle wilt and pods which pass the wilting

stage take about 5 to 6 month from flowering to develop; and cacao has two season, which is classified as the main crop (September to January); and the light crop (February to August) (van Vliet *et al*., 2015).

2.2 Physiology of Cacao

Cacao always displays a major hereditary variability in morphological and physiological characteristics (Lahive *et al*., 2019). When multiplied by seeds, it initially has an orthotropic growth pattern that reveals leaf flushing in cycles, in which the leaf arrangement is alternate and in addition, leaf emergence occurs in a relatively climate-independent manner, suggesting that the growth rhythm is under endogenous influence, but however, as the orthotropic growth ceases, the plant emits plagiotropic branches at a height of approximately 1 to 1.5 metres (Lahive *et al*., 2019). Environmental factors have about 70% impact on the development of the cocoa tree (Almeida and Valle, 2007). Cacao has broad leaves that lose water quickly under high irradiance conditions (Lahive *et al*., 2019).

Cacao trees require a stable warm and humid climate and are vulnerable to climate extremes (Niether *et al.*, 2018). Temperatures should not fall below 15°C during high ambient temperatures, which adversely affect yield and decomposition rates (Niether *et al.*, 2018). High temperature can also cause indirect stress because of higher evapotranspiration air demand (Niether *et al.*, 2018).

Cacao requires maximum rainfall between 1500 to 2000 mm with less than three months of the dry season (Zakariyya and Indradewa, 2018). During the drought period, stomata closure is a strategy of cacao plants for diminishing water loss through the transpiration process (Zakariyya and Indradewa, 2018). Water deficit in cacao trees triggered the shedding of leaves from the cacao tree, a change response to reduce water loss through transpiration (Niether *et al*., 2020). The reduction leaf area and number of stomata help the cacao tree adapt to water deficit (Niether *et al*., 2020). The physiological response of cacao to water-logging, flooding and reduction in Co_2 compensation, which may occur as early as a few hours after the stress is imposed, is paralleled by a reduction in photosynthetic rate, suggesting an increase in stomatal limitation to photosynthesis (Almeida *et al.*, 2016).

Asare *et al*. (2017), studied the influence of shading and fertilization on on-farm yield of cacao in Ghana, and their findings revealed that the most important climatic factors affecting the growth and development of cacao are rainfall and temperature. According to Niether *et al*. (2020), who reviewed the effects of shade on the physiology of cacao and they found out that the decrease of the xylem water potential in cacao tree in response to drought is an indication that cacao tree is susceptible to drought. Almeida and Valle (2007), reviewed the eco-physiology of cacao and their findings revealed that light intensity has a significant effect on leaf development and photosynthetic capability of mature leaves.

Saj *et al*. (2021) also, studied the litterfall and seasonal dynamics in cacao agroforests and they find out that extended dry season, reduced humidity, and lower night temperatures are associated with the stimulation of abscisic acid synthesis in plant foliage which stimulates leaf senescence, this is because cacao is a semi-deciduous tree that is expected to shed its leaves at a certain period of the year in response to environmental changes.

2.3 Litterfall Production in Cacao Agroforestry System

The material fixed in the aboveground biomass is eventually returned to the soil through litterfall (Muoghalu and Odiwe, 2011). Litterfall is an essential process in nutrient cycling and is a primary source of organic and mineral elements transferred from vegetation to the forest floor (Muoghalu and Odiwe, 2011). Litterfall provides an important pathway for returning organic matter and nutrients from the plant's leaves to the soil (Odiwe *et al*., 2016; Pushpa *et al*., 2017 and Berg, 2018). Production of the litter varies according to stand features (tree size, species (type of the tree), age and tree density), geographical location (climate) and site characteristics (soil), season, and management (Negash and Starr, 2013). Aboveground litter is the source of nutrients deposited in the soil's upper layers, and it plays a vital role in nutrient turnover and energy transfer between plants and soil (Siddiqui *et al*., 2009). When leaves fall to the forest floor, they build up a layer of nutrients and debris on top of the soil. This layer is essential not only for the food chain because it provides food for many microorganisms, but it also serves to recycle nutrients back into the soil (Abugre *et al*., 2011). Physical factors such as wind and rain, physiological responses of plants to environmental changes, influence litterfall production (Dawoe *et al*., 2010). Older plantations (>30

years) produce a high quantity of litter than younger plantations (<15 years), so there is a direct correlation between the older and younger plantations (van Vliet *et al*., 2015). Fontes *et al*. (2014), studied the nutrient stocks in litterfall and litter in cacao Agro-forests and their findings revealed that the mean total annual litterfall in cacao ranged from 4.6 and 8.8 Mg DM ha^{-1} yr^{-1}. Dawoe *et al*. (2010), studied the litterfall and litter nutrient dynamics in cacao ecosystem in Ghana and their findings revealed that the mean total annual litterfall in lowland humid Ghana ranged from 5.0 to 10.4 Mg DM ha^{-1} yr^{-1}. According to Hartemink (2005), who reviewed the nutrient stocks and nutrient cycling in cacao ecosystems, and his finding revealed that total annual litterfall ranged from 3.9 to 5 Mg DM ha^{-1} yr^{-1}.

2.4 Litter Decomposition

Litter decomposition can be defined as the process by which energy and nutrients are transferred from plant residues to decomposer organisms (Sanghaw *et al*., 2017). Decomposition is also defined as the physical and chemical breakdown of litter that reduces litter to Co_2, water, and mineral nutrients and is regulated by several abiotic and biotic factors (Baker *et al*., 2001; Triadiati *et al*., 2011). The major process for returning organic matter and nutrients to the soil for reabsorption by plants is decomposition (Sreekala *et al*., 2001). Decomposition involves mass loss, changes in the nutritional content of plant litter and, nutrient release (Murovhi *et al*., 2015). Litter decomposition is critical to biogeochemical nutrient cycling, which determines the productivity of agro-ecosystems (Mohammed *et al*., 2019). Decomposition is essential for the functioning of the ecosystem because if nutrients are released quickly, they can be lost through leaching or volatilization (Mohammed *et al*., 2019). The slow rate of decomposition of cacao litter may cause insufficient nutrients to be available for uptake by the cocoa tree, thus, limiting their growth (Petit-aldana *et al*., 2019). The majority of the nutrients absorbed by plants are the result of decomposition and mineralization of leaf litter (Muoghalu and Odiwe, 2011).

Cacao litter is of low quality (N< 2%, and C/N ratio >25) and exhibits a slow decomposition rate compared to other shade trees found in cacao plantations that are of high quality (Sauvadet *et al*., 2020).

Micro-climate and litter quality controls litter decomposition contributing about 60-70% of litter decomposition rates and soil fauna communities contributes about 7% of litter decomposition rates (Djukic *et al*., 2018). Berg (2014), studied the decomposition pattern of foliar litter and his finding revealed that climate has an indirect effect on decomposition and litter quality is the main factor that determines the rate of decomposition. The most important abiotic factors governing the decomposition rate are humidity and temperature. Litter decomposition is very slow during the period of low humidity (<60%), and it increases during the rainy season when high humidity (>80%) and low temperatures occur (<25°C) (Silva *et al*., 2018). According to Dawoe *et al*. (2010), the decomposition rate of cacao litter for secondary forests in the Ashanti region of Ghana was very slow as a results of poor litter quality (N<2% and C/N ratio>25). The rate of litter decomposition not only increases when the carbon to nitrogen (C/N) ratio is low (<25) but also when the initial nitrogen (N) concentration increases (>2%) (Yang and Chen, 2009). Riggs *et al.* (2015) studied the effects of nitrogen addition on decomposition rate and they found out that nitrogen addition is one of the most critical factors that facilitate decomposition rate. The low litter decomposition rate of cacao litter results in the widespread accumulation of litter in forest floors in cacao plantations (Muoghalu and Odiwe, 2011).

Management practices, such as the widespread use of pesticides to control pests, the use of herbicide, and fungicide in cacao plantation, may cause the disruption of nutrients cycling by adversely affecting the microorganism, especially bacteria and fungi; involved in decomposition processes, thus altering nutrient cycling in these ecosystems (Muoghalu and Odiwe, 2011).

2.5 Nutrient Release from Litter Decomposition

Nutrient release is the process by which organically bound nutrients is released as free ions into the soil solution during the litter decomposition process, making them available for plant uptake (Mohammed *et al*., 2019). Nutrients such as nitrogen, phosphorus, and potassium are released during the decomposition of cacao litter, and they can be absorbed by plants and soil fauna (Odiwe *et al*., 2016).

