EFFECT OF BIOCHAR AMENDMENT ON pH, SELECTED NUTRIENTS AVAILABILITY AND GREENHOUSE GAS EMISSIONS IN A PADDY SOIL FROM YALA BASIN UNDER ANAEROBIC CONDITIONS

BY

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DECLARATION

By Student

I Rogers Omondi Ong'injo, declare that the work presented in this thesis is my original work and has not been presented for the award of a degree or diploma in any other institution of higher learning.

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By Supervisors

With the undersigned confirm that the report in this thesis was carried out under our supervision and that it has been submitted with our approval.

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Finally, thanks to the Almighty God for giving me a peaceful and sound mind for this work.

DEDICATION

I wish to dedicate this work to my parents John Ong'injo and Josephine Ong'injo

ABSTRACT

Although tropical paddy soils have high levels of phosphorous (P), most of it is in forms that are not available for crops. Water logging of paddy soils during rice cultivation leads to anaerobic conditions which could limit the availability of nutrients, alter soil pH and cause greenhouse gas (GHG) emissions. Biochar use for soil amendment is increasingly gaining fame due to its potential benefits of increased nutrient availability and reduced GHG emissions. However, information on the effect of biochar amendment on nutrient availability, soil pH and GHG emissions in tropical paddy soils are scanty. The aim of this study was therefore to establish the effect of biochar amendment on soil pH, phosphorus fractions, nitrogen, organic carbon, and greenhouse gas emissions under anaerobic conditions. A paddy soil collected from the Yala basin of Lake Victoria was used as a representative tropical paddy soil amenable to water logging. Four different treatments including S; unamended soil, SB; soil amended with biochar in the ratio of 99:1, SP; soil amended with KH_2PO_4 in the ratio of 99:1, and SPB; soil amended with biochar and KH_2PO_4in the ratio of 98:1:1 (w/w) respectively were prepared, water logged and incubated at 25^oC in airtight glass containers. Air samples and soil aliquots were sampled periodically. Dissolved organic carbon, P and N were extracted and determined using UV/Vis spectrophotometry, total organic carbon by loss on ignition, pH using a pH meter and GHGs (CO_2, CH_4 and N_2O) using gas chromatography. The data obtained was subjected to ANOVA, regression analysis and correlation analysis using Microsoft excel 2007. Data obtained indicated that biochar amendment increased different soil parameters in the ranges total P (500.11±34.38 - 1709.51±101.40 µg/g of soil), iron bound P (111.402±11.80 - 174.75±9.78 µg/g of soil), initial soil pH (3.96±0.15 - 5.00±0.12), and CH_4 emission (7.52×10^{-6} - 2.33×10^{-5} µg/g of soil), reduced CO_2 emissions(9.54±3.54 - 5.96±2.17 µg/g of soil), and reduced loosely sorbed P (66.11±4.93 – 49.10±2.30 µg/g of soil) and had no effect on N_2O emissions, TOC, DOC, TON, ammoniacal N and organic N. P amendment increased total P(500.11±34.38 - 1001.98±30.34 µg/g of soil), loosely sorbed P (66.11±4.93 - 106.65±5.38 µg/g of soil), labile organic P (89.42±13.15 - 179.34±10.67 µg/g of soil), soil pH (5.22±0.1 - 5.94±0.07) and cumulative N_2O emission. It however, reduced total N (1987.83±345.30 - 502.00±2.36 µg/g of soil), Organic-N (1695.91±118.56 - 340.39±4.30 µg/g of soil), CO_2 (9.54±3.54 - 6.97±2.21 µg/g of soil), and CH_4 emissions (2.33×10^{-5} - 1.14×10^{-5} µg/g of soil) with respect to SB but had no significant difference ($p \leq 0.05$) on TOC, DOC, TON and ammoniacal N. The high CO_2/CH_4 ratio (>2) showed dominance of fermentation over methanogenesis. Biochar amendment, in this study helped stabilize soil nutrients and reduced GHG emissions with the exception on methane although its levels were far much lower than for the carbon dioxide and nitrous oxide. The use of biochar for soil amendment (1% w/w) is thus commendable and would benefit the residents of Siaya County, policy makers and the physical environment.

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LIST OF ABBREVIATIONS AND ACRONYMS

APHA - American Public Health Association
FAO - Food and Agriculture Organisation
SOM - Soil organic matter
DOM - Dissolved organic matter
GHG - Greenhouse gases
pH - Power of hydrogen
kg - Kilogram
UV - Ultra violet
GC - Gas chromatograph
ODS - Oven dry soil
IBI - International Biochar Initiative.
P - Phosphorus
FID - Flame ionization detector
ECD - Electron capture detector
GHG - Greenhouse gases
GWP - Global warming potential
SWC - Soil water content
EC - European Community
SB - Soil amended with biochar
S - Unamended soil
SPB - Soil amended with both biochar and Phosphorous
SP - Soil amended with phosphorous
IPCC - Intergovernmental Panel on Climate Change
LBDA - Lake Basin Development Authority

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CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Biochar is a carbon rich pyrolysed biomass produced from a variety of feedstocks including municipal, agriculture and forestry wastes (Sohi *et al.,* 2010). It has a variety of applications including soil amendment, where it enhances soil aggregation, water holding capacity and organic matter content (Smith *et al.,* 2010; Lehmann *et al.,* 2011). Biochar also increases crop yields, improves soil cation exchange capacity, reduces soil acidity and loss of nutrients thereby lowering demand for mineral fertilizers (Lehman and Joseph, 2009).

Lower River Yala Basin, commonly known as Yala swamp was reclaimed in the 1970's by the Lake Basin Development Authority (LBDA) for agricultural activities to produce cereals, and horticultural crops. The reclaimed region known as area I covers about 2300 ha (KWF, 2006). This area was leased to the Dominion farm, a subsidiary of the Dominion group of companies based in the USA in the year 2003 through an agreement with LBDA (Simonit and Perrings, 2004). The main activity in the agreement was rice production but the firm expanded to engage in sugarcane production, fish farming among other development projects. The soils here being acidic and high in iron content would encourage phosphorus fixation hence depriving crops of the nutrient (Owino *et al*., 2015).

Phosphorus (P) is an essential element for plant and animal nutrition (FAO, 2006) and the second most limiting nutrient after nitrogen for crop production in agricultural areas within the tropics (Fageria and Baliga, 2005). Even though soils may contain several hundreds to thousands kilograms of phosphates per hectare, much of this may not be available for plant uptake (Mahmoud *et al., 2018*). This availability is controlled by sorption, desorption and precipitation processes in the soil (Eghball *et al*., 1996). Phosphorus occurs in soil in both organic and inorganic forms, which vary in their rates of P release (Smeck, 1985). The original sources of soluble P in soil are the primary P minerals, mainly apatite. However, the levels of these minerals decrease in soil with continued weathering.

Most field trials of the agricultural benefits of biochar have been carried out in poor soils from tropical regions with lower rates of nitrogen fertilization (Lehman and Joseph, 2009**).** Little information is available on the use of biochar with phosphorus fertilization and most of these studies focus on crop yield and little information on nutrient fractions and availability which impact on the crop yield in the end. Moreover, most of the studies are conducted under aerobic conditions. In high P fixing soils, such as the case of western Kenya (Okalebo *et al.*, 2003), the application of large amounts of inorganic fertilizers can quench the soil P need (Hue and Amien, 1989; Kisinyo *et al.,* 2014). However, this is impractical for the impoverished small holder farmers in Kenya (Omwonga *et al.*, 2013). It has been established that soils in Siaya County are weakly acidic and contain Al^{3+} and Fe^{3+} ions which are responsible for P fixation (Owino *et al.*, 2015). However, they do not explain how and why the soils are deficient of available P. Furthermore, past research has not explored the effect of ecological conditions and organic amendments on soil nutrient levels. Moreover, how this high P fixation can be overcome still remains unknown.

Biochar research conducted in Kenya by Camilla (2013), Aslund (2012) and Andrew and Abigail (2012) report increase in crop yields with application of biochar. Similar results were reported by Katterer *et al.* (2018) and Almuth (2011).

Whereas the effect of biochar on plant growth is reported, limited information is available on its effect on soil chemistry and nutrient availability. Moreover, the effect on soil fertility parameters with respect to nutrients was not investigated. Soil P dynamics is controlled by pH, $Fe^{3+}$and Al^{3+}oxides (Ige *et al*., 2005) as well as soil moisture through redox processes (Sah and Mikkelson, 1989; Stephens *et al*., 2005). Many researchers like Amand and Beck (2003), Zhang *et al*. (2003), Osafo *et al.* (2017), Kalyani *et al.* (2015) and Mahmoud *et al.* (2018) reported increase in soil available P for anaerobic soils amended with biochar. However, these results are only available for temperate soils with limited reports on tropical soils. Moreover, these studies focus only on plant available P and not the effect on the various P fractions in the soil especially under anaerobic conditions.

Nitrogen (N) is an important nutrient for crop production (Prommer *et al.,* 2015). However, published reports on biochar amendment effects on soil nitrogen give conflicting results. Deluca *et al.* (2015) and Diatta (2016) reported decreased ammoniacal N and nitrate N while Lehman *et al.* (2003) and Zhu *et al*. (2016) reported no effect, with Zhu *et al*. (2016) affirming reduction in organic N with biochar amendment. This conflicting data need to be ascertained for this particular tropical soil more so under anaerobic conditions, since it is mostly subjected to rice production. Biochar amendment has been reported to improve soil organic carbon (SOC) and dissolved organic carbon (DOC) in temperate soils due to its high content of labile carbon and carbon sequestering capacity (Sander and Tarek, 2012; Marlene *et al.*, 2013). However, there are limited reports on tropical soils particularly in Kenya. Atkinson *et al*. (2010), Farrel *et al*. (2013) and Lai *et al*. (2013) reported increased soil pH after biochar amendment, while Ventura *et al.* (2012) and Phy *et al*. (2014) indicated no significant change in soil pH. As a result of this uncertainty, it is necessary to investigate the effect of anaerobic conditions and biochar amendment on the pH of tropical soils.

Developing countries monitoring and reporting on greenhouse gas (GHG) emissions, policies and measures, identify the agricultural sector as the main source of greenhouse gas emissions contributing approximately 56% of the total emission (IPCC, 2010). This fact raises a lot of concern since GHGs pose a great danger of global warming and climate change. The main greenhouse gases identified to be emitted by soil are carbon dioxide, methane and nitrous oxide (Chunxiao, 2014). Rogovska *et al*. (2014) observed that biochar increased CO_2 emissions, reduced N_2O emissions and increased soil organic carbon storage capacity in temperate soils. However, data on the effect of biochar amendment on tropical soils is limited and hence deserve to be studied.

Methane is a key GHG with a global warming potential(GWP) 21 times greater than CO_2 and a lifetime of 12 years (IPCC, 2021). It is largely emitted from anaerobic soils as organic matter decomposes in limited oxygen (Segers, 1998). Dasselar *et al*. (1998) and Scheer *et al*. (2011) report low methane absorption by soils with soil water capacity(SWC) of above 45%. Previous studies have reported conflicting results on the effect of biochar amendment on soil CH_4 emissions under anaerobic conditions. Karhu *et al*. (2011), Liu *et al*. (2011) and Xingguo *et al*. (2010) reported reduced CH_4 emissions in biochar amended soil while Zhang *et al.* (2010), Phy *et al.* (2014) and Liu *et al.* (2016) reported increased CH_4 emissions under high water content (77% - 95%). Rondon *et al*. (2005) reported almost complete suppression of CH_4 emissions while Knoblauch *et al*. (2011) reported no significant changes in CH_4 emissions. Most of these studies were conducted using temperate soils, with little information on tropical soils.

Nitrous oxide is the most potent GHG with GWP 310 times greater than CO_2, a lifetime of 114 years (IPCC, 2021) and has high potential of depleting the ozone layer (Ravinshankara *et al.*, 2009). Zwieten *et al*. (2010), Case *et al.* (2012), Cayuela *et al*. (2013), Sarah *et al.* (2015), Keenan *et al*. (2016), Nguyen, (2016), and Liu *et al.* (2016) reported reduction in N_2O emissions in flooded soils amended with biochar. However, most of these investigations have been conducted using temperate soils with little data on tropical soils.

Previous studies give conflicting results on the effect of soil biochar amendment on CO_2 emissions from the soil. Wang *et al.* (2012) and Liu *et al*. (2013) reported no significant effect on CO_2 emissions, while Case (2013) reported variable levels of emissions. Nguyen *et al.* (2016), and Keenan *et al*. (2016), reported reduced CO_2 emissions from paddy soils amended with biochar. These reports confirm the uncertainty on the effect of biochar amendment on CO_2 emissions from flooded soils. From the foregoing, it was necessary to investigate the effects of biochar amendment on GHGs emissions, phosphorus pools and nutrient availability, from this tropical paddy soils due to its economic importance and the water logging system of farming used in the production of rice.

1.2 Statement of the Problem

Food security is a key pillar of the millennium development goals and Kenya vision 2030 (Kogo *et al*., 2020). However, achieving it remains a challenge due to unavailable forms of soil nutrients. Improvement of soil available nutrients like nitrogen, phosphorous and carbon could help ensure food security for all. Soils have high levels of phosphorus most of which are in forms that are unavailable for plants. It is not known whether anaerobic conditions could lead to more P availability. Furthermore, the effect of organic amendments such as biochar on P pools, carbon sequestration, soil chemistry and nutrients availability on Sub-Saharan African soils have not been established. It is thus important to investigate the effects of biochar amendment under anaerobic conditions on soil quality since this could avail soil nutrients hence good crop yields. Moreover, information on the sustainability of soil treatments remains scanty. For instance, published reports indicate that soils under anaerobic conditions emit greenhouse gases (GHGs) that are responsible for global warming and climate change (IPCC, 2007). However, the effect of anaerobic conditions and biochar amendment on GHGs emissions, as well as on nutrients and other soil properties, is unknown. It was therefore necessary to investigate the effect of anaerobic conditions and biochar amendment on soil quality, nutrients availability, phosphorus pools and GHGs emissions.

1.3. Justification of the Research

The data obtained from this research will show the various fractions of soil phosphorus and confirm if biochar amendment can convert the fixed forms of phosphorus to forms that are available for plant uptake under anaerobic conditions in the tropical climate.

The data will also help show if biochar can help minimize greenhouse gas emissions from tropical soils under reducing conditions, thus contributing to the world's commitment in fighting global warming and climate change as spelt in the UN charter of 2009 (Pandey, 2014).

The results will provide information on the effect of biochar on soil organic matter content and availability of nutrients. For instance, if biochar can promote the sequestration of carbon to the soil, it would result into improved soil fertility.

The data obtained from this research would therefore provide useful information for better, environmentally friendly and sustainable agricultural practices that would ensure food security as spelt out in Africa Union Agenda 2063 on modern agriculture and environmental sustainability. It will also open up a way to better maximize on the utilization of agricultural wastes especially crop residues. If the study is not conducted, the perennial problem of food shortage, expensive farming processes and exposure to harmful greenhouse gases will continue unabated.

1.4 Significance of the Research

The dominion farm is an economic pillar for many residents of Siaya County. The agricultural output from this area supplies food to a large number of people. However, the high cost of production may make it unaffordable. The study provides useful information that can help the residents of Siaya County, and policy makers to institute measures towards low cost farming that is environmentally friendly. If not conducted, the residents of this area would continue using the old farming techniques that deplete the soil nutrients and are harmful to the environment exposing them to the harmful effects of GHGs and famine due to poor yields.

1.5 Aims and Objectives

1.5.1 Broad objective

The aim of this study was to establish the effect of biochar amendment on soil pH, phosphorus pools, availability of nutrients, and greenhouse gas emissions (GHGs) in a tropical paddy soil from Siaya County under reducing conditions.

1.5.2. Specific objectives

The specific objectives were:

1. To determine the effect of biochar amendment on phosphorus pools in a tropical paddy soil from the Yala basin of Lake Victoria under anaerobic conditions.
2. To determine the effect of biochar amendment on the level of nitrogen, organic carbon and pH of a tropical paddy soil from the Yala basin under anaerobic conditions.
3. To determine effect of biochar amendment on GHGs emissions in a tropical paddy soil from the Yala basin under anaerobic conditions.

1.6 Null Hypothesis

1. Biochar amendment has no effect on phosphorus pools in a tropical soil under reducing conditions.
2. Biochar amendment do not affect the soil nitrogen, organic carbon and pH under reducing conditions.
3. Biochar amendment do not affect the level of GHG emissions from a tropical soil under reducing conditions.

CHAPTER TWO

LITERATURE REVIEW

2.1 Biochar

Biochar is a carbon rich pyrolised biomass produced from a variety of organic feed stocks including municipal, agricultural and forestry wastes (Sohi *et al*., 2010). The process of biochar production involves thermal degradation of organic material in the absence of oxygen (Liard *et al.,* 2010). Biochar characteristics are governed by the type of source material and pyrolysis temperature (Chen *et al.,* 2008; Gray *et al*., 2014). For instance, biochar has a higher specific surface area but lower functional groups (H and O containing functional groups are dehydrated) when processed at temperatures higher than $500^{o}C$ (Chen *et al*., 2008).

The high surface area of biochar makes it useful for various applications. It can serve as soil additive to enhance soil aggregation, water holding capacity and organic matter content (Smith *et al*., 2010; Lehmann *et al*., 2011). It can also be used for mediating contaminants from point sources of polluted water (Beesley *et al*., 2010). Biochar can also be added to compost piles where it promotes microbial growth (Jindo *et al.,* 2012), increases aeration (Zhang *et al*., 2014), and reduces NH_3 and N_2O emissions during the composting process (Steiner *et al.*, 2007; Wang *et al*., 2012). It also sorbs nutrients and dissolved organic carbon during the composting process (Dias *et al*., 2010; Prost *et al.,* 2013). Biochar can be applied together with compost as soil amendment and potential benefits include enhanced nutrient use efficiency and long term carbon sequestration (Fischer and Glaser, 2011; Schulz *et al*., 2013).

2.2 Soil pH

The most important chemical change that takes place when soil is submerged is the reduction of iron and the accompanying increase in its solubility. This reduction of iron has important chemical consequences like: the concentration of water soluble iron increases, the pH increases, cations are displaced from exchange sites, the solubility of phosphorus and silica increases, new minerals are formed (Ponnamperuma, 1972). The reduction of iron is a consequence of the anaerobic metabolism of bacteria and appears to be chiefly a chemical reduction by bacterial metabolites, although direct reduction coupled with respiration may also be involved. Approximately 5% to 50% of the free iron oxides present in a soil may be reduced within a few weeks of anaerobic conditions depending on temperature, the organic matter content and the crystallinity of the oxides. The lower the degree of crystallinity, the higher is the reduction percentage. The soil pH is a parameter that greatly affects the concentration of water soluble iron. The bulk of water soluble iron is present in soil as bicarbonate and salts of the lower fatty acids as well as complexes of humic acids (Ponnamperuma, 1972).