The major way by which nutrients are recycled back to the soil is through litterfall (Odiwe *et al*., 2016). The amounts of residues and nutrients returned to the soil

through litterfall is determined by the rate of litter decomposition (Saj *et al*., 2021). The decomposition and nutrients release in cacao leaf litter are vital in ensuring the proper functioning of the biogeochemical cycle processes and encouraging appropriate physical, chemical and biological soil conditions (Petit-aldana *et al*., 2019). The production and breakdown of litter regulate nutrient recycling, which is important for sustaining soil fertility. The amount of nutrients release by decomposing litter is highly dependent on environmental factors, the chemical composition of the litter, and the decomposer organisms (Murovhi *et al*., 2015). These variables work at various geographical and temporal dimensions, with climate prevailing at the regional scale and litter quality dominating at the local scale (Murovhi *et al*., 2015). Since nutrients are translocated before litterfall, nutrient concentrations in litterfall are lower than in fresh leaves and the amount of nutrients released is determined by litterfall and nutrient concentrations (Hartemink, 2005). As plant litter decomposes on the soil surface, nutrients may either remain in the soil in mineral form, be integrated into soil biomass and organic matter (immobilisation), be taken up by plants, or be removed from the system through leaching or gaseous form (Schroth, 2003). van Vliet *et al*. (2015), reviewed the mineral nutrition on cacao and they found out that organic residues offer an advantage over typical NPK fertilizers by supplying extra nutrients to the soil for plant absorption such as Ca, Mg and micronutrients.

2.6 Litter Quality

Litter quality refers to the physical and chemical properties of plant residues which determines the rate of decomposition (Pushpa *et al*., 2017). High-quality litter (>2% N and C/N ratio<25) decomposes fast, whereas low-quality litter (<2% N and C/N ratio> 25) decomposes slowly (Pushpa *et al*., 2017). The potential rate of decomposition is governed by the quality of the litter (Shepherd *et al*., 2005). Mohammed *et al*. (2019), reviewed the leaf litter decomposition and mitigation of Co_2 emissions in cacao ecosystems and their findings revealed that C/N ratio of plant litter is the main factor that regulate the rate of decomposition and nutrient release. Kwabiah *et al*. (1999), studied the effects of inorganic fertilizer on the decomposition of plant litter and they found out that nitrogen and phosphorus deficiencies are common in humid tropical soils and may potentially limit macrofaunal activity, thus reducing the decomposition rate. According to Fontes *et al*. (2014) and Berg (2014), nutrient stocks in litterfall in a cacao

agroforestry system, they found out that the negative correlation between the decomposition rate, and litter quality indices (N, P and C/N ratio), indicate that litter quality is the most important factor controlling the decomposition rate. According to Sanghaw *et al*. (2017), the chemical quality criteria utilised as a robust indicator of decomposition include nitrogen and the carbon/nitrogen ratio. Djukic *et al*. (2018), investigated the early stages of mass loss in two litter types, green tea leaves (Camellia *sinensis*) and Rooibos tea leaves (Aspalathus *linearis*), and discovered that litter quality was the most important determinant of decomposition while climate and land use had negligible effects on the rate of decomposition.

2.7 Effects of Macrofauna on Decomposition Rate

Macrofauna (>2mm in size), especially termites, ant and earthworms are important components of the soil ecosystems and, as ecosystem engineers, they influence soil processes, such as organic matter decomposition and nutrients release (Ayuke, 2010). Macrofauna primarily modify the soil environment and makes nutrients to be available for uptake by plants (Xin *et al*., 2012).

Macrofauna also regulates litter decomposition, nutrient transformation, and primary production in an ecosystem by fragmenting or comminuting litter material and indirectly by altering the structure and function of the microbe population while consuming soil microbial biomass and excreting nutrient-rich wastes (Wang *et al*., 2010). Macrofauna plays a critical role in leaf litter decomposition by dispersing soil microorganisms (Frouz *et al*., 2015). Macrofauna is essential for litter shredding, transformation, and decomposition (Sofo *et al*., 2020). The presence of macrofauna has an immediate impact on litter decomposition by feeding on the litter (Bradford *et al*., 2002). Macrofauna plays a vital role in decomposition by eating residual plants and animals that produce waste in the form of organic matter, which is then released into the soil for eventual uptake by the plant (Tongkaemkaew *et al*., 2018). According to Sanghaw *et al*. (2017), macrofauna directly contributes to decomposition by breaking down plant litter into smaller pieces, which opens up new litter surfaces for a microbial attack. The impact of macrofauna on decomposition varies according to litter type and chemical composition. Furthermore, its influence is stronger in recalcitrant plant litter than in more easily decomposable counterparts (Sanghaw *et al*., 2017). Sanghaw *et al*. (2017), investigated the use of residue quality parameters to predict the effects of larger

soil fauna on decomposition, and they found out that macrofauna has a significant effect on the rate of decomposition. Schmidt *et al*. (2008) reported that macrofauna had a significant ($p \leq 0.001$) effect on the decomposition rate of Tibouchina *pulchra* trees in the Atlantic Rainforests of Southern Brazil. Macrofauna plays an important role in nutrient cycling (Ayuke, 2010). Slade and Riutta (2012) investigated the interactive effects of forest edge, moisture, and soil macrofauna on leaf litter decomposition and discovered that the presence of macrofauna enhanced the rate of decomposition of recalcitrant oak leaf litter. The impact of macrofauna on litter decomposition may vary depending on climate, litter species, and soil microbial population (Berg, 2014). Yang and Chen (2009), studied the impacts of macrofauna on litter decomposition in humid tropical forests in China, and their findings revealed that macrofauna plays an important role in litter decomposition. According to Sanghaw *et al*. (2017), the impacts of macrofauna on the breakdown of resistant litter are particularly evident in the late stage of decomposition. Djukic *et al*. (2018) studied the early-stage mass loss of two typical litter types, green tea leaves (Camellia *sinensis*) and Rooibos tea leaves (Aspalathus *linearis*) and his findings indicated that macrofauna accounts for around 7 per cent of litter decomposition rates.

2.8 Role of Environmental Factors in Decomposition Process

Wang *et al*. (2010) opined that climate is an important factor in predicting large-scale decomposition patterns. Berg (2014) studied the decomposition pattern of foliar litter and he found out that climate has an effect on decomposition rate. Climatic variables such as air temperature, relative humidity, and rainfall have a significant effects on litter decomposition rate (Jeyanny *et al*., 2015).

Chapter 3

Materials and methods

3.1 Site description

This study was carried out from January 2020 to January 2021 on low-shade cocoa plantations in Southwestern Nigeria in three locations: Ago-Owu (longitude: 04° 15.1'E; latitude: 07° 786'N, elevation: 272 meters a.s.l.); Akowonjo-Akoko (longitude: 05° 38.25'E; latitude: 07° 35.73'N elevation: 328 meters a.s.l.), and Ijebu-Itele (longitude: 04° 10.5'E; latitude: 06° 786'N elevation: 295 meters a.s.l.), respectively Fig.3.1. Weather data on temperature, and relative humidity were collected with the aid of GSP data logger while daily rainfall was recorded with the aid of rain gauge that was installed at the experimental site. The average monthly temperature range between 23.5°C (September) to 26.74°C (January) and relative humidity ranges from 55.45% (September) to 98.17% (December) with average annual rainfall which varied from 1128 mm to 1193 mm yr^{-1} with July and September as peaks separated by a dry spell from November to early March respectively. The predominant vegetation in the study area is the semi-deciduous forest type, with most of the trees shedding their leaves in the dry season. The soils of the study area are developed from weathered quartzite schist and classified as Alfisol (Soil Taxonomy, 2010). They are deep, moderately well-drained with a textural class that varies from sandy clay loam to sandy loam and the soil bulk density was very low ranging from 1.11 to 1.15 Mg m^{-3} and the plantation characteristics of the sites are shown in Table 3.1.

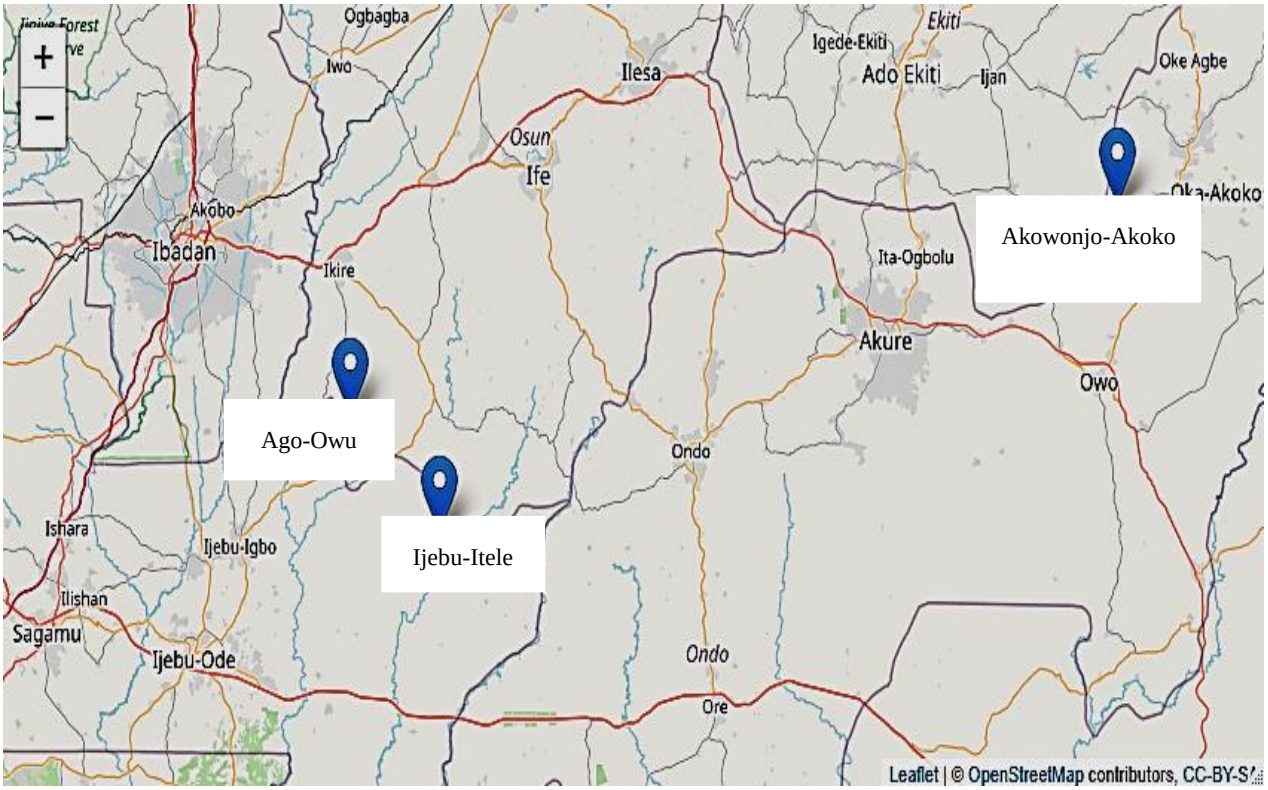

Fig 3.1: Map showing the experimental sites.

Table 3.1: Plantation Characteristics

Locations	Production System	Age (Years)	Management
Ago-Owu	Agroforestry	18	332 grams of NPK 15:15:15 fertilizer was applied in split dose per tree, and the first dose was applied in June while the second dose was applied around September, fungicide was used for controlling black pod disease and herbicide was used for weed control and we have 985 cocoa tree per hectare planted at a spacing of 3.5 × 2.9 meters and we also have 27 shade trees that are planted at 1.8 × 7.6 meters apart.
Akowonjo Akoko	Agroforestry	15	150 grams of NPK 15:15:15 fertilizer; fungicide was used for controlling black pod disease while herbicide was used for weed control. The cocoa tree density was 1087 trees per hectare at a spacing of 3.4 × 2.7 meters apart with 8 shade trees.
Ijebu Itele	Agroforestry	23	Application of 332 grams of NPK 15:15:15 fertilizer was applied in split dose per tree, and the first dose was applied in June while the second dose was applied around September, fungicide was used for controlling black pod disease and herbicide was used for weed control. The cocoa tree density was 1162 trees per hectare planted at a spacing of 3.3×2.6 meters, while the shade tree density was 56 trees planted at a spacing of 3.4 ×3.6 meters.

3.2 Sampling

3.2.1 Litterfall sampling

Litter traps were placed in each site in December 2019, and the litter trap were distributed in each sites using a stratified random sampling, litterfall was collected every 14 and 21 days during the wet and dry seasons from January 2020 to January 2021, but due to the outbreak of the covid-19 pandemic, we were unable to quantify the litterfall in the late dry season from April to June, 2020. Ten litter traps were placed in each plot in two replicate for each of the three plantations, making a sum 60 litter traps or 20 litter traps per plantation (Anderson and Ingram, 1993). To improve the plot's representativeness, litter traps (0.4 m^2) were randomly distributed in a zigzag pattern within the experimental plot (441 m^2) (Plate 3.1) in a direction facing the true south in which one litter trap was placed 0.24 meters close to the crown of the cacao tree while the other litter trap was placed 1.38 meters away from the crown of the cacao tree based on heterogeneity (Schroth, 2003). The litter collected in each trap was oven-dried at 65°C, and then the leaves were sorted according to constituents: cacao leaves, shade tree leaves, and miscellaneous materials (twigs, flowers, and any other unidentified tiny plant part), and the dry biomass was determined using a sensitive laboratory scale. The annual litterfall for each site was calculated by summing the monthly values of litterfall production for each land use (Fig 3.2).

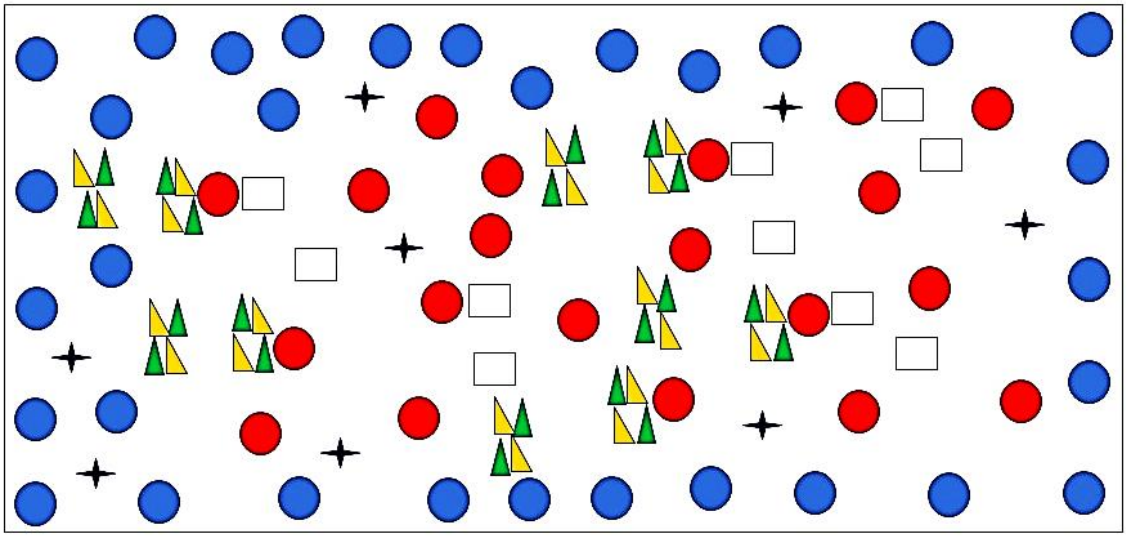

Fig 3.2: Experimental layout with 20 cacao trees at the net plot (red) where the experiment was set up, litter trap (square), litter bag (yellow triangle), wooden frame (green triangle), shade tree (star) and the border cacao tree (blue).

Plate 3.1: Litter Trap used for quantifying litterfall (80 cm × 50 cm)

3.2.2 Decomposition

The litterbag and wooden frame (0.4 x 0.3 m^2) were used to determine the decomposition rate and nutrients release from cacao leaves over time. The experiment was conducted from December, 2019 to January, 2021 on the same cacao plantations. Litterbag was constructed from aluminium netting with 2 mm mesh size to exclude macrofauna, while wooden frames were constructed from wooden materials in which access is prevented from the top with a net of 2 mm mesh size, to avoid additional materials to fall into the wooden frame, but access is given from below and macrofauna could access from the side where the wooden frame is not in tight contact with the soil. In early December 2019, freshly-senesced cacao leaves of the current season were collected from the study sites, the cacao leaves were air-dried for 4-5 days in the screen house, and then a sub-sample was oven-dried at 65°C for 72 hours to determine the initial dry matter content of the fresh cacao leaves before incubation in the field. A cluster of thirty cacao trees were identified in the study sites. Then five cacao trees were selected at random and then a compass was used to identify a true north and then forty grams of fresh cacao leaves were incubated in a net bag and wooden frames (0.4 x 0.3 m^2) in December 2019 in which pairs of litterbag and wooden frames were placed close and away from the cacao trees at random based on heterogeneity (Plate 3.2, A and B). A total of 120 pairs of litterbag and wooden frames (two mesh sizes, two-position, three locations and 20 sampling dates) and the incubated samples were retrieved from the field every 14 and 21days interval during the wet and dry season to observe the pattern of weight loss and nutrients release over time, but due to COVID-19 pandemic, there was a time lag of three month from April to June 2020, were by we could not carry out sampling. Each sampling date, one litter bag and wooden frame were retrieved at random from each site. Samples retrieved at each sampling date were oven-dried at 65°C for 72 hours, and then we carefully brushed the leaves to remove the adhering soil particles attached to the leaves and then weighed the leaves to obtain the final dry matter weight. Loss in weight in the litter samples was calculated from the initial oven-dried weight and the final dry matter weight. The leaf litter's decomposition constant (k) was calculated using the first order exponential model (Olson, 1963).

$M_t = M_o e^{-kt}$

Where;

M_t = mass of the material remaining at time t (days)

M_o = initial mass of the material at time 0

e = base of natural logarithm

k = exponential decay coefficient (day^{-1})

t = time (days)

3.2.3 Macrofauna Species Abundance

Macrofauna counted individuals (termite, ants, and worm cast) in the study sites were observed during the period of this study.