The dynamics of the concentration of Fe^{2+} affects the concentration of other cations like Ca^{2+}, Mg^{2+}, Na^{+}, NH_4^{+} and K^{+} due to the similarity of their kinetics in the soil. The values of exchangeable and water soluble iron (II) is highly dependent on the pH of the extractant as in the equilibrium equation below.

$$Fe_3O_4.nH_2O \rightleftharpoons Fe^{2+} \rightleftharpoons Fe^{3+}$$

The cations displaced may be lost due to leaching while the supply of Fe^{2+} is maintained by dissociation of hydrated ferrous tetra oxide. On drying and oxidizing the soil is acidified hence the need for pH determination (Ponnamperuma, 1972). Atkinson *et al.* (2010), Farrel *et al.* (2013), and Lai *et al*. (2013) reported increased soil pH due to biochar amendment over their varying incubation periods due to the alkaline nature of biochar. In contrast Ventura *et al*. (2012) and Phy *et al.* (2014) reported no significant change in soil pH due to biochar amendment. It is therefore not clear whether biochar alters soil pH, which is a key soil quality parameter that determines P release and fixation in soils.

2.3 Phosphorus

2.3.1 Soil Phosphorus Pools

Phosphorus is essential in plants for photosynthesis, nitrogen fixation, crop growth, produce quality and root development (Ashman and Puri, 2002). It forms part of the DNA that drives biological processes like nutrient uptake, transport in plants and assimilation into different biomolecules (Brady and Weil, 2008).

Many soils in sub – Saharan Africa are characterized by deficient levels of phosphorus (World bank, 1994). Despite diversity in distribution of parent material and conditions affecting soil formation, soil phosphorus deficiencies result from either inherent low levels of soil phosphorus or depletion of soil phosphorus. Most of these tropical soils are derived from acidic parent material that contained low levels of phosphorus (Sanchez *et al.,* 1997). For once fertile soils, soil phosphorus stocks have decreased as increasing population has led to replacement of traditional systems of shifting cultivation with shorter duration, unsustainable fallow systems and sedentary agriculture (World bank, 1994). Many small holder farmers have lacked the financial resources to purchase sufficient fertilizers to either correct inherent low levels of soil phosphorus or replace the phosphorus exported with harvested products (World Bank report, 1994; Sanchez *et al.,* 1997). Phosphorus occurs in the soil in both organic and inorganic forms which vary in their rate of phosphorus release. Inorganic phosphorus has the following forms in the soil as identified by Chang and Jackson (1957); soluble and loosely sorbed phosphorus, aluminium bound phosphorus, iron bound phosphorus, apatite phosphorus, reductant phosphorus.

The original source of soluble phosphorus in soil is dissolution of primary phosphorus minerals mainly apatite. However, these minerals decrease in soil with increasing soil weathering and are relatively unimportant for highly weathered soils (Smeck, 1985). Once in solution, primary phosphorus can either be taken up by plants, or soil biota and converted to organic phosphorus, or sorbed on to soil minerals. Labile soil phosphorus is loosely sorbed and is rapidly equilibrated with soil solution whereas strongly sorbed phosphorus is only slowly released and made available to plants (SSSA, 1997).

In acidic soils, the sorption of phosphorus occurs mainly on hydrous oxides of iron and aluminium at the surface of clay particles (Frossard *et al*., 1989). Phosphorus sorption is directly related to oxides of iron and aluminium in soil (Juo and Fox, 1997) and is adominant process that controls phosphorus availability in soils (Sanchez and Uehara, 1980; Deckers, 1993).

Total organic phosphorus decreases with continuous cropping with phosphorus fertilization (Jones, 1972) but increases with soil age (Walker and Syers, 1976). Only a small fraction of total organic phosphorus is labile in the short term with a majority occurring in stabilized soil organic matter and is not rapidly mineralized. Organic phosphorus exists in three forms named as labile phosphorus, moderately labile phosphorus, non-labile phosphorus (Chang and Jackson, *1957).*

The circulation of these pools in the soil is summarized in Figure 2.1.

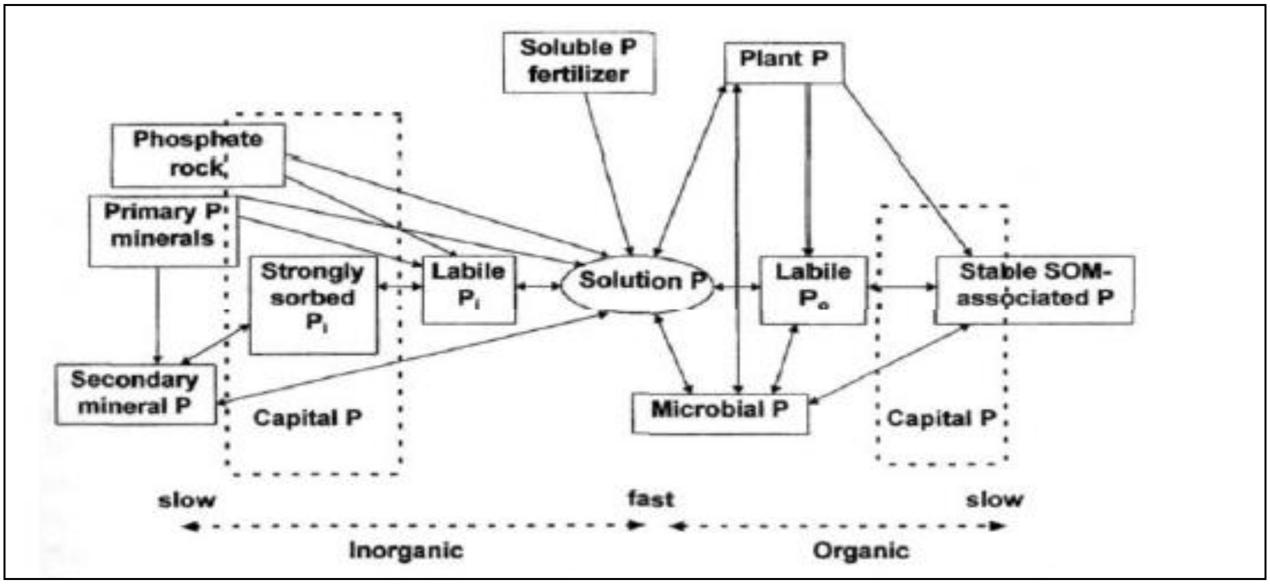

Figure 2.1: Soil P pools and P dynamics following application of phosphate rock and soluble P fertilizer (Adopted from Sharpley, 1995)

Most common phosphorus forms used by plants are the labile and loosely sorbed phosphorus which are readily soluble in water at pH values of between 6 – 7 (Beaton *et al.*, 1960). Organic phosphorus comes from decaying plants and animal wastes while inorganic phosphorus comes from mineral fertilizers and parent rocks for soil formation.

Both commercial and small holder farmers grow crops more so maize (*zea mays)* but in most parts of Kenya the yield is constrained by low soil nitrogen and phosphorus availability (FAO, 2006). Phosphorus deficiency in many of the soils is due to low occurrence of phosphorus containing minerals, high sorption to the acidic soils of Kenya or continous cropping without fertilization (Nyandat, 1984; Obura, 2008). Unlike nitrogen that can be fixed directly from the atmosphere, phosphorus can only be obtained from weathering of rocks hence its identification as a limiting nutrient in soil (Walker and Syers, 1976).

Phosphorus sorption in Kenyan acidic soils ranges from moderate to high (Obura, 2008; Kifuko *et al.,* 2001; Kisinyo *et al*., 2014 and Opala *et al.*, 2012). This sorption leads to low recovery of applied phosphate fertilizers which Kisinyo *et al*. (2014) states to range from 9.6% to 13.5%. Consequently, the soils have low plant available phosphorus resulting in to low crop yields (Okalebo *et al.*, 2003). The low recovery and high sorptivity of soil phosphorus warrants the determination of soil phosphorus fractions with the aim of quantifying those available for plants and the fixed forms which are unavailable for plant uptake.

Condron and Newman (2011) acknowledge the need for fractionation in studying phosphorus dynamics in soils – an area that has not been tackled for river Yala basin, which solely depends on agriculture for subsistence and economically.

In high phosphorus fixing soils, such as is the case of River Yala basin (Okalebo *et al.* 2003), the application of large amounts of inorganic fertilizers can quench the soil phosphorus sorption capacity and avail the excess phosphorus in the soil (Hue and Amien, 1989 and Kisinyo *et al*, 2014). However, this is impractical for the impoverished small holder farmers in Kenya (Omwonga *et al*., 2013). Application of mineral fertilizers also has negative effects on the environment such as nutrient immobilization and ground water pollution (Myint *et al.*, 2010). To improve productivity of the acid soils, rebuilding soil phosphorus fertility in a feasible and environmental friendly manner is thus imperative. Kisinyo *et al*. (2014) outlines liming, use of phosphate fertilizers and organic materials as ways of improving crop yields. This is unsustainable as lime and fertilizers are costly and their sources are exhaustible. Moreover, organic materials take too long to decay and in the long process also release carbon dioxide which contributes to global warming. It is very laborious to burry crop residues into the soil hence the need for better ways of improving soil fertility. This could be achieved by amending the soil with biochar, which is a modified form of plant residues in a way that can easily be assimilated by the soil (Nguyen *et al.*, 2013).

Research has shown that organic and inorganic fertilizers can have a wholesome effect on soil health by increasing soil available phosphorus and other soil properties (Swift and Sherpherd, 2007). This phosphorus release is due to the organic material through increased microbial activity and mineralization of resistant phosphorus fractions (Nzinguheba *et al*., 1998). Combination of phosphate fertilizers with organic materials have also been reported to increase phosphorus availability and crop yields in Kenya (Kisinyo *et al*., 2014 and Okalebo, 2003). However, this has not been quantified and is not so far done in the Yala river basin.
Owino *et al*. (2015), reports that soils in Siaya County are weakly acidic (pH 6.02 – 5.06) and contain oxides of Al and Fe - together with clay and free Fe^{3+} and Al^{3+} ions - which are responsible for P fixation. The same conditions were identified by Tsado *et al*. (2012), for

tropical paddy soils. Owino *et al*. (2015), further recommends increasing organic matter content of these soils to improve P availability for better crop yields. However, they fail to give the ideal additive that would increase the organic matter content with minimal negative impacts on the environment.

Although soil P dynamics is mostly controlled by soil pH, carbonates in calcareous soils and Al^{3+} and Fe^{3+} oxides (Ige *et al.*, 2005), soil moisture also affects P transformations through redox processes (Sah *et al.*, 1989; Stephens *et al*., 2005). Anaerobic soil conditions increase soluble P and Fe in soils due to reduction of iron(III) to the more soluble iron(II) with P being released (Zhang *et al*., 2003; Snyder *et al.*, 2009). Ma *et al.* (2010) reported a decrease in Olsen P of soil after each field water saturation process and increase after each draining process. These results are only available for temperate soils with no information on tropical soils. Moreover, the results focus mainly on the plant available P with no information on how these P transformations affect the various P pools in the soil especially after biochar amendment.

2.3.2 Biochar and Phosphate Amendment on Soil Phosphorus Availability

Biochar is a carbon rich product derived from pyrolysis of organic materials at relatively low temperatures of below 700^{o}C (Lehmann and Joseph, 2009). It stores carbon for a long period, ameliorates degraded soils and reduces soil acidity for better crop yield (IBI, 2012).

In many countries, farmers have been advised to burry crop residues into the soil to restore soil fertility. These organic materials perform two basic functions in restoring soil fertility. They can either supply the nutrients directly via decomposition or indirectly increase the soil organic matter content by improving the soil physical properties that regulate the supply of soil nutrients (Handayanto *et al*., 1994). However, availability of these crop residues is limited due to competition for them with animals that feed on them. Their addition to soil also increase carbon emissions due to decomposition (Widowati *et al*., 2011) hence a balance must be established. Yamoto *et al*. (2006) revealed that biochar improves soil physical and chemical properties enhancing nutrient availability and root colonization by mycchorizal fungi. Lehmann *et al*. (2006) established that biochar application improves crop productivity but for great improvement of soil fertility, it needs to be combined with fresh organic material. Irrespective of all these good qualities of biochar, its application in Kenyan soils is very limited with little sensitization done to inform the public. Chan *et al*. (2007), Asai *et al*. (2009) and Saarnio *et al*. (2013) have shown that biochar application, in addition to fertilizer, can lead to plant growth benefits but negative effects are sometimes observed without fertilization due to reduced bioavailability through sorption of nitrogen. In addition, the effect may be short term (Saarnio *et al*., 2013).

For many agricultural fields in Kenya, application of phosphorus is necessary to maintain high productivity. This continuous and large demand for phosphorus would eventually lead to rapid depletion of phosphorus resources, which are mainly non-renewable (Van Vuuren *et al*., 2010). For this reason, the use of biochar could help mitigate the depletion of P, if found to be effective. Atkinson *et al*. (2010) found that biochar affected soil phosphorus availability and plant uptake of phosphorus indirectly by changing the soil environment. Deluca *et al*. (2009) observed that biochar altered soil available phosphorus through biochar anion exchange capacity or by influencing the availability of cations that interact with phosphorus. Moreover, Liard *et al*. (2010) found that addition of biochar reduced phosphorus leaching from manured soils due sorption of both orthophosphate and organic phosphorus by biochar. However, Sionne *et al*. (2014) observed that biochar can even act as a source of soluble phosphorus. Auxtero, *et al.* (2013), observed that biochar amendment in P fertilized ferrasols increased all soil fractions and improved plant nutrient content, soil pH, SOM content and basic cations like Ca^{2+}, Mg^{2+} and K^{+}. However, all these results were obtained from temperate soils with scanty information on tropical soils more so under anaerobic conditions.

Transformation of inorganic and organic forms of phosphorus are governed by microbial activity, moisture content, physico-chemical and mineralogical soil properties (Tiecher *et al*., 2012). Sharpley, (1995), outlines that sometimes soluble P gets sorbed on active organic compounds to remain as organic P in the soil. However, Fink *et al.* (2016), clarifies that negatively charged functional groups in organic substances can interact with positively charged minerals like iron oxides and alter P adsorption as a result. He further explains that organic additives increase P availability by producing organic acids while decaying. These acids compete with P for adsorption sites but if already adsorbed they alter the specific surface charge of iron oxides and cause P ions to be electrostatically repelled. This result in to P desorption from soils hence availability. Yan and Maschner, (2015) and Souza *et al*. (2014) also found similar results.

Biochar has high number of phenol, hydroxyl, carboxyl and quinine groups which increase the negative surface charge (Cohen-Ofri, *et al.,* 2006) and decrease P adsorption as a result. Ywi *et al*. (2014), states that biochar is able to fix Al^{3+} and Fe^{3+} to prevent them from being further precipitated to fix P onto the soil and also due to its alkaline nature it raises the soil pH causing dissolution hence the release of P.

2.4 Biochar and Soil Organic Carbon

SOM is the organic component of soil consisting of three primary parts including small fresh plant residues and small living soil organisms, decomposing active organic matter and stable organic matter (Marlene *et al.,* 2013). SOM serves as a reservoir of nutrients for crops, provides soil aggregation, increases soil nutrient exchange, retains moisture, reduces compaction, reduces surface crusting and increases water infiltration into the soil (Marlene *et al.,* 2013). European Community report (2010) outlines climate, soil texture, soil hydrology, land use (tillage) and vegetation as factors contributing to decline in SOM content. Because of the above enlisted roles, increasing SOM content is a necessary measure for boosting crop productivity.

Sander and Tarek (2012) observe that mineralization of biochar in the soil occurs much more slowly than plant litter and is hence more stable. They also observed that biochar does not decrease the stability of SOM making it a suitable soil additive for agricultural production.
Camilla (2013) studied biochar amendment of soils in Kisumu and found that there was no improved crop yield. This was due to use of nutrient deficient soils and the treatments did not include fertilizer additions. Aslund (2012) noted an increase in soil carbon when amended with biochar using soil samples from Embu in Kenya. Similarly, biochar has been extensively used in Bungoma County and Matungu sub-county with positive crop yields. Andrew and Abigael (2012) observed reduction in SOM with biochar application hence long term stability. Organic carbon is observed to have improved on biochar amendment and the improved level maintained for long durations (Almuth, 2011).

All these reports on biochar amendment on SOM content show an improvement in quantity. However, this improvement is not expressed in mathematical terms and more emphasis is given to the increase in crop yields. It is thus vital to quantify this change since the importance of SOM to agriculture and environmental conservation are very profound. Anaerobic degradation of SOM takes place in four key stages namely; hydrolysis, acidogenesis, acetogenesis and methanogenesis aided by microorganisms present in each stage to form lower molecular weight organic compounds, CO_2, H_2, and CH_4. The equations of reactions for the various stages are as presented: (Anukam *et al*., 2019)

Hydrolysis

$$(C_6H_{10}O_5)_n + nH_2O \longrightarrow nC_6H_{12}O_6 + nH_2$$

Acidogenesis

$$C_6H_{12}O_6 \longleftrightarrow 2CH_3CH_2OH + 2CO_2$$

$$C_6H_{12}O_6 + 2H_2 \longleftrightarrow 2CH_3CH_2COOH + 2H_2O$$

$$C_6H_{12}O_6 \longrightarrow 3CH_3COOH$$

Acetogenesis

$$CH_3CH_2COO^- + 3H_2O \longleftrightarrow CH_3COO^- + H^+ HCO_3^-$$

$$C_6H_{12}O_6 + 2H_2O \longleftrightarrow CH_3COOH + 2CO_2 + 4H_2$$

$$CH_3CH_2OH + 2H_2O \longleftrightarrow CH_3COO^- + 3H_2 + H^+$$

Methanogenesis

$$CH_3COOH \longrightarrow CO_2 + CH_4$$

$$CO_2 + 4H_2 \longrightarrow CH_4 + 2H_2O$$

$$4CH_3OH \longrightarrow 3CH_4 + CO_2 + 2H_2O$$

2.5 Nitrogen

The impact of biochar on soil nitrogen(N) transformation processes are extremely important given the dominant role N nutrition plays in regulating crop production (Prommer *et al.,* 2015). Singh *et al.* (2010), states that N mineralization is not only a step in soil organic N transformation, but also one of the most essential processes in N cycling. Inorganic N, particularly ammoniacal N from soil by mineralization is crucial for paddy crops N supply.