3.2.4 Nutrient Release

The amount of nutrients remaining in the litterbag at each sampling date was determined by multiplying the mass of residue remaining by their respective concentrations. We assumed that the amount of nutrients released or immobilized at each sampling date was the difference between the amount of nutrients in the initial leaf materials and the amounts in the materials at the given sampling date.

A

B

Plate 3.2: Litter Bag (A) and Wooden Frame (B) used for the incubation study (40 cm × 30 cm)

3.3 Laboratory analysis

3.3.1 Soil chemical analyses

Ten soil auger samples at 0 to 30 cm depth were collected using a stratified random sampling method per duplicate. The samples were bulked to obtain one composite sample per duplicate. The collected samples were air-dried, grounded, mixed thoroughly, and sub-sampled were sent to the analytical laboratory of the International Institute of Tropical Agriculture (IITA) for chemical analyses. Particle size distribution were determined by the hydrometer method according to the procedure of Gee and Or (2002). Bulk density was determined by core methods, according to Grossmans and Reinch (2002). pH was determined in 1:2.5 (w/v) soil: water suspension, according to McLean (1982). Exchangeable basic cations, Ca^{2+}, Mg^{2+} and K^{+} were extracted by the Mehlich-3 procedure. Cations were determined by atomic absorption spectrophotometer and phosphorus by the molybdate blue procedure described by (Murphy and Riley, 1982). Organic carbon was determined using the chromic wet oxidation method according to Nelson and Sommers (1982). Total nitrogen was determined by the Kjeldahl digestion method using concentrated H_2SO_4 and Sodium Copper sulphate catalyst mixture.

3.3.2 Leaf chemical analysis

Composite samples of leaves from each plantation were analyzed for carbon and major nutrients (N, P, and K) in duplicate from June, 2020 to January, 2021. All samples were oven-dried at 65°C for 72 hours and grounded to pass through 0.5 mm mesh. Carbon was determined by the wet oxidation method (Walkley and Black, 1934). Total nitrogen was determined by the standard micro-digestion method (Kjeldahl) with colorimetric determination by spectrophotometer. Potassium was analyzed by atomic absorption spectrophotometer while phosphorus was determined by Murphy and Riley method (1982).

3.4 Statistical analysis

Data collected on litterfall were subjected to analysis of variance (ANOVA) and significantly different means were separated using Tukey's Honestly Significant Difference (HSD) at $P \leq 0.05$ probability level. The assumption of normality and

homogeneity of variance was checked using the Shapiro-Wilk test and Levene's test, respectively. Where these assumptions were not met, the data on litterfall where log transformed to meet the normality and homoscedasticity of the residual assumptions. Before the analysis of variance and after the transformation, and if this assumption is not met, the data on litterfall was analyze using Kruskal-Walis one-way ANOVA. Non-linear mixed-effect models were used to evaluate the effects of macrofauna and litter bag position on the rate of decomposition, where position and incubation are used as fixed effect while location was used as a random effect and residual weight was used as the response variable in the model (Pinheiro *et al*., 2020). Step-wise regression was used to select all the predictors to include in the model, where Incubation, Position and macrofauna where used as explanatory variables and residual weight was used as the response variable in the model. The Pearson correlation was used to assess the relationship between litter quality and the rate of decomposition. We analysed all data using R (R Core Team, 2020) and the dplyr package was used for data management (Wickham *et al*., 2020) and the results were visualized using ggplot2 (Wickham, 2016).

Chapter 4

RESULT

4.1 Meteorological data

The average annual rainfall in the study sites ranged from 1128 mm to 1193 mm (blue), average temperature ranged from 23.5°C to 26.75°C (red) while relative humidity at the study sites ranged from 55.45% to 98.17% (black) respectively (Fig 4.1).

4.2 Initial soil physical and chemical properties at 0 to 30 cm depth at the study sites after residue incubation

Some of the selected physico-chemical properties of the soils in the study sites are: pH which ranged from 6.20 to 6.85 showing that the soils were slightly acidic; organic carbon (g kg^{-1}) ranged from 8.53 to 11.80; total nitrogen (g kg^{-1}) ranged from 0.79 to 1.21; available phosphorus (mg kg^{-1}) ranged from 8.24 to 35.07; potassium content (cmol kg^{-1}) ranged from 1.36 to 1.81; magnesium content (cmol kg^{-1}) ranged from 8.67 to 6.70; sand fraction (g kg^{-1}) ranged from 680 to 750; silt fraction were 90 (g kg^{-1}); and clay fraction (g kg^{-1}) ranged from 160 to 230; the soil bulk density (Mg m^{-3}) varied from 1.11 to 1.15 and the soil textural class ranged from sandy clay loam to sandy loam respectively (Table 4.1).

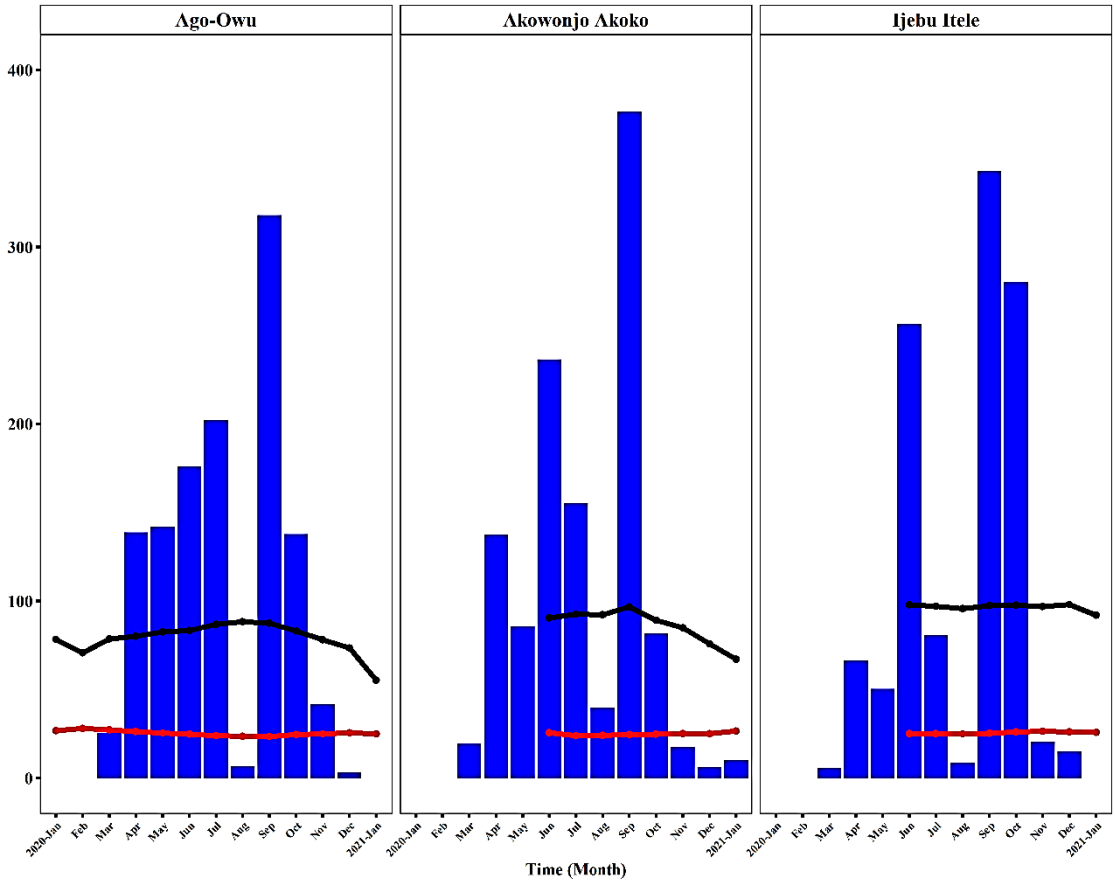

Fig 4.1: Monthly weather pattern at the study sites

Source: (Field data, 2021)

Table 4.1: Initial soil Physical and Chemical properties at 0 to 30 cm depth at the study sites after residue incubation

Parameters	Locations		
	Ago-Owu	**Akowonjo Akoko**	**Ijebu Itele**
Sand (g kg^{-1})	680±0	730±50	750±30
Silt (g kg^{-1})	90±10	90±10	90±10
Clay (g kg^{-1})	230±10	180±40	160±20
Bulk density (Mg m^{-3})	1.11±0.04	1.15±0.06	1.12±0.04
Textural class (USDA)	Sandy clay loam	Sandy loam	Sandy loam
pH (H_2O)	6.85±0.05	6.20±0.20	6.70±0.50
Organic carbon (g kg^{-1})	11.80±0.47	8.53±0.49	9.60±1.53
Total nitrogen (g kg^{-1})	1.21±0.06	0.79±0.06	0.86±0.08
Available Phosphorus (mg kg^{-1})	35.07±9.88	8.24±9.88	28.01±2.82
Potassium (cmol kg^{-1})	1.81±0.25	1.36±0.10	1.51±0.05
Calcium (cmol kg^{-1})	$64.67±5.57^{a}$	$52.55±3.48^{ab}$	$16.31±3.21^{b}$
Magnesium (cmol kg^{-1})	$8.67±0.05^{a}$	$6.20±0.20^{b}$	$6.70±0.50^{b}$

Mean values (±SEM) in the same row followed by the same superscript for different locations were not significantly different at $P \leq 0.05$ according to Tukey's HSD test;
SEM = Standard Error of Means

4.3 Litterfall production

The monthly pattern of litterfall production follows a similar pattern among the study locations with dips during the wet season (July to October) and peaks during the dry season (November to March) respectively (Fig 4.2). There were significant differences in cacao leaves ($p \leq 0.050$), shade tree leaves ($p \leq 0.00$) and total litterfall ($p \leq 0.001$) but however, there was no difference in the miscellaneous fraction ($p \geq 0.590$) between the study locations respectively (Table 4.2). Significant ($p \leq 0.05$) difference was observed between shade tree leaves and total annual litterfall between seasons while there was no difference ($p \geq 0.05$) in the cacao leaves and miscellaneous fraction produced between season; and a similar pattern ($p \geq 0.05$) was also observed between position (close and away from the cacao tree) respectively (Table 4.2). The contributors to total annual litterfall were in the following order; cacao leaves > miscellaneous fraction > shade tree leaves respectively (Table 4.2). Total annual litterfall production between seasons is dependent on the study locations (Fig 4.3).

4.4 Chemical quality of cacao litter

There were significant differences in nitrogen content ($p = 0.001$) between the study locations, potassium content and Carbon/Nitrogen ratio also follows similar pattern ($p \leq 0.05$), respectively (Table 4.5). There was no significant difference in phosphorus content ($p = 0.560$) between study locations, Carbon/Phosphorus ratio also follows a similar pattern ($p \geq 0.05$) (Table 4.3).

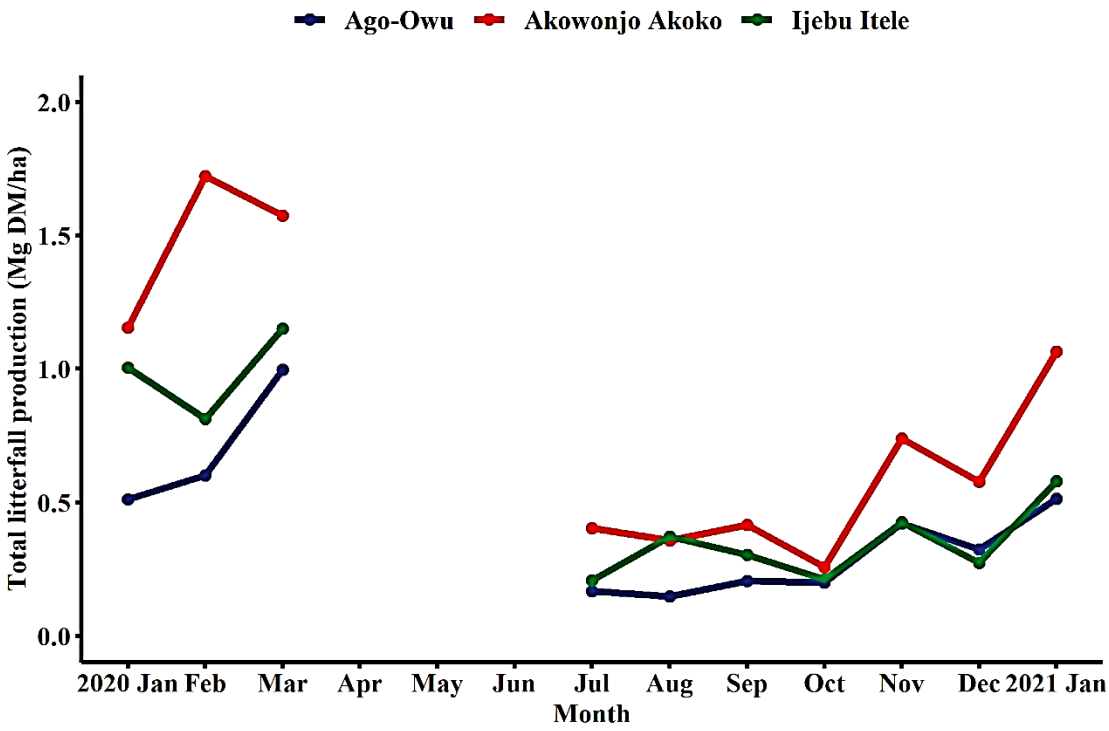

Fig 4.2 Monthly pattern of litterfall production at the study sites

Table 4.2: Quantity and seasonal pattern of litterfall fractions production at the study sites

Treatment	Cacao leaves	Shade tree leaves	Miscellaneous	Total litterfall
	(Mg DM ha $^{-1}$ yr^{-1} ±SEM)			
Locations				
Ago-Owu	3.20±0.30^{b}	0.01±0.00^{b}	0.29±0.06^{a}	3.50±0.32^{b}
Akowonjo Akoko	7.24±0.73^{a}	0.12±0.03^{a}	0.34±0.06^{a}	7.71±0.76^{a}
Ijebu Itele	4.71±0.48^{b}	0.02±0.00^{b}	0.26±0.05^{a}	4.49±0.48^{b}
Seasons (S)				
Dry season	5.65±0.51^{a}	0.09±0.02^{a}	0.36±0.06^{a}	6.10±0.55^{a}
Wet season	4.55±0.45^{a}	0.01±0.00^{b}	0.25±0.03^{a}	4.81±0.45^{b}
Positions (P)				
Close to cacao tree	5.14±0.49^{a}	0.06±0.02^{a}	0.30±0.04^{a}	5.50±0.50^{a}
Away from cacao tree	4.96±0.47^{a}	0.04±0.01^{a}	0.29±0.05^{a}	5.30±0.50^{a}
Location × Season	*	***	NS	*
Location × Position	NS	NS	NS	NS
Season × Position	NS	NS	NS	NS
L × S × P	NS	NS	NS	NS

Mean values (±SEM) in the same column followed by the same superscript for different locations were not significantly different at $P \leq 0.05$ according to Tukey's HSD test; NS= Not Significant; Legend: S = Season; L = Location; P = Position; SEM = Standard Error of Means

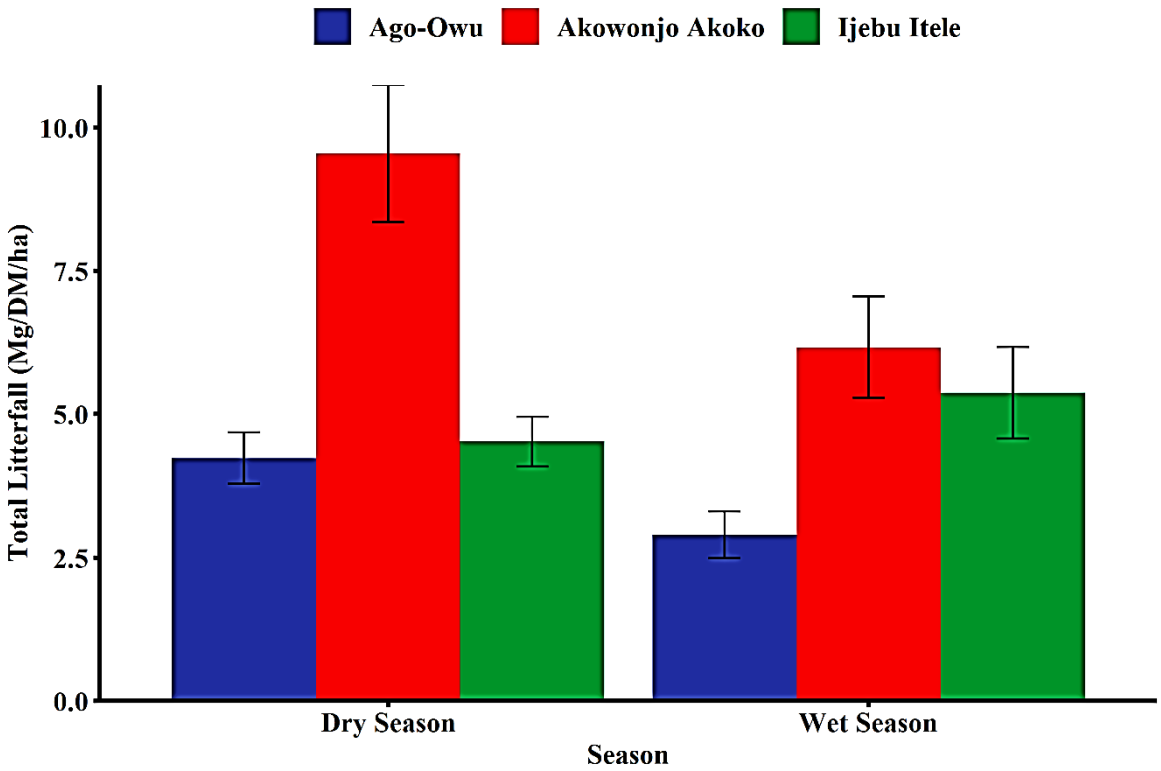

Fig 4.3 Effects of season and location on total litterfall production at the study sites