Once a plant is converted to biochar, N obtained from the plant biomass does not improve soil fertility in the short term. This is caused by formation of heterocyclic compounds such as pyrroles, imidazoles and pyridines during the heating process (Knicker, 2010).

Deluca *et al.* (2015), and Diatta (2016), report decreased ammoniacal N and nitrate N with biochar amendment. They attributed this to N sorption by biochar due to its high cation exchange capacity (CEC), volatization of ammonia and losses as nitrogen gas due to nitrification and denitrification. Clough *et al.* (2013), underscored the fact that biochar pyrolised at 600^{o}C adsorbs nitrate, reducing N leaching and ensuring constant release of N in the long term due to its high CEC. However, Lehman *et al.* (2003) and Zhu *et al*. (2016) report no effect on inorganic N fractions with biochar amendment. Zhu *et al.* (2016) however reports a decrease in organic N with biochar amendments. These conflicting results though from different soils need to be ascertained for this paddy soil.

2.6 Biochar and Soil Green House Gas Emissions

Greenhouse gases are those gases that absorb and emit radiations within the thermal IR range (IPCC, 2007). They include water vapour, ozone, and the three primary anthropogenic gases – nitrous oxide, carbon dioxide and methane. Given the current projection on global population growth and food consumption patterns, agricultural production will need to increase by at least 70% to meet food demands by 2050 (Metz *et al*., 2007). At the same time, the agricultural sector is responsible for an estimated 10 – 14% of total greenhouse gas emissions worldwide (Smith *et al.*, 2007). In Kenya, agriculture is the leading producer of greenhouse gases contributing 56% of the total national greenhouse gas emissions (IPCC, 2010). Majority of these greenhouse gas emissions are expected to increase in low and middle income countries where small holder farmers predominate (Smith *et al*., 2007).

As negative impacts of global warming became more pressing, massive and immediate climate change mitigation measures are urgently needed to prevent the dangerous and irreversible consequences of global warming. Therefore, greenhouse gas emissions need to be cut by 85% (Metz *et al*., 2007); a target that is too ambitious due to high carbon footprint of developed countries and increasing greenhouse gas emissions by developing countries (Michaela, 2013).
Global greenhouse gas emissions have increased steadily in the last decade due to fossil fuel use, land use and land use change (Metz *et al*., 2007). Kenya is no exception as more forests are being destroyed for human settlement and agriculture, causing an imbalance in the ecosystem. The temperature rise and climate change that would result from the greenhouse gas emissions would greatly affect the agricultural sector which is the pillar of Kenya’s economy. Carbon (iv) oxide, methane and nitrous oxide are considered the main greenhouse gases in the earth’s atmosphere (Chunxiao, 2014). A general summary of each greenhouse gas, their lifetime in the

atmosphere, their global warming potential over a period of 100 years and annual emissions from agriculture is presented in Table 2.1.

Table 2.1: The global warming potentials and agricultural emissions of carbon dioxide, nitrous oxide and methane.

Greenhouse gas	**Lifetime in years**	**GWP in 100 years**	**Emissions from agriculture (CO_2eq.yr^{-1})**
Carbon dioxide	5 – 20	1	0.04
Nitrous oxide	114	310	2.8
Methane	12	21	3.3

Adapted from IPCC (2021) and Smith *et al.* (2007).

***GWP:** Global warming potentials

Continuous measurement of these gases would provide meaningful information to track greenhouse gas emission trend and help in the fight against the negative effects of climate change. Agricultural land occupies 40% - 50% of the world's surface and accounted for 10% - 12% of anthropogenic greenhouse gases in 2005 as summarized in Table 1(Smith *et al.,* 2007). In Kenya agriculture, being the main producer of greenhouse gases holds a significant potential in climate change mitigation through reduction of greenhouse gas emissions from the soil as well as the sequestering of carbon in agricultural landscapes. This therefore justifies the need to study the effect of biochar amendment on soil greenhouse gas emissions in Kenya and particularly the Yala basin. Rogovska *et al*. (2014) observed that biochar increased carbon dioxide emissions, decreased nitrous oxide emissions and increased soil organic carbon storage capacity, benefits that need to be verified before biochar can be applied to soils in Kenya.

2.6.1 Methane

Methane is a key greenhouse gas with a GWP 25 times greater than carbon dioxide and a lifetime of 12 years (IPCC, 2007). It is produced as part of carbon cycle in anaerobic soil conditions via methanogenesis process (Verheijen *et al.,* 2010). It is largely emitted from anaerobic soils as organic matter decomposes in limited oxygen. These sources contribute 15% - 45% of global methane emissions (Segers, 1998). In anaerobic conditions, the first step in the degradation of organic carbon is fermentation, in which the organic matter is the electron acceptor, producing low molecular weight organic acids and alcohols that are further degraded to acetate, formate and CO_2 (Torres-Alvarado *et al.*, 2005). These substrates form raw materials for methanogenesis with the following methane production reactions.

$$CH_3COOH \longrightarrow CO_2 + CH_4$$

$$CO_2 + 4H_2 \longrightarrow CH_4 + 2H_2O$$

$$4CH_3OH \longrightarrow 3CH_4 + CO_2 + 2H_2O$$

$$4CH_3NH_3 + 2H_2O \longrightarrow 3CH_4 + CO_2 + 4NH_4^+$$

Kengara *et al*. (2019) observe that reduction of CO_2 by hydrogen is the prevalent methanogenesis process in anaerobic soils with limited organic matter. However, where micro-organisms are not limiting, methanogenesis from acetate predominate. Methane oxidation is exploited in reducing CH_4 emissions from soils under aerobic conditions. This process is affected by temperature and soil water content (Dasselar *et al.*, 1998; Bogner, 2011).

Methane absorption by soils increases when soil water content (SWC) increases from 22.5% to 37.5% but decreases when it exceeds 45% (Dasselar *et al.*, 1998). Similar results were found by Scheer *et al*. (2011) in sub – tropical pasture soils. These reports indicate increase in CH_4 emissions and reduced absorptions during very wet soil conditions. Spokas *et al.* (2009) attributes the increased CH_4 emission from anaerobic soils to the inhibitory effect of chemicals in the biochar on the soil methanotrophs. Ekabafe and Asueni, (2015) attribute the high CH_4 emissions under anaerobic conditions to warm temperature and presence of soluble carbon which promote high activity of methanogenic microorganisms.

Previous studies have reported reduced methane emissions from soils amended with biochar (Karhu *et al.*, 2011; Liu *et al.*, 2011). Liu *et al.,* 2011 reports a reduction of 51.1% - 91.2% from paddy soils amended with bamboo and straw biochar while Karhu *et al.* (2011) registers increased methane consumption of 96% compared to the control without biochar. However, none of these tests were conducted using tropical soils. Cumulative methane production was highly increased in biochar amended soil incubated under anaerobic conditions with a range of 5.63 – 19.4 g CH_4/kg soil (Phy *et al.,* 2014). Rondon *et al.*, (2005) reported almost complete suppression of methane emission from biochar amended acidic soils in the eastern Colombian plains. In contrast, Knoblauch *et al.* (2011) reported no significant changes in CH_4 production from a calcarid fluvisol amended with biochar as Zhang *et al.* (2010) registered increased methane emissions from biochar amended paddy soils. It is thus not clear whether biochar amendment under anaerobic conditions reduces or increases CH_4 emissions from anaerobic soils. Moreover, most of these studies were conducted using temperate soils and not tropical soils hence the need for this study.

2.6.2 Nitrous Oxide

Nitrous oxide is among the most potent greenhouse gases with a global warming potential 298 times greater than carbon dioxide and remains in the atmosphere for much longer than the other gases (Solomon *et al*., 2007; Felber *et al.*, 2012). It also has a very high potential of ozone layer depletion (Ravinshankara *et al.*, 2009). Nitrous oxide emissions from agriculture accounts for 62% of the anthropogenic nitrous oxides (Duxbury, *et al.*, 1994). The denitrification reaction that occurs in soils under anaerobic conditions is responsible for converting NO_3^- into nitrous oxide gas (Kool *et al.*, 2011; Deng, 2013). Denitrification and nitrous oxide production are dependent on water content and temperature (Kool *et al.*, 2011) with maximum nitrous oxide production occurring at over 90% soil water holding capacity. Inglett *et al.* (2005) report that nitrate reduction occurs in wetlands according to two main pathways (i) dissimilatory nitrate reduction to ammonium and (ii) denitrification as in the reactions below.

$NO_3^- \rightarrow NO_2^- \longrightarrow NH_4^+$

$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \longrightarrow N_2$

Yang *et al.* (2014), asserts that oxidation of ammonium is a key step in the nitrogen cycle, regulating the production of nitrate, nitrous oxide and nitrogen gas. He further notes that anaerobic oxidation of ammonium to nitrogen under iron reducing conditions called Feammox provides basis for nitrogen loss from wetland soils.

$6Fe(OH)_3 + 5H^+ + NH_4^+ \rightarrow 6Fe^{2+} + 9H_2O + 1/2\ N_2$ (-245 kJ/mol)

This reaction remains energetically favourable over a wide pH range.

Feammox to nitrite mainly occurs in pH below 6.5 and yields less energy as in the reaction below.

$6Fe\ (OH)_3 + 10H^+ + NH_4^+ \rightarrow 6Fe^{2+} + 16H_2O + NO_2^-$ (-164 kJ/mol)

The nitrite produced can be a substrate for denitrification resulting into production of nitrous oxide. Feammox to nitrate is also possible but produces -207 kJ/mol energy per reaction.

Anaerobic ammonium oxidation (Anammox) is a microbial process of the nitrogen cycle where nitrite and ammonium ions are converted directly into nitrogen and water.

$NH_4^+ + NO_2^- \longrightarrow N_2 + 2H_2O$.

Zwieten *et al.* (2010) reported that 5% biochar amendment of acidic ferralsol soils reduced N_2O emissions by about 84% compared to the control without biochar in flooded soils. Case *et al.* (2012) mixed biochar into topsoil at the rate of 2% w/w and found at least 47% suppression in N_2O emissions after each wetting event. However, all these investigations were conducted with temperate soils with scanty information on tropical soils.

One of the explanations for the reduction of N_2O emissions from biochar amended soils includes reduction in the amount of nitrogen(N) available for denitrification as adsorption of NH_4^+ is much enhanced in soils containing biochar (Singh *et al.*, 2010; Steiner *et al.*, 2007; Bai *et. al.*, 2015). Furthermore, it facilitates the transfer of electrons to the denitrifying microorganisms which enhances further reduction of N_2O to nitrogen (Cayuela *et al.*, 2013). This is however further enhanced with increased biochar rates (Yanghui *et. al*., 2017). Moreover, the degree to which N_2O emission can be reduced also have been shown to vary depending on the feedstock used to produce biochar (Zwieten *et al*., 2009) as well as the type of soil, biochar application rate and soil moisture conditions. The above mentioned studies were conducted using straw, bamboo and wood chip biochar. None was done using maize stock biochar which was used in this study.

2.6.3 Carbon Dioxide

Carbon dioxide is a greenhouse gas with a global warming potential of one and a lifetime of between five to twenty years (Solomon *et al.,* 2007). Its emission from soils is derived from native SOM, the mineralization of added carbon compounds (like dead plants), the mineralization of dead roots and the direct respiration from plant roots (Hanson *et al.,* 2000; Luo and Zhou, 2006). Tans and Keeling (2009), reported atmospheric carbon dioxide concentration of 386ppm with an increasing rate of 1.9 ppm/year from 1995-2005. CO_2 emissions from soils are primarily controlled by soil temperature, moisture conditions and the available substrate (Raich and Tufekciogul, 2000). Soil CO_2 emissions may be affected by biochar amendment with some authors suggesting a co-benefit of biochar amendment in reducing CO_2 emissions and long term increase in soil organic carbon (Lehmann *et al.,* 2011). However, few reports support this statement. Wang *et al.* (2012) reports no significant change in CO_2 emissions with biochar amendment from paddy soils under anaerobic conditions. Lui *et al.* (2013) supports Wang *et al.* (2012) but adds that high amounts of biochar reduces CO_2 emissions. Zimmerman *et al*. (2011) and Case (2013) on the other hand reported variable CO_2 emissions from soils amended with more saturated biochar. It is therefore not certain whether biochar reduces CO_2 emissions from soils. It is also not known if CO_2 emissions from tropical soils is affected by biochar amendment especially when under anaerobic conditions.

2.6.4 CO_2/CH_4 Ratio

In wetland soils organic molecules are initially degraded by a series of hydrolysis and fermentation reactions that generate lower molecular weight products like acetate, hydrogen and carbon dioxide which are used for microbial respiration (Keller *et al.*, 2009). The inorganic terminal electron acceptors (TEAs) for microbial respiration in the absence of oxygen in the order of decreasing thermodynamic yield are NO_3^-, Fe^{3+}, Mn^{4+}, and SO_4^{2-}. Reduction of these TEAs coupled with oxidation of SOM to CO_2 and competitively suppresses CH_4 production. Once these TEAs are depleted CH_4 is either produced through splitting acetate to CO_2 and CH_4 or reducing CO_2 using H_2 (Keller *et al.*, 2009). Most of the CO_2 produced in anaerobic systems cannot be accounted for by microbial reduction of the inorganic TEAs. Many fermentation processes produce CO_2 hence CO_2/CH_4 ratio > 1 in wetland soils (Keller *et al.*, 2009). The ratio is even higher where humic substances are used as TEAs because they are thermodynamically favorable and also directly inhibits methanogenesis (Keller *et al.*, 2009).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Soil Material

3.1.1. Sampling Area for Soil Material

The soil material was collected from Dominion farm in the lower Yala River basin area of Siaya County. The Yala swamp is located in western Kenya with coordinates lattitude 0^{o} 02’ 10.80’’ N and longitude 34^{o} 04’ 0.06’’ E. The soil samples were collected from Area I presented in Figure 3.1.

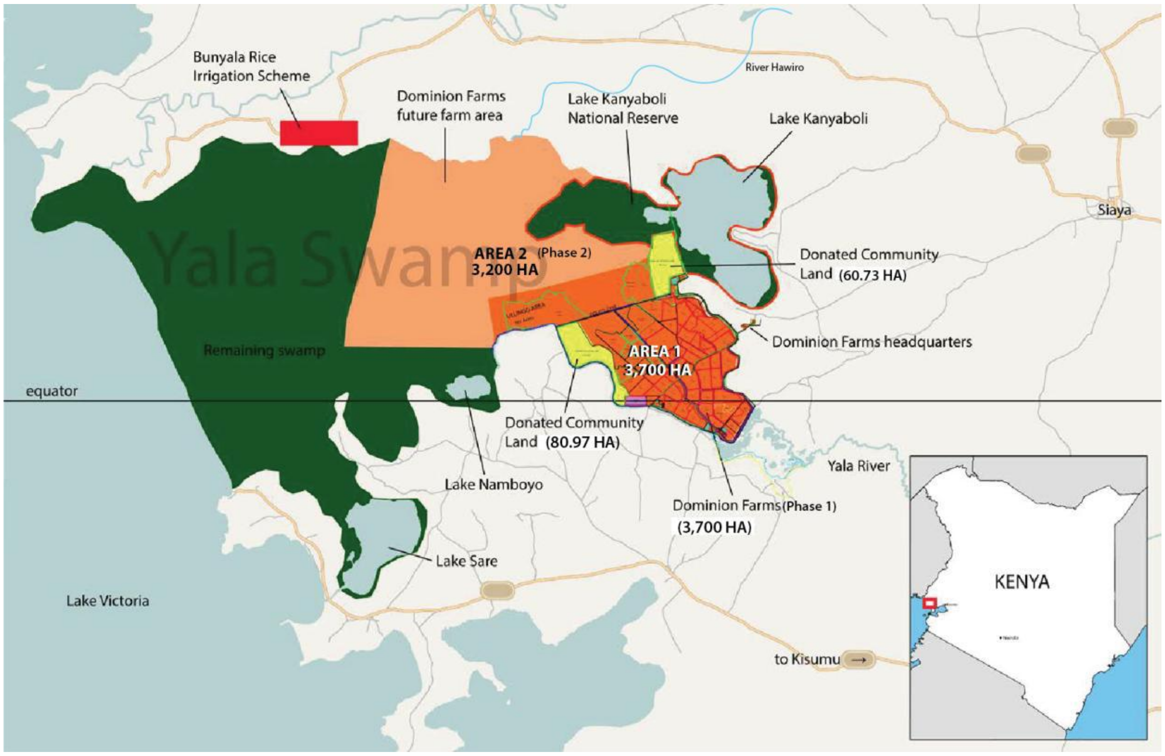

Figure 3.1: A map of Yala Swamp in Kenya showing the area where the large scale agricultural project is situated. Adopted from Abila (2002).

3.1.2 Sample Collection and Characterization

Ten soil samples (approximately 1 kg) were collected from different sites selected randomly within the Dominion Farm in Yala Swamp. Each sample was obtained by digging the soil to a depth of 30 cm using a soil auger (Zhai *et al*., 2014). The samples were packed in cellulose bags and labeled, after which they were transported to the laboratory. The soil samples were air dried in the laboratory for one week. Each sample was then ground using a pestle and mortar then sieved using a 2 mm diameter stainless steel sieve. 200 g of each sample was transferred into a mixer operating at 200 rpm to form a composite sample. The soil was characterized at Kenya Forestry Research Institute(KEFRI) in Maseno and Kenya Agricultural and Livestock Research Organization (KALRO) in Kisumu and the data were as shown in Table 3.1.

Table 3.1: Characterization of the soil used for the study

Parameter	Quantity
Sand (%)	68.04
Silt (%)	7.4
Clay (%)	24.56
Textural class	Sandy clay loam
pH ($CaCl_2$)	4.2
CEC (me/100g soil)	9.3
Calcium (ppm)	1694.2
Iron (ppm)	406.2
Sodium (ppm)	823.9

3.2. Biochar

The maize-derived biochar was obtained from and characterized by the Institute of Soil Science, Chinese Academy of Sciences in Nanjing China and the data presented in Table 3.2.

Table 3.2: Biochar characterization

Parameter	Quantity
pH	6.98
Carbon(%)	60
Hydrogen (%)	2.87
Nitrogen(%)	2.49
Silicon (%)	0.62
Calcium (%)	0.16
Magnesium (%)	0.11
Potassium (%)	0.12
Phosphorous (%)	0.09
Iron (%)	0.09
Aluminium (%)	0.08
Sulphur (%)	0.07
H/C ratio	0.57
O/C ratio	0.37

Adopted from Jia *et al*. (2013)

3.3 Chemicals and Reagents

The chemicals and reagents used in the extraction and analysis of phosphorus, nitrogen, carbon, pH and greenhouse gases are listed in Table 3.3. All the reagents were analytical grade.

Table 3.3: Chemicals and reagents used in the study

Reagent	% Purity	Manufacturer
Ascorbic acid	98.0	Unichem, India
Potassium persulfate	99.0	Unichem, India
Potassium antimony tartarate	99.5	Loba chemie PVT, India
Ammonium molybdate	98.0	Loba chemie PVT, India
Sodium hydrogencarbonate	99.9	Rankem, India
Sodium carbonate	99.9	Finar limited, India
Ammonium fluoride	99.0	Finar limited, India
Cupric sulphate	99.5	Starchem, India
Sodium chloride	99.5	Unichem, India
Ammonium chloride	99.0	Loba chemie PVT, India
Tri-sodium citrate dehydrate	99.0	Rankem, India
P-nitrophenol indicator		Rankem, India
Potassium dihydrogen phosphate	99.6	Central drug house limited, India
Potassium nitrate	99.5	Unichem, India
Copper turnings		Rankem, India
Cadmium turnings		Loba chemie PVT, India
Sodium hydroxide pellets	98.0	Loba chemie PVT, India
Sodium nitroprusside	99.5	Riedel-de Haen, India
Calcium chloride	99.5	Rankem, India
Sodium diothinite	99.0	Loba chemie PVT, India
N-1- napthylethylene diamine dihydrochloride	99.0	Loba chemie PVT, India
Potassium chloride	99.5	Unichem, India
Sulphuric acid	98.07	Loba chemie PVT, India
Hydrochloric acid	38	Loba chemie PVT, India
Phenol	98.47	Loba chemie PVT, India
Sodium hypochlorite	14	Di avin enterprises limited, Kenya

3.4 Incubation Experiments

3.4.1 Experimental Design

The experimental design was adapted from Zhai *et al.* (2014). A two factor randomized complete block design was used with incubation duration as one factor and soil amendment with biochar (treatment) as the other factor, with the different treatments as sub factors.

3.4.2 Experimental Set-up

The set up was done according to the method used by Zhai *et al.* (2014) with slight modifications. The composite sample was subdivided into four portions and subjected to the various treatments to form S, SP, SPB, and SB as shown in table 3.4.

Table 3.4: Composition of the various treatments

Treatment	Soil (g)	P fertilizer KH_2PO_4 (g)	Biochar (g)	Total mass (g)
S	100.0	0.0	0.0	100.0
SP	99.0	1.0	0.0	100.0
SPB	98.0	1.0	1.0	100.0
SB	99.0	0.0	1.0	100.0

The samples were prepared in triplicate. Each replicate of the samples consisting of SP, SPB and SB was thoroughly mixed in a mixer at 200 rpm and then transferred into an airtight glass flask, with a septum in the lid (Figure 3.2). Deionized water was then added to each flask up to 2 mm above the compacted soil surface to induce anaerobic conditions. Each flask was tightly closed and then incubated at 25^{o}C in a Panasonic cooled incubator MIR-154-PE. There were two replicate set-ups: one for sampling greenhouse gases and the other for sampling soil aliquots.

Figure 3.2: Incubation Flasks. (Photo Courtesy of Rogers)

3.5 Analysis of Soil Aliquots

The soil aliquots were sampled and analysed after 1, 7, 14, 21, 28, 35, 50, 60, 74, 90 and 100 days.

3.5.1 Determination of soil pH

Soil pH was determined according to the method used by Kengara (2010). A mass of 5 g of soil was placed into a 50 mL centrifuge tube and 0.01 M calcium chloride solution added to make a final soil to calcium chloride solution ratio of 1:2. The tubes were shaken for 1 hour, allowed to stand for another 1 hour then vigorously shaken for a minute and the pH immediately determined using a pH meter.

3.5.2 Determination of Total Phosphorus

The soil samples were digested using the alkaline persulphate oxidation (APHA, 1995). A mass of 2.0 g of each sample was weighed and transferred into 25 mL conical flask. To each flask 1.0 g of potassium persulphate, 1 mL of 3.75 M sodium hydroxide and 40 mL deionised water were added. These were shaken gently and transferred into a steam sterilizer autoclave digester at 121^{o}C for 15 minutes. After cooling and settling, each sample was filtered into 100 mL volumetric flasks. A volume of 10 mL mixed reagent (antimony-molybdate) was added to each flask which was then topped up to the mark using deionised water. Calibration standard solutions of KH_2PO4 were made in the range of 0, 5, 10, 20, 40, 60 µg/L. The standards and samples were then allowed to stand for 1 hour for the blue colour to form prior to spectroscopic analysis using a Jasco V-630 UV/Vis Spectrophotometer. Both calibration standards and samples were analysed at 885 nm. The phosphorous level obtained was then converted to total phosphorous using the expression

$$T.P = \frac{y \text{ x } 100}{24.5}$$

where y is the phosphorous level obtained from this analysis. This conversion is based on the efficiency of alkaline persulfate digestion of 24.5% as reported by Dayton *et al*. (2017).

The reagents were prepared as follows; ammonium molybdate was prepared by dissolving 15 g in 500 mL of deionised water, ascorbic acid by dissolving 27 g in 500 mL of deionised water, potassium antimony tartarate by dissolving exactly 0.34 g in 250 mL of deionised water and sulphuric acid by adding 140 mL to 600 mL of deionised water and making it to 1 litre. Mixed reagent was made by combining 100 mL of ammonium molybdate, 250 mL of sulphuric acid, 100 mL of ascorbic acid and 50 mL of potassium antimony tartarate without altering the order. Exactly 10 mL of the mixed reagent was added to 100 mL of each sample.

The stock solution (0.1g PO_4-P/L) was made by dissolving exactly 0.439 g of potassium dihydrogen phosphate in 1 litre of deionised water. A sub stock solution (100 µg/L) was made by diluting 1.0 mL of the stock solution to 100 mL using deionised water. Standard solutions were made in the range of 0, 5, 10, 20, 40, 60µg/L by diluting to 50 mL the following volumes of the substock solution 0, 0.25, 0.5, 1.0, 2.0, and 3.0 mL respectively. The standards were treated in the same manner as the samples.

3.5.3 Inorganic Phosphorus Fractions

The extraction was based on the difference in solubility of various phosphorus fractions in different extracting solvents as outlined by Pierzynski (2000). The reagents were prepared as follows: ammonium chloride was made by dissolving 53.3 g in 1 litre of deionised water, ammonium fluoride by dissolving 18.5g in 1 litre of deionised water and adjusting the pH to 8.2 using 3.75 M sodium hydroxide. 0.1 M sodium hydroxide was made by dissolving 4 g in 1 litre of deionised water, 0.25 M sulphuric acid by diluting 14 mL to 1 litre of solution. Saturated sodium chloride solution was made by dissolving 58.5 g in a litre of deionised water and sodium EDTA made by dissolving 0.372 g in 1 litre of deionised water.

A volume of 40 mL ammonium chloride solution was added to each 2 g wet soil samples and then shaken in an orbital shaker (Stuart orbital shaker SO1) at 200 rpm for one hour followed by centrifugation at 3000 rpm for 5 minutes using Beckman Coutter Allegra X-30R centrifuge. The supernatant was then filtered into 100 mL volumetric flask and made up to the mark with deionised water to get loosely bound phosphorus. To get aluminium bound phosphorus, the residue was mixed with 40 mL of ammonium fluoride, shaken for 30 minutes, centrifuged for 15 minutes and filtered. The residue was rinsed twice with 25 mL portions saturated sodium chloride and the supernatant added to the extract above. The residue was then mixed with 40 mL sodium hydroxide, shaken for 17 hours, centrifuged at 5000 rpm then vacuum filtered to avoid re-oxidation of Fe^{2+} to Fe^{3+} due to long exposure to air. The residue was rinsed twice with 25 mL portions of sodium chloride and the supernatant added to sodium hydroxide extract to give iron bound phosphorus. 40 mL 0.25 M sulphuric acid was added to the residue and the mixture shaken for 1 hour, centrifuged and filtered to obtain calcium bound phosphorus. The pH of the extract was adjusted using 3.75 M sodium hydroxide before addition of mixed reagent. The residue was then mixed with 40 mL of sodium EDTA, shaken for 1 hour, centrifuged and then filtered to obtain occluded phosphorus. The phosphorus level in each of the extracts was determined as in 3.5.2.

3.5.4 Organic Phosphorus Fractions

Organic phosphorus separates into labile, moderately labile and non-labile pools (Chang and Jackson, 1957). The reagents used for this extraction were prepared as follows; 0.5M sodium hydrogen carbonate was made by dissolving 42 g in 1 litre of deionised water, and the pH adjusted to 8.5 using 3.75 M sodium hydroxide. 2 M and 1 M hydrochloric acid was made by diluting 168 mL and 84 mL concentrated acid to 1 litre respectively. P-nitophenol indicator was prepared by dissolving 0.25 g in 100 mL of deionised water and 2.5 M sulphuric acid made by diluting 141 mL to 1 litre of solution. 0.2 M sodium hydroxide was made by dissolving 8 g in 1 litre of deionised water.

3.5.4.1 Labile Organic Phosphorus

Masses of 2 g of wet soil sample mixtures were each placed into a centrifuge tube and 40 mL of 0.5 M sodium hydrogen carbonate added. The mixture was shaken for 16 hours then centrifuged at 5000 rpm for 15 minutes, filtered through Whatman number 41 filter paper into 100 mL volumetric flask and brought to volume. The supernatant was divided into two equal portions of 50 mL. To the first portion, 5 drops of p-nitrophenol indicator was added and the pH adjusted using 2M hydrochloric acid until it turned colourless. The phosphorus content was then determined as in 3.5.2. A mass of 1 g potassium persulphate and 1 mL 3.75 M sodium hydroxide were added to the second portion and the mixture digested in an autoclave steam stereliser at 121^{o}C for 15 minutes. The sample was then allowed to cool, filtered and phosphorus determined as in 3.5.2. The difference in phosphorus levels between the two portions gave the labile organic phosphorus.

3.5.4.2 Moderately Labile Organic Phosphorus

A volume of 40 mL of 1 M hydrochloric acid was added to the residue from the above process and the mixture shaken for 3 hours, centrifuged and filtered into 100 mL volumetric flask. The supernatant was brought to volume, mixed thoroughly and divided into two equal portions. Phosphorus level in the first portion was determined directly as in 3.5.2., while the second portion was subjected to alkaline persulphate digestion to give total moderately labile phosphorus. The difference between the two gave part of the moderately labile phosphorus.

The residue was rinsed with deionised water and the supernatant discarded. The residue was mixed with 40 mL of 0.5 M sodium hydroxide and shaken for 3 hours, centrifuged and vacuum filtered. The pH of the supernatant was adjusted to 0.2 using 2 M hydrochloric acid then centrifuged at 5000 rpm for 15 minutes followed by filtration. Phosphorus was determined in 50 mL of this extract and added to the hydrochloric acid extracted moderately labile phosphorus to give the total moderately labile organic phosphorus.

3.5.4.3 Non-labile Organic Phosphorus

The above residue was rinsed with deionised water and the supernatant discarded. A volume of 40 mL of 2.5 M sulphuric acid was added to the residue followed by 1 g potassium persulphate then digested in a steam sterilizer for 15 minutes at 121^oC. The mixture was then allowed to cool, filtered and the pH adjusted using 3.75 M sodium hydroxide. Phosphorus was then determined as in 3.5.2.

3.5.5 Determination of Total Organic Carbon (TOC)

TOC was determined by loss on ignition as described by Ball, (1964). The crucibles to be used were heated for 1 hour at 375^oC in a furnace, allowed to cool to 150^oC in the open, placed in a desiccator for 30 minutes then weighed and recorded. 10 g of each of the samples were oven dried at 105^oC for 24 hours. 5 g of each sample was placed in a crucible from the desiccator, then heated for 2 hours at 375^oC in a furnace. The samples were allowed to cool to 150^oC and the post ignition weight measured and recorded. The difference between the pre-ignition weight and post ignition weight gave the total organic matter (TOM) in the soil samples. The total organic carbon was determined using the equation $y = 0.52x - 0.55$ as outlined by Bojko and Kabala (2015), where x is the TOM and y is the TOC.

3.5.6 Determination of Dissolved Organic Carbon

The determination of dissolved organic carbon was done in accordance with the technique used by Carter *et al*. (2012). Masses of 5 g of soil sample mixtures from each treatment were each placed into a 50 mL centrifuge tube and 0.01 M calcium chloride solution added to make a final soil to calcium chloride solution ratio of 1:2. The tubes were shaken for 1 hour, followed by centrifugation at 5000 rpm for 10 minutes. The supernatant was vacuum filtered using 0.45 µm filter paper and dissolved organic carbon analyzed at two wavelengths of 340 nm and 355 nm.

This is a triple component DOC with optical absorbance in terms of linear sum of two components A and B each with its own fixed absorbance spectrum and a third component C that does not absorb light and is present at the same concentration in all the water samples.

Component A absorbs UV light strongly and is presumed to be hydrophobic while component B absorbs UV light weakly and is assumed to be hydrophilic. The DOC concentration in a given sample is given by the formula

$$DOC = DOC_{AB} + DOC_{C} \qquad (1)$$

Where DOC_{AB} referring to light absorbing components.

Therefore

$$DOC = \frac{A_{\lambda}}{E_{AB\lambda}} \quad + \quad DOC_{c} \qquad (2)$$

where A_{λ} is the absorbance of the sample at 340 nm and E_{AB} is the extinction coefficient of the light absorbing DOM given as

$$\begin{aligned} E_{AB\lambda} &= f_{A}E_{A\lambda} + f_{A}E_{A\lambda} \\ &= f_{A\lambda} + (1 - f_{A})E_{B\lambda} \qquad (3) \end{aligned}$$

where f_A and f_B are the fractions of components A and B that comprise the light absorbing DOM and $E_{A\lambda}$ and $E_{B\lambda}$ are the extinction coefficients at 340 nm. The above equation can be written for the two different wavelengths (340 nm ($_{\lambda1}$) and 355 nm ($_{\lambda2}$)) and the ratio R defined as

$$\begin{aligned} R &= E_{AB\lambda1}/E_{AB\lambda2} \\ &= A_{\lambda1}/A_{\lambda2} \end{aligned}$$

The combination of R and the extinction coefficients of A and B yields;

$$F = (E_{B\lambda1} - RE_{B\lambda2})/(R(E_{A\lambda2} - E_{B\lambda2}) + (E_{B\lambda1} - EE_{A\lambda1}))$$

Therefore, if the values of $E_{A\lambda1}$, $E_{A\lambda2}$, $E_{B\lambda1}$ and $E_{B\lambda2}$ are known, f_A can be calculated and then substituted in equation 3 to obtain E_{AB} at either of the wavelengths and DOC determined as in Equation 2. The extinction coefficients and DOC_c are as in Table 3.5.

Table 3.5: Extinction coefficients of components A and B at different wavelengths.

Extinction Coefficient	**Component A**	**Component B**
E_{270nm}	69.3	15.4
E_{350nm}	30.0	0.0
E_{254nm}	77.1	21.3
E_{280nm}	63.9	12.0
E_{285nm}	61.1	10.6
E_{310nm}	47.6	4.7
E_{340nm}	34.1	0.7
E_{355nm}	27.9	0.0
DOC_C	0.80	

The extinction coefficients (E) have units of L/gcm, DOC_c is in mg/L. (Adopted from Carter *et al*. 2012).

3.5.7 Determination of Nitrogen

The method prescribed by APHA (1995) was adopted for the total nitrogen and total oxidized nitrogen.

3.5.7.1 Total nitrogen (TN)

Masses of 0.2 g of the wet soil samples from each treatment were digested in alkaline persulfate oxidation (APHA, 1995), by adding 1g potassium persulphate, 1 mL 3.75 M sodium hydroxide and 40 mL deionised water to each sample. The samples were then digested in an autoclave steam sterilizer at 121^{o}C for 15 minutes, allowed to cool then filtered. The filtrate was passed through copper cadmium column to be reduced to nitrites. To each of the samples 1 mL sulphanilamide was added and shaken thoroughly. The sample was allowed to rest for 5 minutes and then 1mL of N-1- Naphthylethylene diamine dihydrochloride and mixed completely. The samples were allowed to settle for 1 hour before analysis. A 1000 µg/L stock solution was made by dissolving 7.218 g of anhydrous potassium nitrate in deionised water and made up to 1 litre. A sub stock solution was made by diluting 1 mL of the stock solution to 1 litre. Standard solutions were made in the range of 0, 5, 10, 15, 20, and 30 µg/L by diluting to 50 mL the following volumes of the sub stock solution: 0, 0.25, 0.5, 0.75, 1.0, and 1.2 mL respectively. The standards were treated the same way as the samples. The spectrophotometer was calibrated using the NO_3^- nitrogen and absorbance measured at 543 nm using deionised water as a blank. The copper–cadmium column was made by reacting 100 g 0f cadmium turnings with 2% copper sulphate (w/v) that is 10 g in 500 mL of solution. Copper turnings were used to plug the bottom of the column together with cotton wool. The column was filled with dilute ammonium chloride solution and copper–cadmium mixture to produce a 30 cm length.

3.5.7.2 Total Oxidised Nitrogen (TON)

A volume of 30 mL of 2 M potassium chloride was added to 2 g wet soil sample from each treatment and the mixture shaken for 30 minutes, centrifuged then filtered. The supernatant was then passed through the copper-cadmium column and nitrogen determined as in 3.5.7.1.

3.5.7.3 Ammoniacal Nitrogen

The method used for ammonium determination was the indophenols blue photometric determination (APHA, 1995). Ammonium is converted to monochloroamine in an alkaline condition resulting to the formation of the indophenols blue complex. The blue colour forming reaction is called Berthelot reaction and it is not a rapid reaction hence the 24 hours wait. Nitroprusside was used as a catalyst and precipitation of hydroxides was prevented by metal complexing sodium citrate.

Ammonium nitrogen was extracted by centrifuging 2 g moist soil samples with 30 mL of 2 M potassium chloride for thirty minutes. Each sample was treated with 3 mL hypochlorite solution, 3 mL phenol reagent and the mixture thoroughly shaken and the samples kept in the dark for 24 hours at 4°C for the blue colour to form before analysis. Phenol solution was made by dissolving 17.5 g of phenol, and 0.2 g of sodium nitroprusside in 500 mL of deionised water and refregirated to be used when required. Hypochlorite was prepared by first dissolving 140 g of tri-sodium citrate- dihydrate and 11 g of sodium hydroxide in 300 mL of deionised water. After complete dissolution, 20 mL of sodium hypochlorite was added and made to 500 mL with deionised water. The reagent was refrigerated until time of use.