Table 4.3: Chemical quality of cacao litter

Chemical quality	Locations		
	Ago-Owu	**Akowonjo Akoko**	**Ijebu Itele**
		(mg g^{-1} DM ±SEM)	
Nitrogen (mg g^{-1} DM)	13.29±0.19[a]	13.09±0.87[c]	13.22±0.85[b]
Phosphorus (mg g^{-1} DM)	1.11±0.01	1.05±0.07	1.11±0.08
Potassium (mg g^{-1} DM)	4.93±0.84[a]	3.08±0.36[b]	4.68±0.82[a]
Carbon (mg g^{-1} DM)	549.08±24.80	561.38±25.80	522.76±34.70
Carbon/Nitrogen ratio	40.74±1.71[b]	47.10±3.87[a]	40.74±0.57[b]
Carbon/Phosphorus ratio	665.31±14.70	693.15±17.20	629.85±8.09

Mean values (±SEM) in the same row followed by the same superscript for different locations are not significantly different at P≤0.05 according to Tukey's HSD test;

SEM = Standard Error of Means

4.5 Litter decomposition

The decomposition of cacao litter expressed as mass remaining (%) follows a similar pattern across the three study locations, but the decomposition rate in the wooden frames was more rapid than the decomposition bag (Fig 4.4). The decomposition constant between the wooden frames and decomposition bag ranges from 0.0015 to 0.0019 day^{-1}, (Fig 4.4) and there was a significant difference ($p \leq 0.001$) in the weight loss between litterbags and the wooden frames and this difference is attributed to macrofauna. There was no correlation between species abundance of macrofauna and decomposition rates.

4.6 Effects of spatial and temporal patterns on mass remaining between the decomposition bags and wooden frames

The residual weight expressed as mass remaining (%) showed significant ($p \leq 0.001$) spatial and temporal patterns and the contribution of macrofauna to mass loss in the wooden frames varied over the sampling period ($p \leq 0.001$) (Table 4.4 and Fig 4.4).

4.7 Pearson correlation between decomposition constant and parameters of litter quality

Nitrogen initial concentrations has a weak positive correlation with decomposition rate ($p \geq 0.05$) while Phosphorus initial concentration has a strong positive correlation with the decomposition rate ($p \geq 0.05$); Carbon/Nitrogen ratio and Carbon/Phosphorus has a weak negative correlation with decomposition rate ($p \geq 0.05$), respectively (Table 4.5).

4.8 Nutrient release pattern from cacao litter after incubation

There was a significant difference ($p \leq 0.001$) in nitrogen release pattern in some certain periods across the study locations (2020 June, Aug, November and 2021 Jan) (Fig 4.5a), while at the other sampling periods, there was no significant difference ($p \geq 0.05$) in nitrogen release pattern and nitrogen was released more slowly across the sampling period (Fig 4.5a).

There was a significant difference ($p \leq 0.05$) in phosphorus release pattern in only one study location (Akowonjo Akoko), but phosphorus release was more stable across the study locations and was release more slowly than nitrogen (Fig 4.5b), but at the other

study periods, there was no significant difference in phosphorus release pattern ($p \geq 0.05$) (Fig 4.5b) in the study locations.

Potassium release follows a similar pattern to nitrogen release, but potassium was release quickly than nitrogen. The pattern of nutrients release was in the following pattern potassium release > nitrogen release > phosphorus release (Fig 4.5 a, b, c).

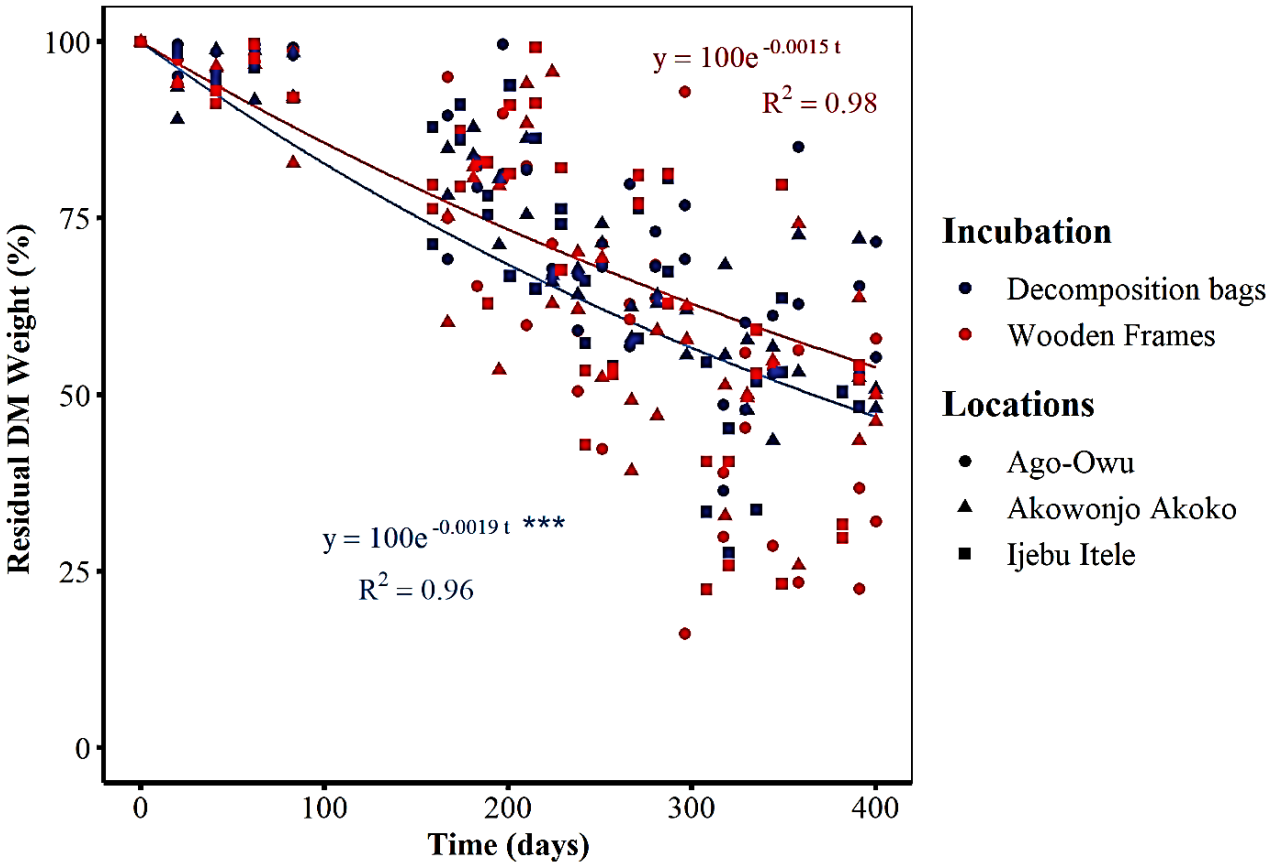

Fig 4.4: Decomposition pattern of cocoa litter after incubation
Legend: DM = Dry matter

Table 4.4: Non-linear mixed-effects models to examine the variation in the spatial and temporal pattern of mass remaining: full model with all interactions fitted and minimum adequate model (lowest AIC score)

Term	NumDF	DenDF	F-value	P-value
Full-model				
Intercept	1	246	1101.011	<.0001***
Incubation (I)	1	246	10.481	0.0014**
Position (P)	1	246	7.034	0.0085**
Incubation: Position	1	246	1.634	0.2024ns
Minimum adequate model				
Intercept	1	247	1100.044	<.0001***
Incubation	1	247	11.201	0.0009***
Position	1	247	7.102	0.0082**

Legend: NumDF = Numerator Degree of Freedom; DenDF = Denominator Degree of Freedom; AIC = Akaike Information Criterion

Table 4.5: Pearson Correlation Coefficients between decomposition rate and parameters of litter quality

Parameters	R	P-Value
Nitrogen (mg g^{-1})	0.05	0.7098^{ns}
Phosphorus (mg g^{-1})	0.79	0.9078^{ns}
C/N ratio	-0.14	0.2921^{ns}
C/P ratio	-0.07	0.5923^{ns}

NS = Not Significant ($p \geq 0.05$); C/N = Carbon/Nitrogen; C/P = Carbon/Phosphorus