A stock solution 1000 mg/L was made by dissolving 3.821 g of anhydrous ammonium chloride in 1 litre of solution. A substock solution (10 mg/L) was prepared by diluting 10mL of the stock solution to 1 litre. Standard solutions were prepared in the range of 0, 10, 20, 40, and 60 µg NH_4^+ nitrogen/L by diluting to 50 mL the following volumes of the substock solution: 0, 0.05, 0.1, 0.2, and 0.3 mL respectively. The standards were treated like the samples and the absorbance measured at 630 nm using deionised water as a blank.

3.5.7.4 Organic Nitrogen

Organic nitrogen was determined by substracting both TON and ammoniacal N from the total N (APHA, 1995).

3.6 Determination of Greenhouse Gas Emissions

GHGs were determined using gas chromatography as described by Wang *et al.* (2012) with slight modifications. A volume of 5 mL of GHGs were sampled after 1, 2, 4, 8, 12, 14, 18, 21, 25, 40, 46, 50, 54, 60, 65, 67, 70, 73, 76, 81, 83, 87, 90, 95 and 100 days from the headspace of each airtight glass container by inserting syringes with thin needles through the septum on the glass lids and analysed immediately using GC. The sampled gases were analysed for methane, carbon dioxide and nitrous oxide using a gas chromatograph (SRI 8610C, USA). Methane and carbon dioxide (converted to methane) were detected using a hydrogen flame ionization detector (FID) and nitrous oxide was detected using an electron capture detector (ECD). Carbon dioxide was reduced to methane by hydrogen in a nickel catalytic converter at $375^{o}C$. Argon-methane (5%) and nitrogen were used as carrier gas at a flow rate of 40 mL/minute for nitrous oxide, carbon dioxide and methane analysis respectively. The temperatures of the column and ECD were maintained at $40^{o}C$ and $300^{o}C$ respectively. The oven and FID were operated at temperatures of $50^{o}C$ and $300^{o}C$ respectively. The concentrations of the gases were determined by comparing their peak areas to those of the gas standard, obtained from Zusammensetzung-Germany with a composition of 0.0004% methane, 0.00004% nitrous oxide, 0.04%`carbon dioxide and 99.9556% synthetic air.

To obtain the concentration of the greenhouse gases in micrograms/ gram of soil, the total mole of all the gases in the headspace of the incubation flask was calculated from the ideal gas law as;

$$N_{tot} = PV/RT$$

where P is atmospheric pressure (101325 Pa), V is volume of headspace (0.00035) in m^3, R is a proportional constant (8.314 J/mol.K) and T is the incubation temperature of 298 K.

Moles of greenhouse gas $(GHG)_A$ = (ppm value/1000000) x N_{tot}

The concentration of GHG in µg/g of soil was then given as

(GHG) µg/g of soil = $\{(GHG)_A$ x RFM x $10^6)\}$/Mass of soil used (100 g)

The cumulative greenhouse gas emissions were sequentially cumulated from two adjacent measurements.

3.7 Quality Assurance

Prior to GC analysis, standard was injected to check for degradation and analysis only undertaken when the system was stable. The standard was also injected after every 3 samples. The highest and lowest concentration standard mixtures were analyzed after every 10 samples to confirm the integrity of the calibration curve. For the other quantities, the spectrophotometer was calibrated every day before the quantities were measured. Replication assisted on monitoring consistency of the results.

3.8 Statistical Analysis

The data was analysed using Microsoft Excel version 2010. The means and ranges of the data collected were determined at confidence limits of 5% to test the significance of the analytical results. One-way analysis of variance (ANOVA) without replication was used to determine significant differences at 95% confidence interval and T- test ($p \leq 0.05$) used to check phosphorus, nitrogen and greenhouse gas variations with each treatment as well as the duration of incubation.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Effect of Anaerobic Conditions and Biochar Amendment on Soil pH

The results for variations in pH over time for the various treatments are in Figure 4.1. The mean pH for S, SP, SPB, and SB are 5.22±0.1, 5.73±0.1, 5.94±0.07 and 5.56±0.08 respectively. There was a significant increase ($p \leq 0.05$) between S and SP, S and SPB as well as between SB and SPB. However, with SP as the control, no significant difference was registered with the other treatments. In the first 21 days, the pH of SB and SPB rose almost uniform. SPB remained stable after the 21 days while the pH of SB dropped. The drop in the pH of SB could be due to organic acids produced during mineralization of biochar. Similarly, the pH of S and SP also rose and remained relatively steady after the 21st day till end of incubation period. SP, SPB and SB had a higher initial pH than S showing that both P and biochar raised the initial pH.

The lack of significant difference between S and SB shows that biochar amendment had no influence on the soil pH. This result is in agreement with data obtained by Ventura *et al*. (2012) and Phy *et al*. (2014) but differs with Atkinson *et al*. (2010), Farrel *et al*. (2013) and Lai *et al*. (2013) who recorded increased soil pH with biochar amendment. However, P addition (SP and SPB) – as shown by the mean values - increased the soil pH hence the significant difference, although biochar reduced the P influence on pH by a smaller margin as in SPB.

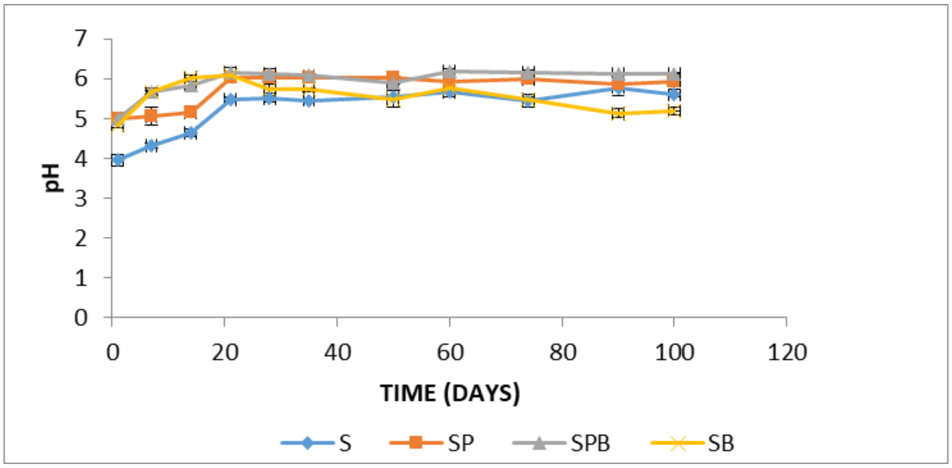

Figure 4.1: Variations in soil pH over time

4.2 Effect of Anaerobic Conditions and Biochar Amendment on Soil *Phosphorus Pools*

4.2.1 Total Phosphorus

The total phosphorus content of the various treatments is shown in Figure 4.2. The total P for S, SP, SPB and SB were 500.11±34.38, 1001.98±30.34, 1709.51±101.40, and 978.90±47.2 µg/g of soil respectively. There was a significant increase in total P ($p \leq 0.05$) between S and the other treatments while between SP and SB there was no significant difference. These results indicate that addition of biochar increased total phosphorus. This implies that the biochar contained phosphorus that it added to the soil. This is not surprising given that the biochar had a P content of 0.09% (Table 4.2). Similar results were reported by Lehmann and Joseph (2009), Chen *et al*. (2016) and Kim *et al*. (2018), who further claimed that charring of woody tissues during biochar manufacture was the main source of phosphorus found within biochar.

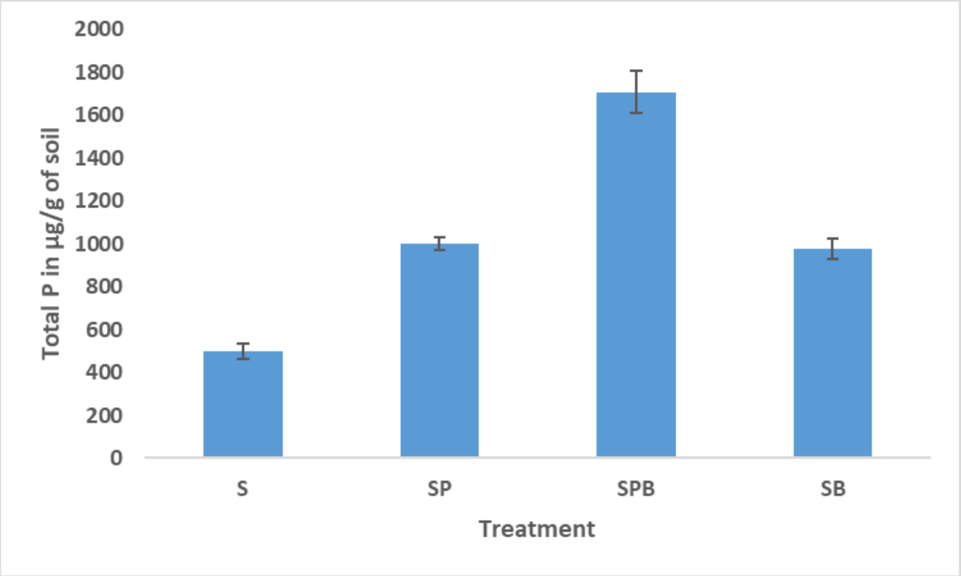

Figure 4.2: Initial Total P content in the different treatments

4.2.2 Inorganic Phosphorus Fractions

4.2.2.1 Loosely Sorbed Phosphorus

The levels of loosely sorbed P over time are shown in Figure 4.3. The mean loosely sorbed phosphorus levels recorded were 66.11±4.93, 106.65±5.38, 84.92±5.91, and 49.10±2.30 µg/g for S, SP, SPB, and SB respectively. There was a significant change ($p \leq 0.05$) in the levels of loosely sorbed phosphorus over the incubation period.

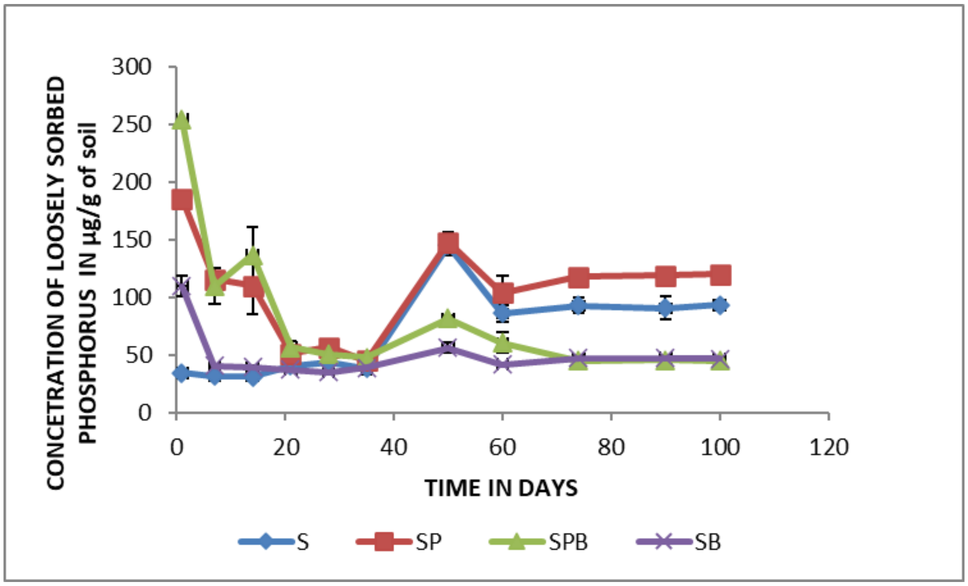

Figure 4.3: Levels of loosely sorbed phosphorus levels over time in the different treatments

With unamended soil (S) as the control, a significant increase ($p \leq 0.05$) was observed with SP, but not with SPB and SB. With SP as the control, a significant decrease ($p \leq 0.05$) was noted with SB but not with SPB. SP, SPB and SB had higher initial loosely sorbed P than S. The loosely sorbed phosphorus level in S rose to a high of 145.42±9.08 µg/g of soil in the 50th day and remained higher (compared to the initial days) for the remaining incubation period. Reduction of Fe(III) to Fe (II) leads to a higher pH because of decreased ability of Fe(II) for hydrolysis. In SP, the phosphorus level was high for the first 14 days then it reduced as more of it got fixed until the 35th day. After that, it increased and remained steady.

In SPB, the phosphorus level was initially high but dropped from the 14th day to the 35th day before rising again. The variation was significant and can be attributed to changes in the soil pH (Ponnamperuma, 1972). Biochar, being neutral (pH = 6.98, Table 3), but more alkaline relative to the soil (pH = 4.2, Table 2), could raise the pH. Furthermore, reducing conditions could also result in increased pH. In any case, increased pH resulted in decreased phosphorus levels. Soil amended with biochar (SB) also started with high phosphorus levels, which dropped during the incubation period. It is also notable that SB, SP, and SPB had very high initial loosely sorbed P levels that decrease with time during incubation. The results for S and SP agree with those reported by Gangxu *et al*. (2014), and Deluca *et al. (*2009), that showed increased loosely sorbed phosphorus in flooded acidic soils. This was attributed to increased soil pH on flooding, facilitated by reduction of Fe(III) to Fe(II), releasing the fixed phosphorus (Ponnamperuma, 1972). The mean loosely sorbed phosphorus levels for SPB and SB were lower than SP and S respectively. This could be because of the introduction of biochar (pH 6.98*)* which is neutral and therefore raises the pH enhancing sorption of LSP. Biochar addition in this study reveals reduction in loosely sorbed phosphorus. Biochar surface could be carrying organic molecules involved in chelation of Al^{3+}, Fe^{3+} and Ca^{2+} ions, which are then sorbed on the charged biochar surface as organo-biochar complexes or organo-mineral-biochar complexes hence decreasing P solubility in the short term (Deluca *et al.*,2015). This result contradicts the results reported by Kalyani *et al.* (2015) and Kim *et al*. (2018) of increased loosely sorbed phosphorus with biochar amendment, although they used alkaline soils.

4.2.2.2 Aluminium Bound Phosphorus

Figure 4.4 shows the variation in Al-P within the treatments over the incubation period. The mean aluminium bound phosphorus levels recorded for the various treatments were 34.14±1.94, 39.74±9.39, 32.38±1.39, and 33.84±1.28 µg/g for S, SP, SPB, and SB respectively. There was no significant difference ($p \leq 0.05$) in aluminium bound phosphorus levels over time and among the treatments. This means that the levels were constant over time and were not affected by biochar amendment or phosphorus application. This could be due to aluminium forming organic complexes with organic molecules from biochar thus having no space for P precipitation (Deluca *et al*., 2009).

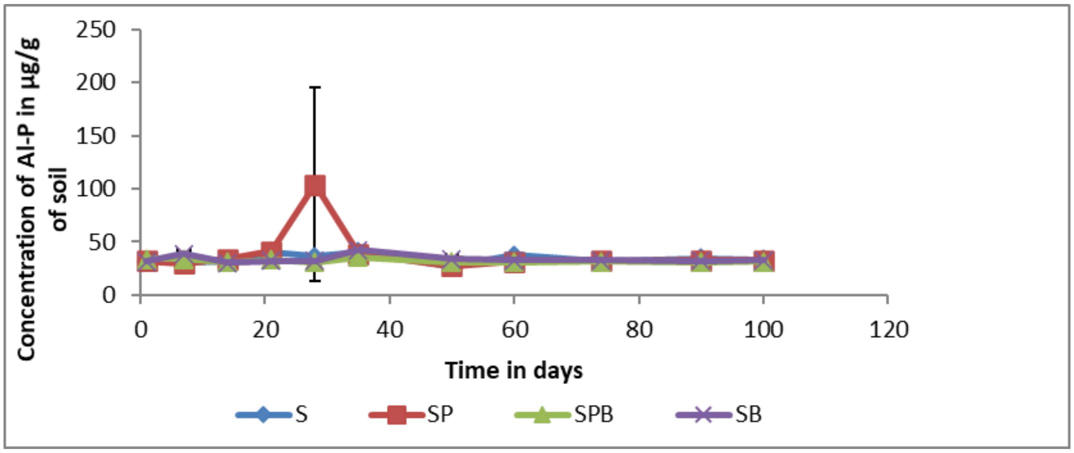

Figure 4.4: Levels of aluminium bound phosphorus over time in the different treatments

This result differs with the findings of Osafo *et al*. (2017) who noted a reduction in aluminium bound phosphorus with biochar treatment. The decrease was attributed to the fact that addition of biochar increased the cation exchange capacity of soil, hence causing increased electrostatic anion repulsion between the negative charges in soil-biochar surfaces and phosphorus ions. (Osafo *et al.,* 2017). However, Gangxu *et al.* (2014) recorded an increase in aluminium bound phosphorus with biochar addition in acidic soil, although the study was not conducted under anaerobic conditions.

4.2.2.3 Iron Bound Phosphorus

Figure 4.5 shows the variations in Fe-P over time in the various treatments.

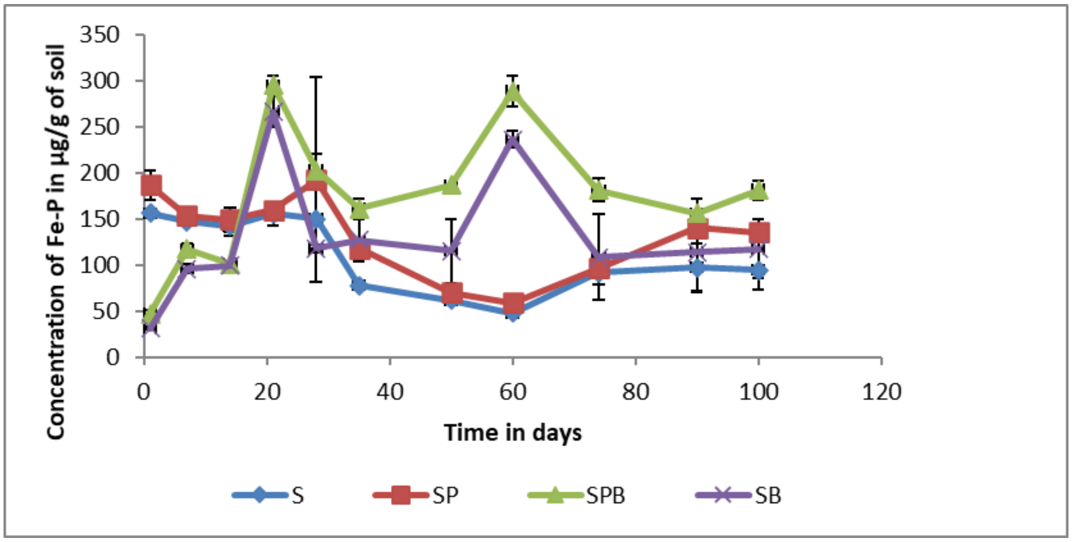

Figure 4.5: Levels of iron bound phosphorus over time in the different treatments

The mean iron bound phosphorus levels for S, SP, SPB, and SB were 111.40±11.79, 133.08±17.51, 174.75±9.78 and 130.24±20.12 µg/g of soil respectively. There was a significant difference ($p \leq 0.05$) in the various phosphorus levels recorded within each treatment over the incubation period. With S as the control, there was a significant increase ($p \leq 0.05$) with SPB while with SB and SP there was no significant difference ($p \leq 0.05$). With SP as the control, there was no significant difference ($p \leq 0.05$) in phosphorus levels with any of the other treatments. SPB and SB had low initial Fe-P levels than S and SP. This could be due to hydrolysis of iron directly added from biochar precipitating most of the P making them unavailable as in the following equation.

$Fe^{3+} + H_2PO_4^- + 2H_2O \longleftrightarrow 2H^+ + Fe(OH)_2H_2PO_4$(Insoluble).

It was also noted that for S, the level of iron bound phosphorus decreased with time. This could be attributed to more of it being released to the loosely sorbed fraction due to reduction of iron(III) to iron(II) causing the increase in the soil pH. From the mean phosphorus levels, it was noted that biochar treatment increased iron bound phosphorus. This result agrees with that of Gangxu *et al.* (2014) and Kalyani *et al.* (2015) that reported increased iron bound phosphorus with biochar amendment. The increase is attributed to the fact that biochar directly provides some amount of iron and aluminium oxides to the soil. These cations first precipitate as soil pH increases with biochar addition and submergence into water (Yuan *et al*., 2012). This result however differs with those reported by Osafo *et al*. (2017), although the study was not conducted under anaerobic conditions and used alkaline soils.

4.2.2.4 Calcium Bound Phosphorus

The levels of calcium bound P over the experimental period are presented in Figure 4.6.

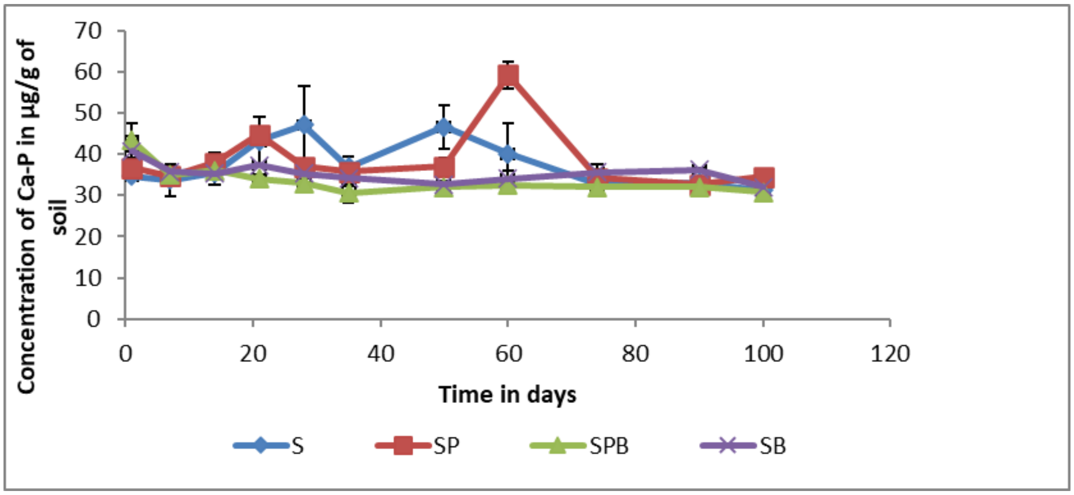

Figure 4.6: Levels of calcium bound phosphorus over time in the different treatments

The mean calcium bound phosphorus levels for S, SP, SPB, and SB were 37.72±3.44, 38.60±2.15, 33.76±1.18 and 35.37±1.43 µg/g of soil respectively. There was a significant change ($p \leq 0.05$) in phosphorus levels over the 100 days' incubation period. However, there was no significant difference ($p \leq 0.05$) among the treatments. This result agrees with those reported by Kalyani *et al*. (2015). The soil being acidic with a pH of 4.2, addition of biochar raises it to between 5 and 6.2. These values do not favour phosphorus precipitation on calcium hence no effect in P bound on calcium. The organic anions in biochar also repel phosphate ions from calcium making them available for plant uptake (Yuan *et al*., 2012). This however differs from the results reported by Osafo *et al.* (2017) and Gangxu *et al.* (2017) where calcium bound phosphorus increased.

4.2.2.5 Occluded Phosphorus

The levels of occluded P over time in the various treatments are presented in Figure 4.7.

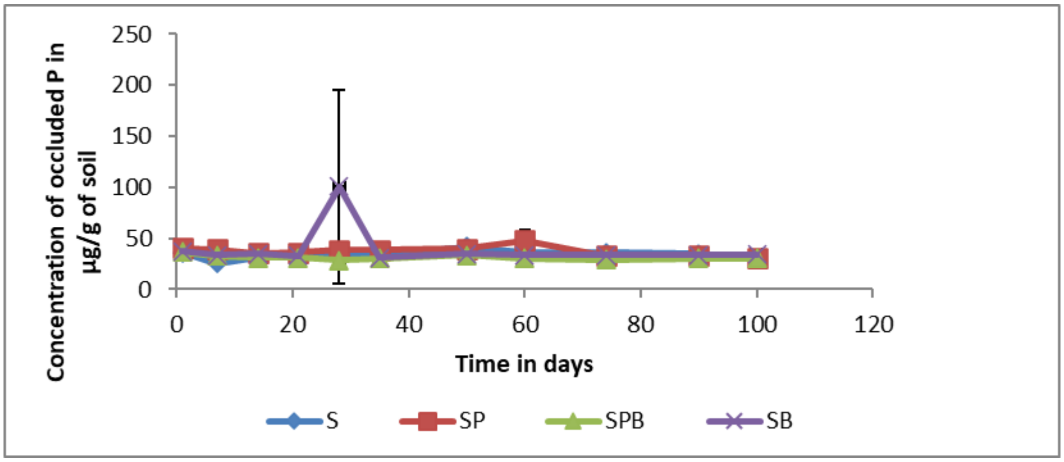

Figure 4.7: Levels of occluded phosphorus over time in the different treatments

The mean occluded phosphorus levels for S, SP, SPB, and SB were 33.83±2.59, 37.45±2.18, 31.13±0.74 and 39.90±10.17 µg/g of soil respectively. There was a significant increase ($p \leq 0.05$) in the mean occluded P in SP and SB with respect to S. However, no significant difference was recorded among the treatments. This means that neither amendment with biochar nor with P has an effect on the levels of occluded phosphorus. This result differs with Abolfazli *et al*. (2012) and Kalyani *et al.* (2015) who reported increased occluded P. This could be attributed to biochar providing more sites for phosphorus sorption making them unavailable.

4.2.3 Organic Phosphorus Fractions

4.2.3.1 Labile Organic Phosphorus

Figure 4.8 presents the variations in labile organic phosphorus with the various treatments. The mean labile phosphorus levels for S, SP, SPB, and SB were 89.42±13.15, 179.34±10.69, 186.19±28.94 and 97.24±26.36 µg/g of soil respectively. There were significant changes ($p \leq 0.05$) in labile phosphorus levels over time. There was a significant increase ($p \leq 0.05$) in labile organic P in SP and SPB, but not in SB using S as the control. With SP as the control, there was a significant decrease ($p \leq 0.05$) in SB but not in SPB. Therefore, the treatments can be divided into two sets: S/SB and SP/SPB. This suggests that biochar does not affect the labile phosphorus content in the soil. This differs with the results reported by Osafo *et al.* (2017) on soils of Ghana. He attributes the outcome to the increase in cation exchange capacity of the soil that facilitate electrostatic anion repulsion between the negatively charged soil-biochar surface and the phosphate ions, hence increased labile P.

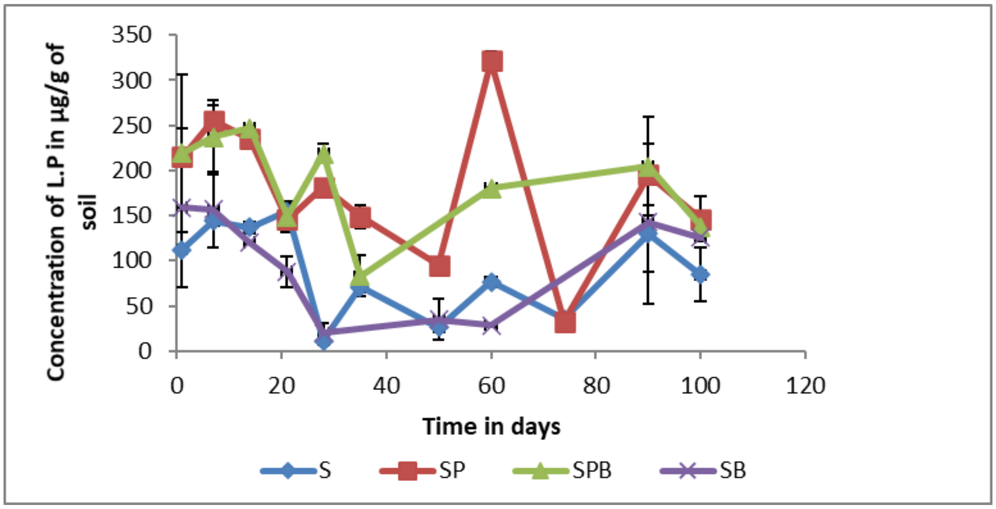

Figure 4.8: Levels of labile organic phosphorus over time in the different treatments

4.2.3.2 Moderately Labile Organic Phosphorus

The levels of moderately labile organic phosphorus in the various treatments over the incubation period are shown in Figure 4.9. The mean moderately labile phosphorus levels for S, SP, SPB, and SB were 63.63±3.50, 78.65±4.07, 72.46±5.58 and 76.89±7.37 µg/g of soil respectively. There were significant changes ($p \leq 0.05$) over time in all the treatments, but there were no significant differences in levels among the treatments. The moderately labile organic phosphorus levels decreased rapidly in the initial days and leveled off after the 28th day until the end of the incubation period. This result agrees with Osafo *et al*. (2017) that reported no effect of biochar amendment on moderately labile phosphorus.

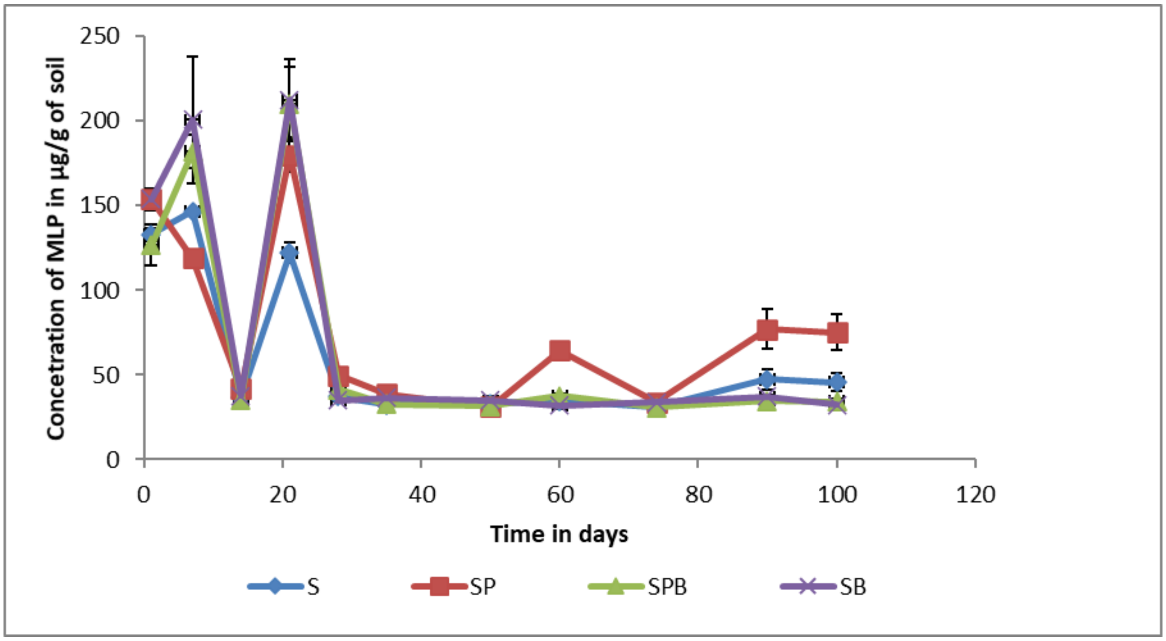

Figure 4.9: Levels of moderately labile organic phosphorus over time in the different treatments

4.2.3.3 Non-labile Organic Phosphorus

The levels of non-labile organic P in the various treatments over time are presented in Figure 4.10.

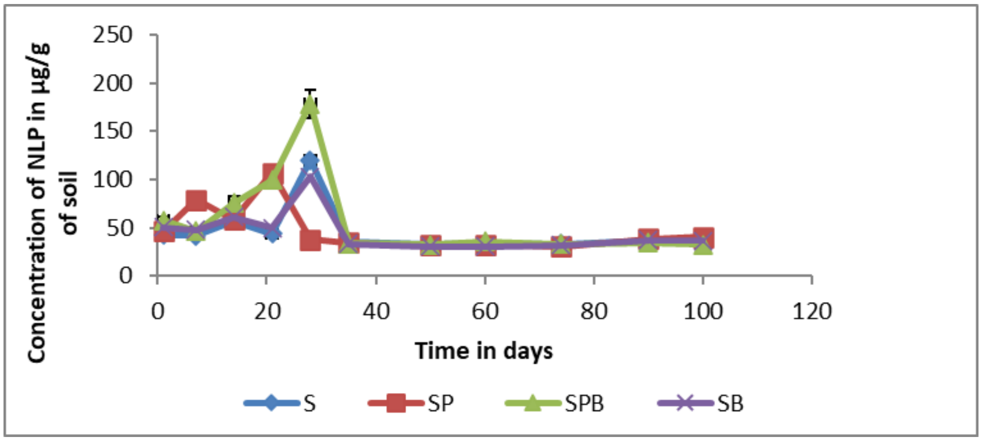

Figure 4.10: Levels of non-labile organic phosphorus over time in the different treatments

The mean non- labile phosphorus levels for S, SP, SPB, and SB were 46.94±2.31, 48.86±1.11, 60.12±3.84 and 46.56±6.49 µg/g of soil respectively. There were significant changes ($p \leq 0.05$) over time in all the treatments, but there were no significant differences in levels among the treatments. The result agrees with what was reported in Osafo *et al.* (2017) where biochar amendment had no significant effect on non-labile organic phosphorus. However, when combined with a phosphorus inorganic fertilizer, an increase was recorded.

4.3 Effect of Anaerobic Conditions and Soil Amendment on Other Soil Nutrients and Parameters

4.3.1 Soil Organic Carbon

4.3.1.1 Total Organic Carbon (TOC)

Figure 4.11 shows the initial TOC for the various treatments. The mean TOC values for S, SP, SPB and SB, were 2.83±0.37%, 3.02±0.45%, 3.03±0.18% and 2.89±0.35%, respectively. There was no significant difference in TOC in all the treatments showing that biochar had no impact on the TOC content. This result differs with Aslund (2012) and Almuth (2011) who reported increase in organic carbon, although the studies were conducted under aerobic conditions. It also differs with Andrew and Abigael (2012) that reported reduction in TOC.

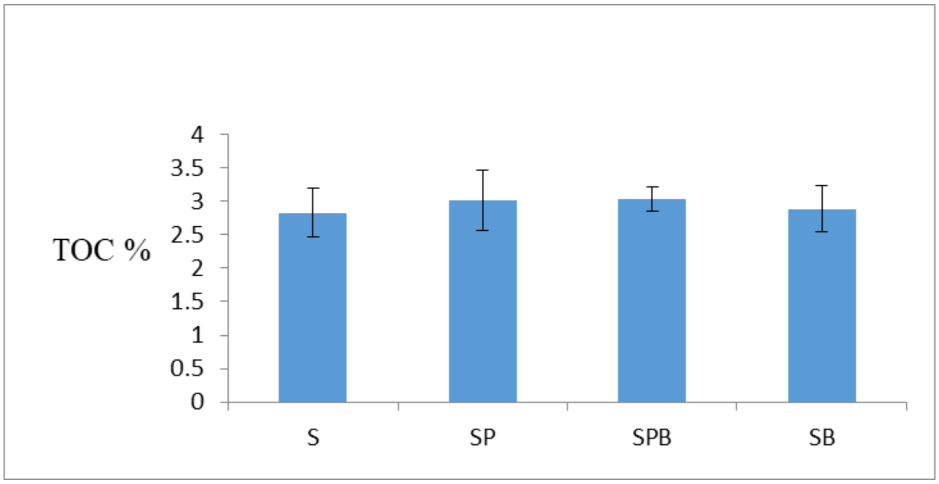

Figure 4.11: Initial TOC for the various treatments.

4.3.1.2 Dissolved Organic Carbon (DOC)

The results for DOC are shown in the Figure 4.12. The mean DOC for S, SP, SPB and SB were 0.80±0.02, 0.77±0.02, 0.77±0.01 and 0.78±0.03 mg/L respectively. The DOC level for S started from a high value, reduced, then rose steadily to a maximum of 0.87±0.03 mg/L. S, SPB and SB attained the highest DOC level on the 35th day then reduced before rising slightly between 74 - 100 days. With S as the control, there were no significant differences ($p \leq 0.05$) in DOC levels with the other treatments. Similarly, with SP as the control, there was no significant difference with the other treatments. However, comparing the means with S as the control, a significant decrease was noted in the other treatments. From these results, P and biochar reduced DOC levels in the acidic soil under anaerobic conditions. These results agree with the data obtained by Angela *et al.* (2015), who reported a reduction in DOC with biochar amendment. At the beginning of incubation, biochar provides high DOC levels as reported by Major *et al.* (2010) and further explains that since biochar raises the cation exchange capacity (CEC) of the soil, it stabilizes the DOC hence low mineralization to CO_2 (Kalbitz *et al.*, 2005) as most of it is sorbed on to the soil-biochar surface.