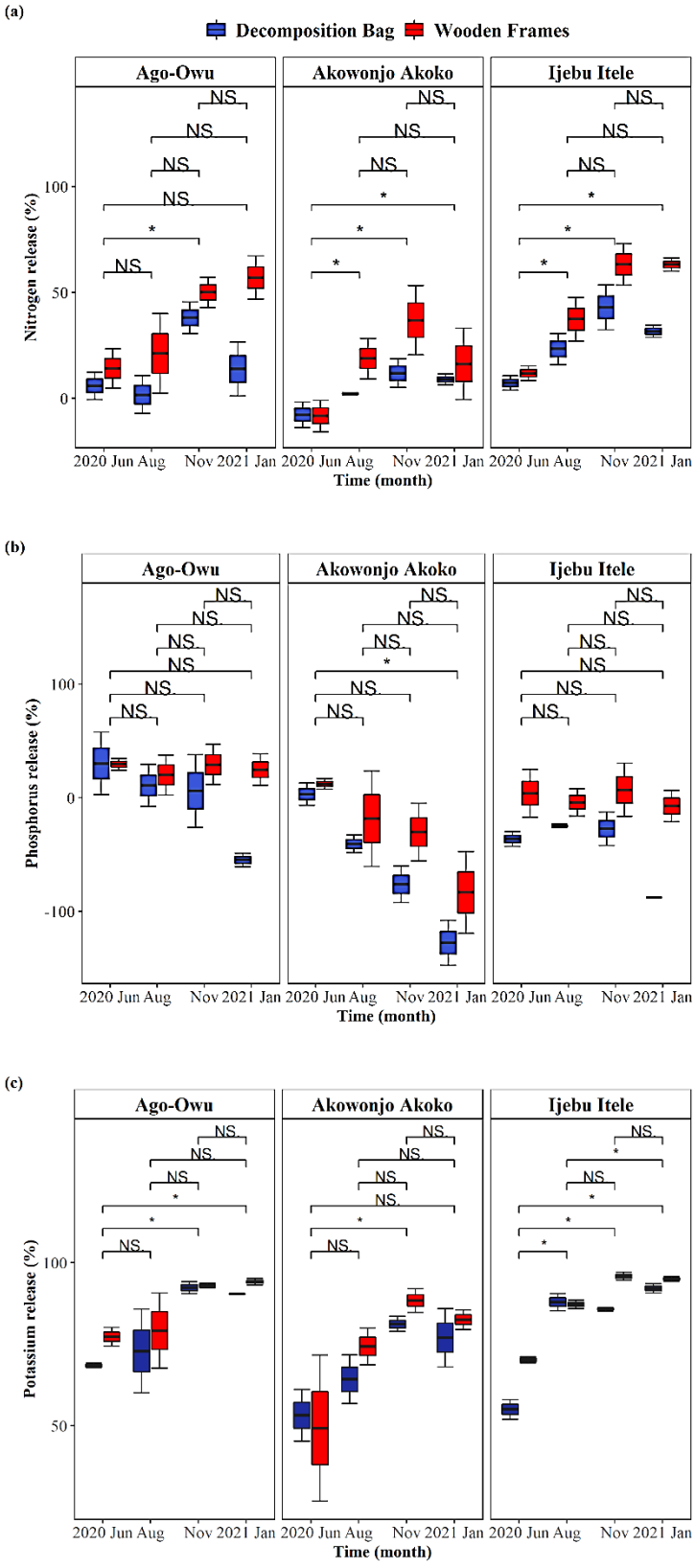

Fig 4.5: Nutrient release pattern: Nitrogen release (a); Phosphorus release (b); Potassium release (c) in cacao litter after incubation. Horizontal bold lines of the box plot indicate the median; the lower and upper bounds of the box represent the 25th and 75th percentiles, respectively. The vertical bars indicate the maximum and minimum values. One asterisk (*) indicates ($p \leq 0.05$) and NS = Not significant.

Chapter 5

DISCUSSION

5.1 Litterfall production

The annual litterfall production in the study locations ranged from 3.50 to 7.71 Mg DM ha^{-1} yr^{-1}, and this values are comparable with those found by other studies in low-shade cacao agroforestry system (Hartemink, 2005; Dawoe *et al*., 2010; Fontes *et al*., 2014; and Saj *et al*., 2021). This study revealed that litterfall production is subject to the seasonal pattern, which increases in the dry season, showing that the physiological response to drought, reduced humidity and lower night temperatures during the dry season plays a significant role in this process because cacao is a semi-deciduous tree which is expected to shed its leaves at certain period in the year. These factors stimulate abscisic acid synthesis in plant foliage which stimulates leaf senescence (Dawoe *et al*., 2010). Litterfall components except miscellaneous fraction showed higher shedding during the dry season than the wet season, and we can attribute this to seasonal variations. The high amount of litterfall produced in the study locations between January and March is associated with the high temperatures and low rainfall that is observed during the dry season.

5.2 Chemical quality of cacao litter

The chemical quality of cacao litter in the study locations is comparable to and fall within the values reported by Dawoe *et al*. (2010) under cocoa ecosystems in humid lowland Ghana; Carbon/Nitrogen ratios reported in this study are also comparable to those reported by Dawoe *et al*. (2010). High C/N ratio (>25) and C/P ratio (>300) and low N content (<2%) obtained in this study suggest that the slow decomposition rate of cacao litter observed at the study sites were strongly regulated by litter quality.

5.3 Litter decompositions

The decomposition constant ranged from 0.0015 for litterbags to 0.0019 for wooden frames for low-shade cacao plantations in Southwestern Nigeria, and this is in contrast with the decomposition constant (k= 0.35 day^{-1}) reported by Dawoe *et al*. (2010) for secondary forests in the Ashanti region of Ghana and 0.46 to 1.11 reported by Fontes *et al*. (2014) for cacao agroforestry systems in Brazil. Mass loss (%) in the study locations followed an exponential decay pattern where the residual mass-gradually decreases with an increase in days after incubation (DAI), and this is in line with the findings of Yang and Chen (2009); Muoghalu and Odiwe (2011) and Silva *et al*. (2018). According to Muoghalu and Odiwe (2011), the rate of decomposition of leaf litter in cacao plantation were influenced by litter quality. Macrofauna increased the decomposition rate in the wooden frames, but we could not detect which species of macrofauna was responsible for this increased weight loss that was observed in the wooden frames.

5.4 Effects of spatial and temporal patterns on decomposition

This study highlights the differences among spatial (far and close to the cacao tree) and temporal patterns of decomposition between the litter bags and wooden frames. The wooden frames had a faster rate of decomposition than the litter bags and this might be attributed to the macrofauna being able to easily access the wooden frames. This is also in agreement with the findings of Maynard *et al.* (2016) and Ge *et al*. (2017), who reported that macrofauna prefers litter of high quality (N>2% and C/N ratio < 25) and is thought to feed on the litter of low quality only in late stages of decomposition.

5.5 Correlation between decomposition constant and parameters of litter quality

The negative and non-significant difference of the decomposition rate and litter quality parameters observed for C/N ratio and C/P ratio in the study sites suggest that the chemical parameters are the key driver of the decomposition and nutrients release rates. This was also in line with the findings of (Dawoe *et al*., 2010).

5.6 Nutrient release pattern from cacao litter after incubation

The slower rate of nutrients release (NPK) observed in this study suggest that micro-climate (temperature and relative humidity) and litter quality might be the dominant factor controlling the decomposition, and nutrients release rate because nutrients release rates depend on mass-loss rates. The slower rates of nutrients release were attributed to the high C/N, and C/P ratio observed in this study, and this is in line with the findings of Fontes *et al.*, (2014) and Mohammed *et al*. (2019).

CHAPTER 6

6.1 Summary and Conclusion

This study aims to quantify the composition, and the seasonal pattern of annual litterfall production in low-shade cacao plantations found in Southwestern Nigeria and to determine the effect of macrofauna on the rate of decomposition and nutrients (NPK) release and the findings of this study reveal that litterfall composition in the study sites depends on variation in both season and total annual litterfall production in the study sites ranges from 3.50 to 7.71 Mg DM ha^{-1} yr^{-1}. Macrofauna ($p \leq 0.001$) increase the rate of decomposition and nutrients release but we were unable to detect which species of macrofauna were actually responsible for the increased weight loss and this might set the direction for future studies. The nutrients cycled through litterfall could help to sustain the nutrition of the cacao tree.

6.2 Recommendation

1. Cacao should be planted with shade trees and these will help to increase the amounts of nutrients cycled in the cacao plantation.