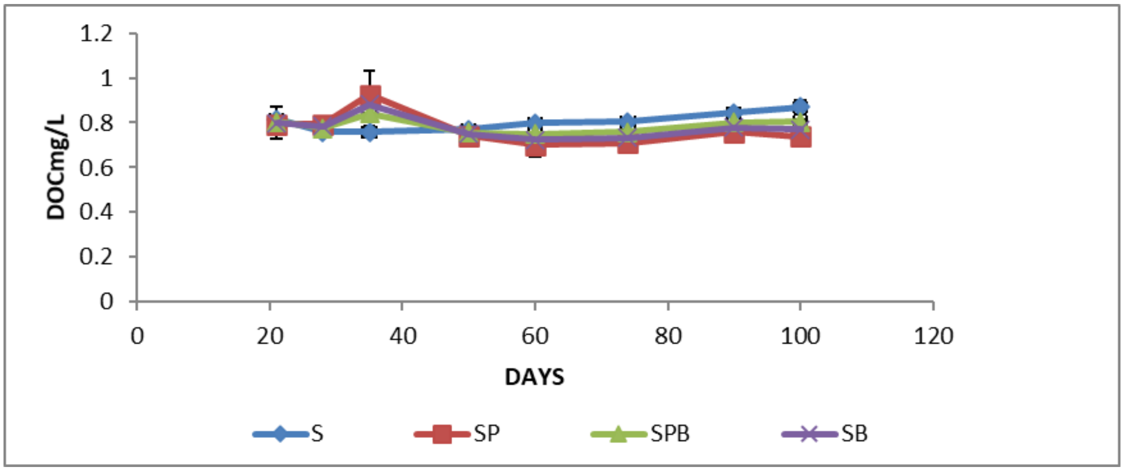

Figure 4.12: Variations in DOC over time for the various treatments.

4.3.2 Nitrogen

4.3.2.1 Total Nitrogen

Figure 4.13 shows the initial total nitrogen in the various treatments. The average total nitrogen for S, SP, SPB and SB were 1987.83±345.30, 502.00±2.36, 952.83±269.88 and 1736.17±425.44 µg/g of soil respectively. The mean total nitrogen was highest in S followed by SB then SPB and lastly SP. The result suggests that phosphatic fertilizers could suppress the availability of nitrogen in soils as seen in the significant difference between S and SP. This result differs with Naiz *et al.* (2017) that reported increased total nitrogen. The lack of a significant difference between S and SB shows that biochar had no effect on total nitrogen. This agrees with Knicker (2010) who reported that once plant biomass is converted to biochar, nitrogen inherent in the plant does not improve soil fertility in the short term because of formation of heterocyclic compounds like pyrrole, imidazoles and pyridines during pyrolysis. The difference between SP and SPB shows that biochar minimizes the effect of P on total nitrogen suppression.

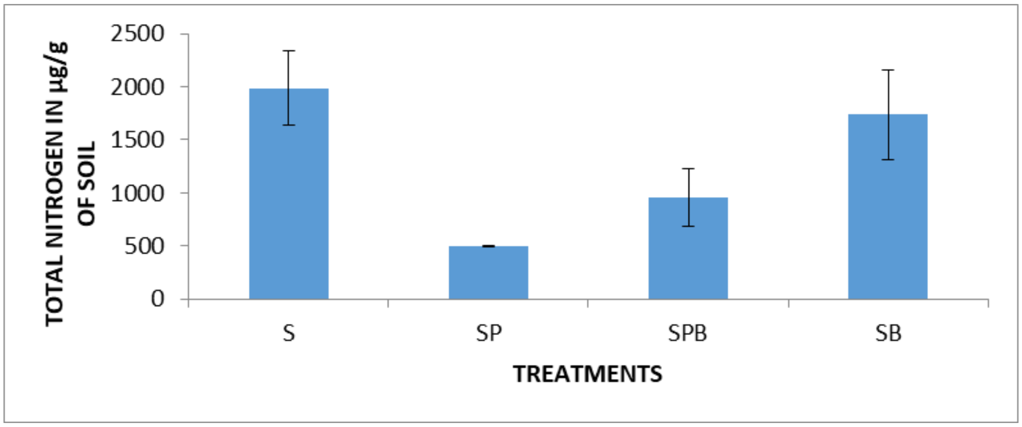

Figure 4.13: Initial total nitrogen in the various treatments.

4.3.2.2. Total Oxidized Nitrogen (TON)

The variations of TON over time for the various treatments are as in Figure 4.14. The mean total oxidized nitrogen levels for S, SP, SPB, and SB were 41.31±1.25, 42.44±1.74, 43.60±5.11 and 45.56±3.98 µg/g of soil respectively. Within the treatments there was significant increase ($p \leq 0.05$) in TON levels over the incubation period. With S as the control, there was a significant increase ($p \leq 0.05$) in SP. However, with the other treatments, no significant difference ($p \leq 0.05$) was noted. With SP as the control, there was also no significant difference ($p \leq 0.05$) in total oxidized nitrogen levels with other treatments. This result differs with those reported by Lehman (2003) and Zhu *et al.* (2016) who reported increased TON with biochar amendment. It also disagrees with Diatta (2016) and Deluca *et al.* (2015) who reported a decrease in TON.

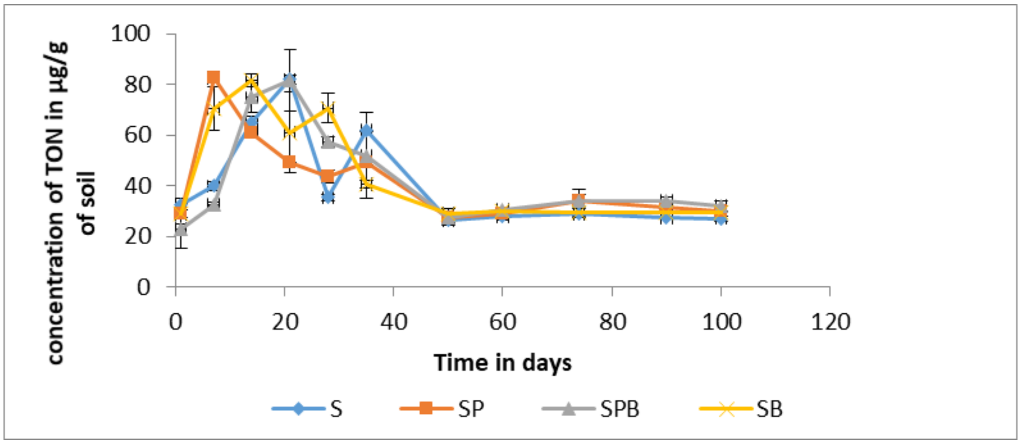

Figure 4.14: Variations in TON over time for the various treatments.

4.3.2.3 Ammoniacal Nitrogen

Figure 4.15 shows the variations in ammoniacal N with time for the various treatments. The mean ammoniacal nitrogen levels for S, SP, SPB, and SB were 250.56±9.13, 255.78±8.81, 271.56±20.31 and 192.55±38.96 µg/g of soil respectively. With S as the control, there was no significant difference ($p \leq 0.05$) in ammonical nitrogen levels with the other treatments. Similarly, with SP as the control there was no significant difference ($p \leq 0.05$) in ammoniacal nitrogen with the other treatments. It is also notable that S, SP, and SPB had high ammoniacal nitrogen in the first week of incubation before reducing drastically in the second week. Biochar amendment and anaerobic conditions therefore had no effect on ammoniacal nitrogen. This result agrees with the findings of Zhu *et al.* (2016) and Lehman (2003). However, it differs with the results reported by Diatta (2016) and Deluca *et al.* (2015) who reported decreased ammoniacal nitrogen

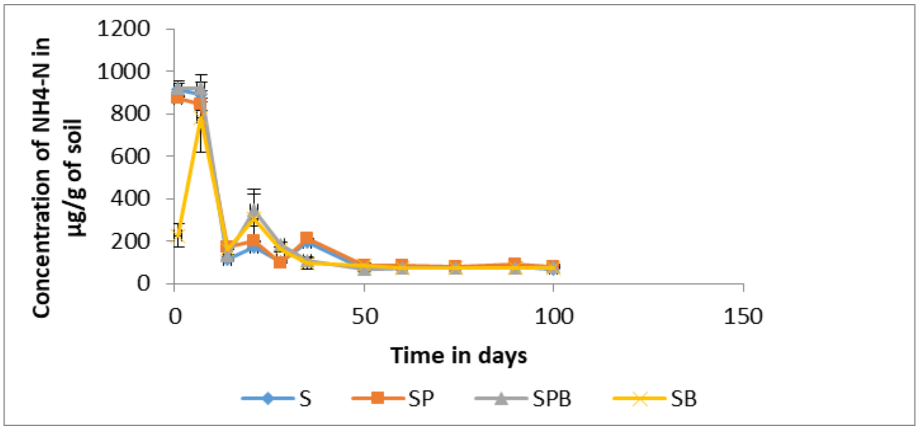

Figure 4.15: Variations in ammoniacal N with time for the various treatments

4.3.2.4 Organic Nitrogen

Figure 4.16 shows the variations in organic nitrogen over time for the various treatments. The mean organic nitrogen levels for S, SP, SPB, and SB were 1695.91±118.56, 340.39±4.30, 637.68±98.43 and 1498.06±156.12 µg/g of soil respectively. This result suggests that addition of phosphatic fertilizers suppresses organic nitrogen in paddy soils. With S as the control there was a significant decrease ($p \leq 0.05$) in organic nitrogen levels in SP and SPB. However, there was no significant difference ($p \leq 0.05$) in nitrogen levels with SB. Using SP as the control, a significant difference ($p \leq 0.05$) in organic nitrogen levels with the other treatments was noted. The result showed a decrease in organic N with P addition. However, biochar amendment and anaerobic conditions had no effect on organic N but, when biochar was combined with P, it reduced the organic N (SPB and SP). This disagrees with the findings of Zhu *et al.* (2016) who attributes the decrease in organic N to more sorption sites for N provided by the biochar surface.

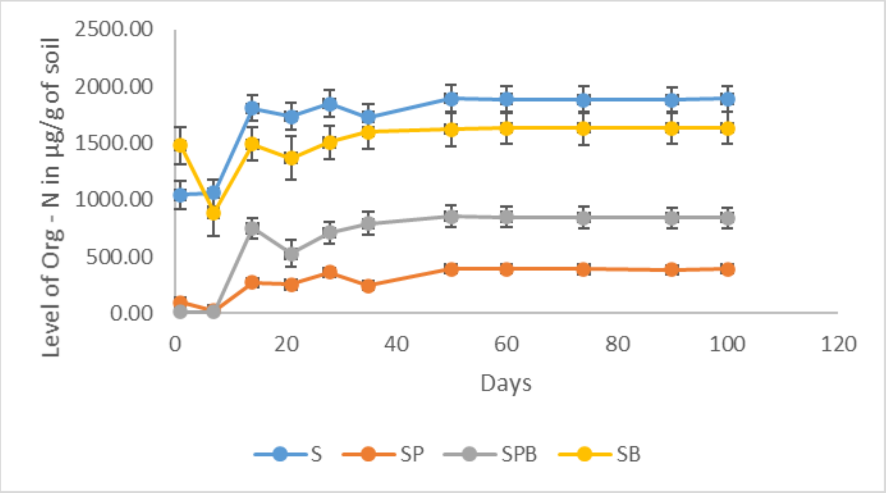

Figure 4.16: Variations in O-N over time for the various treatments.

4.4 Effect of Anaerobic Conditions and Soil Amendment on Greenhouse Gases Emissions

4.4.1 Nitrous Oxide

Figures 4.17 and 4.18 shows the variations in the levels of N_2O emission and cumulative N_2O emission over time for the various treatments. The mean nitrous oxide emissions from treatment S, SP, SPB, and SB were 20.91±2.45, 19.97±2.94, 18.59±2.10 and 16.47±1.09 µg/g of soil respectively. With S as the control, no significant difference ($p \leq 0.05$) with the other treatments was recorded. Similarly, with SP as the control, there was no significant difference with the other treatments. The lack of significant difference among the various treatments shows that both biochar amendment and anaerobic conditions had no effect on N_2O emission. This result agrees with Xie *et al*. (2013) which reported no significant effect of biochar amendment on acidic ultisols with maize stalk biochar. However, it differs with Kamman *et al.* (2012) and Yanai *et al.* (2007) who reported increased N_2O emissions. Zwieten *et al*. (2010) reported that 5% biochar amendment reduced N_2O emissions from flooded acidic ferralsol soils by about 84%. Case *et al*. (2012), Taghizadeh – Toosi *et al.* (2011), Wang *et al.* (2016), Sarah *et al*. (2015), Nguyen *et al.* (2016) and Xingguo *et al*. (2010) all reported reduced N_2O emissions from soils amended with biochar. The initial low nitrous oxide emissions in all the treatments with high levels of NH_4-N could mean dominance of dissimilatory reduction of nitrate before being overtaken by denitrification (Kengara, 2010).

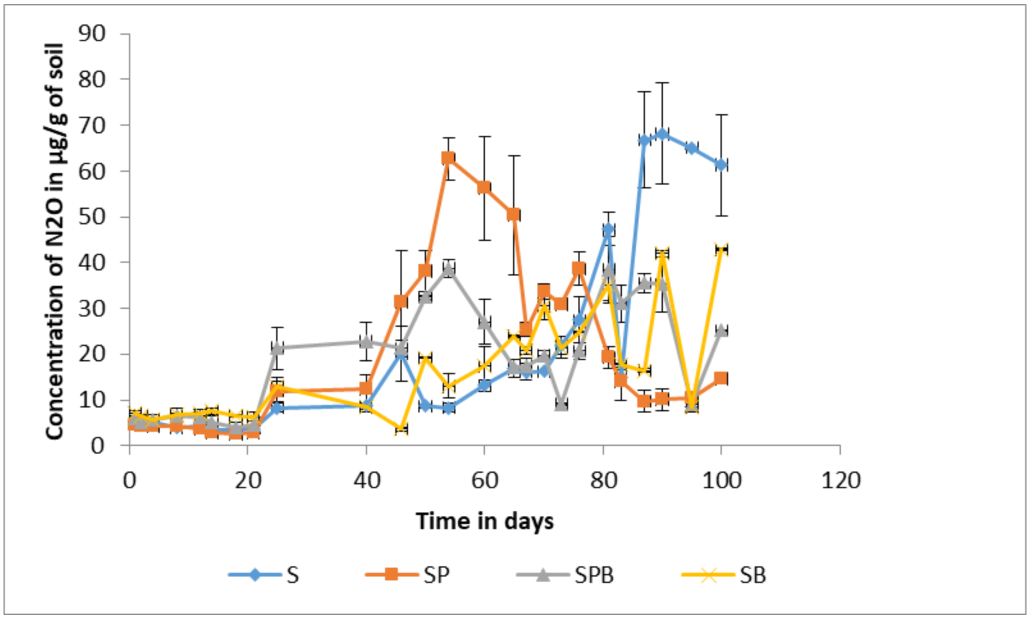

Figure 4.17: Levels of N_2O emission over time for the various treatments.

From Figure 4.18, there was a sudden rise in the concentration of N_2O emitted from the 40th day, in all the treatments. A significant difference ($p \leq 0.05$) was recorded between S and SP, as well as between SP and SB, from the 40th day onwards. The rise was steep in SP but finally it was surpassed by S, SPB and SB had relatively low N_2O emissions. From Figure 21, SP shows higher cumulative emission than S, SB and SPB. This could be an indication that P enhances N_2O emission, while biochar suppresses it. When biochar and P are added together, the effect of P on N_2O emissions is minimized. Anaerobic conditions also tend to increase the emissions, although after a long period of time.

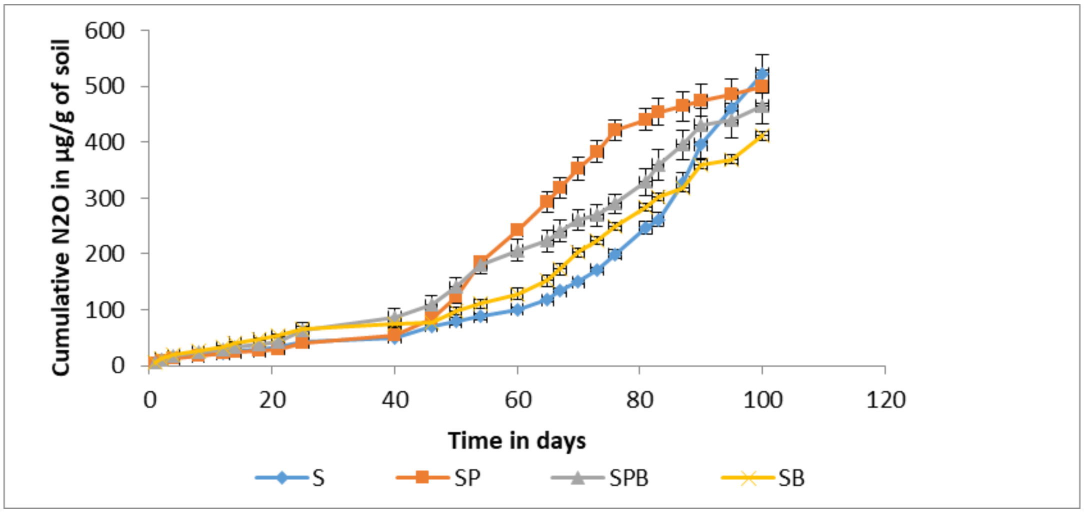

Figure 4.18: Cumulative N_2O emission over time for the various treatments

4.4.2 Carbon Dioxide

Figures 4.19 and 4.20 show the variations in the concentration of CO_2 emission and cumulative CO_2 emission over time, for the various treatments. The mean CO_2 emissions from treatments S, SP, SPB and SB were 9.54±3.54, 6.97±2.21, 7.11±2.48 and 5.96±2.17 µg/g of soil, respectively. The intermittent emissions showed greater variance hence the effect of biochar amendment on CO_2 emissions could not be directly determined. However, the graph of cumulative CO_2 emissions (Figure 4.20) shows reduction in CO_2 emissions from treatments SPB and SB compared to S. The means show a reduction in CO_2 emissions with respect to S of 26.99%, 25.53% and 37.58% for SP, SPB and SB respectively. There were significant differences ($p \leq 0.05$) between S and SP, and also between S and SPB. However, between S and SB there was no significant difference. With SP as the control, there was no significant difference ($p \leq 0.05$) SPB but there was a significant difference with SB.

Cumulative CO_2 emissions showed significant differences ($p \leq 0.05$) between S and SP, and also between S and SPB. However, between S and SB there was no significant difference ($p \leq 0.05$). With SP as the control, there were significant differences ($p \leq 0.05$) with both SPB and SB. The significant difference ($p \leq 0.05$) between SPB and SB suggests that P influences CO_2 emissions from soils, under anaerobic conditions. Therefore, biochar and P suppressed CO_2 emissions while anaerobic conditions increased the emission of CO_2. This reduced CO_2 emission by biochar and P could be attributed to inhibition of microbial activity caused by changes in the soil environment and the adsorptive component of biochar protecting soil organic matter from mineralization (Zimmerman *et al*., 2011). The results of this study agree with those published by other researchers: Liu *et al.* (2011), Liu *et al*. (2016) and Spokas *et al.* (2009) all reported reduced CO_2 emissions from paddy soils amended with biochar.