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Appendix

1 R Script for visualizing the monthly pattern of litterfall production

```
library(tidyverse)
library(agricolae)
library(dplyr)
data<-read.csv(file.choose(),header=TRUE)
data<-data %>% filter(Location%in%c('Ago-Owu','Akoko','Ijebu Itele'))
M<-data%>% select(-10) #%>% filter(Month !='Jun')
M$Month<-factor(M$Month,levels = c('2020 Jan','Feb','Mar','Apr','May','Jun',
  'Jul','Aug','Sep','Oct','Nov','Dec','2021 Jan'))
M=M%>%group_by(Month,Location)%>%
summarise(Total=sum(Total.LF*10/0.4*1/1000))
N=M %>% filter(Month%in%c('Mar','Jul'))

ggplot(M,aes(Month,Total,col=Location,group=Location))+geom_point()+geom_line(
size=1.5)+
theme_classic()+ylab(expression(Litterfall~Production~~(Mg~DM~ha^{-1}~yr^{-
1})))+
xlab('')+theme(legend.title = element_blank())+theme(legend.position = 'top')+
geom_line(data=N,aes(x=Month,y=Total,col=Location),linetype=3,size=1)+
scale_color_brewer(palette = 'Set1')
```

2 R Script for Visualizing the Decomposition Pattern of Cocoa Litter

```
require(nlme)
require(ggplot2)
#clear environment
rm(list=ls(all.names=T))
#For a nice background in ggplot
cleanup <-theme(panel.grid.major=element_blank(),
                panel.grid.minor=element_blank(),
                panel.background=element_blank(),
                axis.line=element_line(color="black"))
# set working directory and have your data ready (is called mydata)
mydata<-read.csv('mydata.csv',header=T)
mydata$Locations=as.factor(mydata$Locations)
mydata$Incubation=as.factor(mydata$Incubation)
mydata$Position<-as.factor(mydata$Position)
#Discarding values above 105% residual weight
mydata$Residual.Weight[mydata$Residual.Weight>100]=NA

res0=nlme(Residual.Weight~100*exp(-k*Days),
          fixed=list(k~Incubation+Position),
          random=k~1|Locations,
          data=mydata,
          start=c(0.1,0,0,0))
summary(res0)
anova(res0)
```

```
anyname=as.data.frame(predict(res0))
anyname$observed=mydata$Residual.Weight
anyname$incubation=mydata$Incubation
colnames(anyname)=c('predicted','observed','Incubation')
View(anyname)
library(dplyr)
bag=anyname %>% filter(Incubation=='Decomposition bags')
frame=anyname %>% filter(Incubation=='Wooden Frames')
res2=lm(predicted~0+observed,bag)
res3=lm(predicted~0+observed,frame)
summary(res2)
summary(res3)
View(res2)
View(res3)
rsq_bags=as.data.frame=summary(res2)[["adj.r.squared"]]
rsq_frame=as.data.frame=summary(res3)[["adj.r.squared"]]
rsq_bags=round(rsq_bags,2)
rsq_frame=round(rsq_frame,2)
res1=lm(predicted~0+observed,anyname)
summary(res1)
label_rsq=summary(res1)[["r.squared"]]
label=summary(res1)[["adj.r.squared"]]
label_rsq_bag=paste('R^2==',rsq_bags)
label_rsq_frame=paste('R^2==',rsq_frame)
T2=data.frame(Days=rep(0:400,2),
 Incubation=rep(c('Decomposition bags','Wooden Frames'),each=401),
```

```
 Locations='Ago-Owu')
pred2=predict(res0,newdata=T2,level=0)
pred2=cbind.data.frame(T2,pred2)
colnames(pred2)=c('Days','Incubation','Locations','Residual.Weight')
k_bag=as.data.frame(summary(res0)[["tTable"]])[1,1]
k_frame=k_bag+as.data.frame(summary(res0)[["tTable"]])[2,1]
k_bag=round(k_bag,4)
k_frame=round(k_frame,4)
p_bag=as.data.frame(summary(res0)[["tTable"]])[1,5]
p_frame=as.data.frame(summary(res0)[["tTable"]])[2,5]
label_bag=paste0(-k_bag,' t')
label_frame=paste0(-k_frame,' t')
```

#---------- **Visualization Decomposition Pattern** ----------

```
P01=ggplot(mydata,aes(x=Days,y=Residual.Weight,
                      colour=Incubation,
                      shape=Locations))+
  geom_line(data=pred2[pred2$Incubation=='Decomposition bags',],
            aes(x=Days,y=Residual.Weight),colour='red')+
  geom_line(data=pred2[pred2$Incubation=='Wooden Frames',],
            aes(x=Days,y=Residual.Weight),colour='steelblue')+
  geom_point(size=1.75)+
  scale_color_brewer(palette='Set1')+
  scale_alpha(0.2)+
  labs(x='Time (days)', y='Residual DM weight (%) ')+
  annotate('text',x=150,y=30,size=4,colour='steelblue',
           label=bquote(y== 100* e ^~.(label_frame)))+
  annotate('text',x=217,y=31,size=5,colour='steelblue',
           label=' ***')+
  annotate('text',x=150,y=23,size=4,colour='steelblue',
           label=label_rsq_frame,parse=T)+
  annotate('text',x=330,y=98,size=4,colour='red',
           label=bquote(y== 100* e ^~.(label_bag)))+
  annotate('text',x=370,y=92,size=4,colour='red',
           label=label_rsq_bag,parse=T)+
cleanup
P01
```

3 R Script for Visualizing the Nutrient Release Pattern over Time

```
library(tidyverse)
library(ggsignif)
library(patchwork)
library(cowplot)
T1<-data %>% filter(`Time(Month)`!='2019 Dec')
T1$`Time(Month)`<-factor(T1$`Time(Month)`,levels= c('2020 Jun','Aug','Nov',
 '2021 Jan'))
```

Nitrogen Release

```
p1<-ggplot(T1,aes(`Time(Month)`,`Nitrogen.Released.(%)`,fill=Incubation))+
  stat_boxplot(geom='errorbar')+geom_boxplot()+
  scale_fill_brewer(palette = 'Set1')+
  facet_grid(~Locations)+theme_test()+
  #theme(axis.title = element_text(size=33))+
  #theme(axis.text.x = element_text(size=33,hjust=1,angle = 45,vjust=1))+
  #theme(axis.text.y = element_text(size=35))+
  #theme(strip.text.x = element_text(size=35,face='bold'))+
  #theme(legend.title = element_text(size=35,face='bold'))+
  #theme(legend.text = element_text(size=33))+
  ylab('Nitrogen release (%)')+xlab('Time (month)')+
  geom_signif(comparisons = list(c('2020 Jun','Aug'),
                                 c('2020 Jun','Nov'),
                                 c('2020 Jun','2021 Jan'),
                                 c('Aug','Nov'),
                                 c('Aug','2021 Jan'),
```

```
                    c('Nov','2021 Jan')),
          map_signif_level = TRUE,step_increase = 0.18,textsize = 10,
          y_position =55,vjust=.3,hjust=.4)+
theme(legend.position = 'top')
```

#Phosphorus Release

```
p2=ggplot(T1,aes(`Time(Month)`,`Phosphorus.Released.(%)`,fill=Incubation))+
  stat_boxplot(geom='errorbar')+geom_boxplot(show.legend = FALSE)+
  scale_fill_brewer(palette = 'Set1')+
  facet_grid(~Locations)+theme_test()+
  # theme(axis.title = element_text(size=28))+
  # theme(axis.text.y = element_text(size=28))+
  # theme(axis.text.x = element_text(size=28,vjust=1,hjust=1,angle=45))+
  # theme(strip.text.x = element_text(size=28,face='bold'))+
  # theme(legend.title = element_text(size=35,face='bold'))+
  # theme(legend.text = element_text(size=28))+
  ylab('Phosphorus release (%)')+xlab('Time (month)')+
  geom_signif(comparisons = list(c('2020 Jun','Aug'),
                                 c('2020 Jun','Nov'),
                                 c('2020 Jun','2021 Jan'),
                                 c('Aug','Nov'),
                                 c('Aug','2021 Jan'),
                                 c('Nov','2021 Jan')),
              map_signif_level = TRUE,step_increase = 0.2,textsize = 9,
              y_position=60,vjust=.3,hjust=.4)
```

Potassium Release

```
p3=ggplot(T1,aes(`Time(Month)`,`Potassium.Released.(%)`,fill=Incubation))+
  stat_boxplot(geom='errorbar')+geom_boxplot(show.legend = FALSE)+
  scale_fill_brewer(palette = 'Set1')+
  facet_grid(~Locations)+theme_test()+
  # theme(axis.title = element_text(size=28))+
  # theme(axis.text.x = element_text(size=28,hjust=1,vjust=1,angle=45))+
  # theme(axis.text.y = element_text(size=28))+
  # theme(axis.title = element_text(size=28))+
  # theme(strip.text.x = element_text(size=28,face='bold'))+
  # theme(legend.title = element_text(size=35,face='bold'))+
  # theme(legend.text = element_text(size=28))+
  ylab('Potassium release (%)')+xlab('Time (month)')+
  geom_signif(comparisons = list(c('2020 Jun','Aug'),
                                 c('2020 Jun','Nov'),
                                 c('2020 Jun','2021 Jan'),
                                 c('Aug','Nov'),
                                 c('Aug','2021 Jan'),
                                 c('Nov','2021 Jan')),
              map_signif_level = TRUE,step_increase = 0.2,textsize = 9,
              vjust=.3,hjust=.4,y_position = 95)
p1/p2/p3+plot_annotation(tag_levels = c(('A')))
```