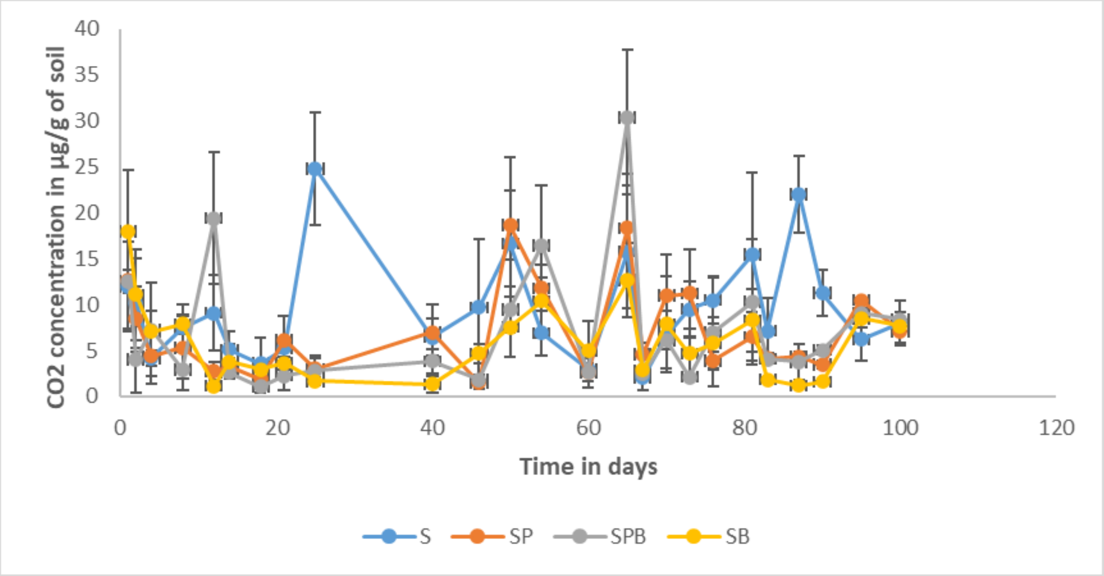

Figure 4.19: Level of CO_2 emission over time for the various treatments

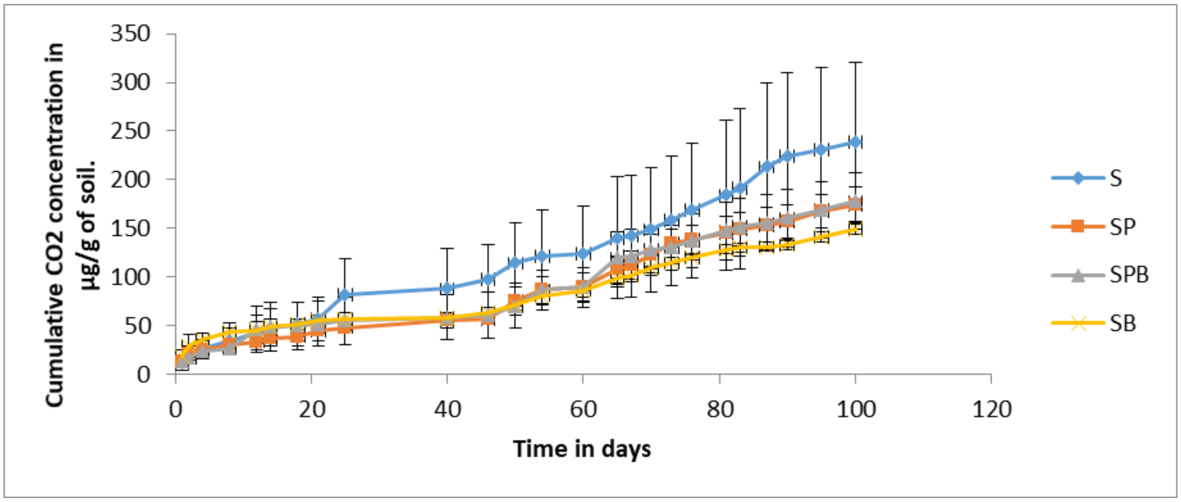

Figure 4.20: Cumulative CO_2 emission over time for the various treatments.

4.4.3. Methane emission

The levels of methane emission in the various treatments over time are presented in Figure 4.21 (concentration of methane emissions) and Figure 4.22 (cumulative methane emissions). The mean methane emissions from S, SP, SPB and SB were 7.52 x 10^{-6}±1.05x 10^{-6}, 1.14 x 10^{-5}±2.97 x 10^{-6}, 1.21 x 10^{-5}±2.61 x 10^{-6} and 2.33 x 10^{-5}±3.4 x 10^{-6} µg/g of soil, respectively. There was a significant difference ($p \leq 0.05$) in the concentration of methane emitted by the control S and the other treatments SP, SPB, and SB. The order of increasing methane emission was S<SP<SBP<SB. The concentrations of treatments SPB and SB increased to a peak after 40 days then dropped gradually as shown in Figure 4.21. Biochar amendment significantly increased cumulative methane emission.

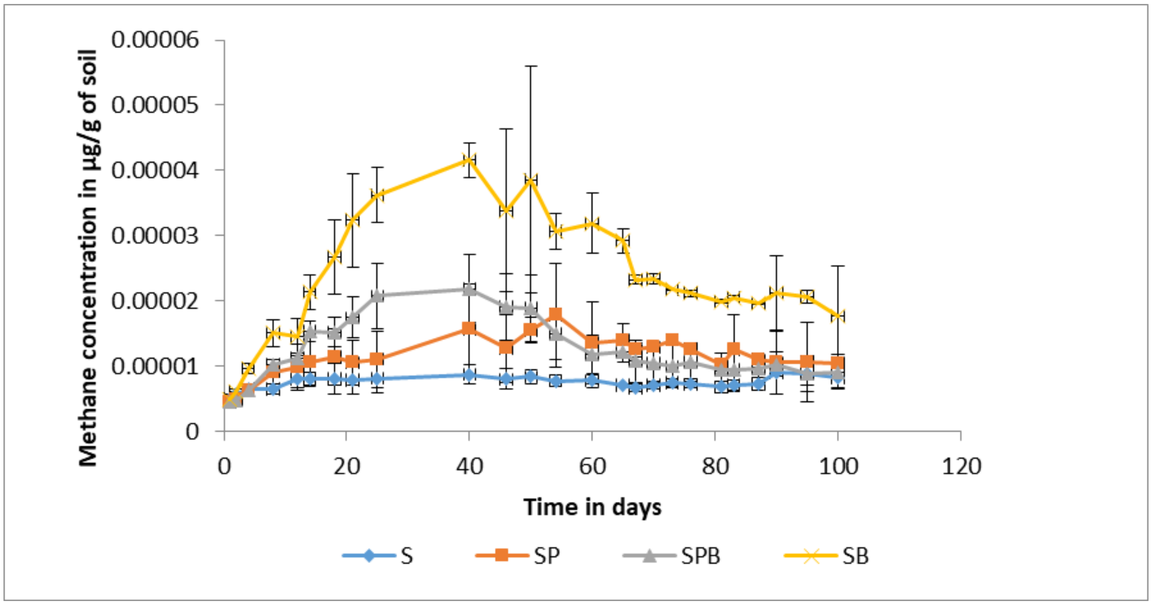

Figure 4.21: Concentration of methane emitted in the various treatments over time.

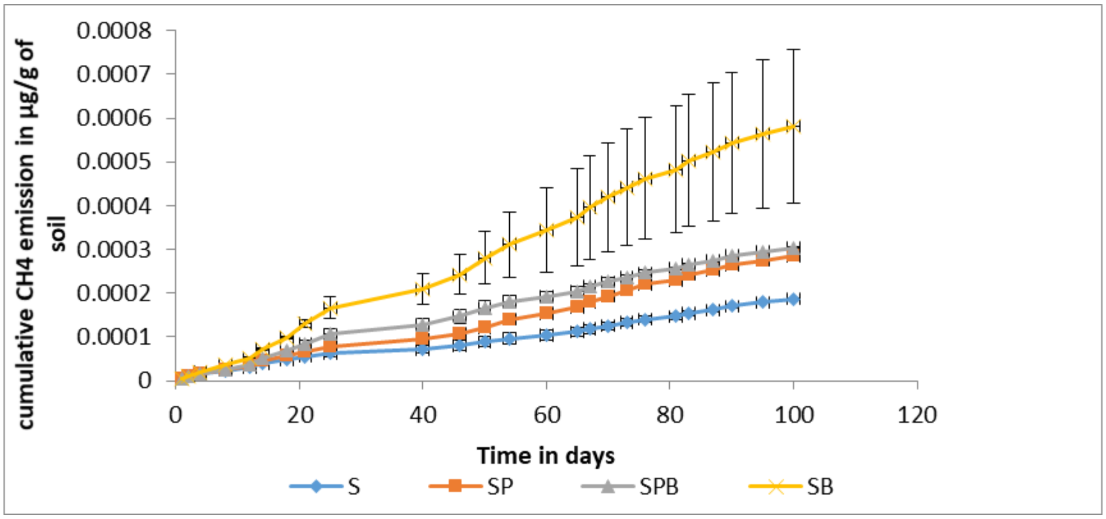

Figure 4.22: Cumulative methane emissions in the various treatments over time.

Generally, there was significant increase ($p \leq 0.05$) in the concentration of methane emitted in SP, SPB, and SB compared to S. This increased emission could be attributed to the increased labile carbon due to addition of biochar (Phy *et al*, 2014; Wang *et al*., 2012), which provides some substrate for methanogenic activity. Methanogens produce methane as a metabolic by-product of organic matter mineralization under anaerobic conditions with the primary pathways being via reduction of carbon dioxide using hydrogen or via acetotrophy (Le Mer and Roger, 2001). This production is enhanced by temperatures above $30^{o}C$ and pH levels below 6 (Harrison and Aiyer, 1915). Though the temperature of incubation was fixed at $25^{o}C$, the soil pH varied within the period of incubation with the means of 5.22, 5.73, 5.94 and 5.56 for S, SP, SPB, and SB respectively.

There was a significant difference ($p \leq 0.05$) between control SP and SB. However, between SP and SPB there was no significant difference. The lack of significant difference between SP and SPB shows that the inclusion of phosphatic fertilizer lowers methane emission in biochar-amended soil. However, how the phosphatic fertilizer reduces methane production is not clear. Spokas *et al.* (2009) attributes the increased methane emission to the inhibiting effect of the chemicals in the biochar on soil methanotrophs. It was noted that there was no significant difference between cumulative methane emission between SP and SPB (Figure 4.22).

It was also noted that methane emission from all the treatments was significantly lower than nitrous oxide and carbon dioxide from the same treatments. This low methane emission could be due to availability of readily degradable organic matter as terminal electron acceptors (TEAs), low population of methanogens, or unsuitable conditions for methanogenesis. The high concentration of nitrous oxide could also imply that the denitrification intermediates which are toxic to methanogens were present hence suppressing CH_4 emission (Dalaal *et al*., 2008).

The results obtained from this study agree with similar studies published by other scholars: Phy *et al*. (2014) reports increased methane production with biochar amendment to the tune of 54.03 g of CH_4/kg of soil compared to the control at 8.42 g of CH_4/kg of soil in rice paddy soil. Wang *et al.* (2012), Zhang *et al*. (2012) and Knoblauch *et al*. (2011) also report increased methane production in rice paddy soils amended with biochar under high water content. Zhang *et al.* (2012) clarified that methane emission increases with the rate of biochar application. Yu *et al.*

(2012) also confirmed that under high moisture content - 80% - 100% water filled pore space - biochar enhanced methane production.

Figure 4.23 shows the variations in CO_2/CH_4ratio with time for the various treatments. S had the highest CO_2 production with the lowest methane emission hence high CO_2 /CH_4 ratio confirming dominance of fermentation over methanogenesis under anaerobic conditions. P on the other hand suppressed CH_4 production to a greater extent than CO_2 production as indicated by the ratio exhibited by SP and SPB. However, biochar (in SB) suppressed CO_2 production but greatly enhanced methane production hence the lowest ratio. Despite the variations in all the treatments, the CO_2/CH_4 ratio was far much greater than 2, hence fermentation predominated over methanogenesis. Keller *et al.* (2009) attributes this high ratio to predominance of humic substances acting as electron acceptors hence strongly limiting production of methane. This could signify that utilization of the more thermodynamically favorable dissolved organic matter as terminal electron acceptors was the main anaerobic decomposition pathway hence resulting in the dominance of fermentation over methanogenesis.

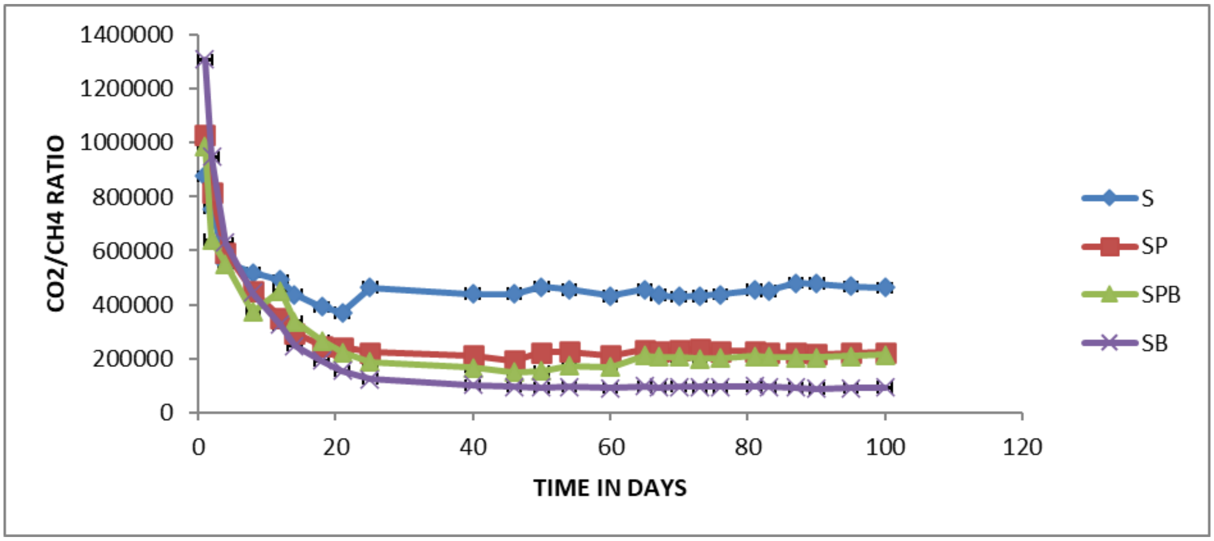

Figure 4.23: Variations in CO_2/CH_4 ratio over time for the various treatments

4.5 Relationships among Analysed Soil Parameters

Tables 4.1-4.4, show the relationships among the analysed soil parameters for the various treatments.

Table 4.1: Correlation coefficients for GHGs, P fractions, N fractions and other soil parameters in S. (n = 10, df = 8, critical value for Pearson, Crit.= 0.632, (P = 0.05))

	CH4	***pH***	***N2O***	***CO2***	***DOC***	***TON***	***NH4-N***	***O-N***	***LSP***	***Al-P***	***Fe-P***	***Ca-P***	***OC-P***	***LP***	***MLP***	***NLP***
CH4	1															
pH	0.858a	1														
N2O	0.483	0.536	1													
CO2	0.088	0.124	-0.089	1												
DOC	0.268	0.464	0.875a	-0.586	1											
TON	0.016	-0.181	-0.43	-0.358	-0.002	1										
NH4-N	-0.893b	-0.881b	-0.418	-0.06	-0.073	0.001	1									
O-N	0.891a	0.890a	0.441	0.080	0.048	-0.057	-0.99b	1								
LSP	0.510	0.644a	0.477	0.144	0.015	-0.591	-0.547	0.579	1							
Al-P	0.237	0.395	0.026	-0.212	0.133	0.464	-0.293	0.267	-0.36	1						
Fe-P	-0.464	-0.619	-0.424	0.143	-0.05	0.605	0.544	-0.577	-0.879b	0.182	1					
Ca-P	0.250	0.305	-0.506	0.518	-0.848b	0.197	-0.287	0.276	0.137	0.219	-0.026	1				
OC-P	0.181	0.351	-0.109	0.356	-0.671b	-0.332	-0.4	0.418	0.640a	-0.191	-0.535	0.486	1			
LP	-0.215	-0.422	-0.041	-0.669b	0.657a	0.595	0.415	-0.447	-0.556	0.246	0.459	-0.445	-0.6	1		
MLP	-0.717b	-0.679b	-0.386	-0.216	0.221	0.373	0.858a	-0.878b	-0.608	0.061	0.655a	-0.157	-0.503	0.638a	1	
NLP	0.096	0.020	-0.277	0.693a	-0.512	0.125	-0.093	0.086	-0.429	0.283	0.485	0.489	-0.102	-0.352	-0.139	1

The letters a and b represent significant positive and negative correlations respectively.

Table 4.2: Correlation coefficients for GHGs, P fractions, N fractions and other soil parameters in SP. (n = 10, df = 8, critical value for Pearson, Crit.= 0.632, (P = 0.05)

	CH4	*pH*	*N2O*	*CO2*	*LP*	*MLP*	*NLP*	*DOC*	*LSP*	*AL-P*	*Fe-P*	*Ca-P*	*OC-P*	*TON*	*NH4-N*	*O-N*
CH4	1															
pH	0.718a	1														
N2O	0.322	0.501	1													
CO2	0.157	0.044	-0.059	1												
LP	-0.363	-0.471	-0.299	-0.618	1											
MLP	-0.695b	-0.329	-0.368	-0.068	0.225	1										
NLP	-0.344	-0.255	-0.507	-0.232	0.138	0.756a	1									
DOC	-0.616	0.413	-0.536	-0.216	-0.089	0.547	0.624	1								
LSP	-0.297	-0.498	0.095	0.618	-0.016	-0.040	-0.425	-0.684b	1							
AL-P	-0.032	0.272	-0.262	-0.346	-0.009	-0.129	-0.074	0.620	-0.589	1						
Fe-P	-0.797b	-0.439	-0.403	-0.297	0.110	0.515	0.429	0.864a	-0.169	0.509	1					
Ca-P	0.255	0.219	-0.161	-0.290	0.534	0.107	0.069	-0.295	-0.277	-0.056	-0.447	1				
OC-P	0.062	-0.130	-0.458	-0.010	0.621	0.078	-0.097	-0.283	0.105	0.008	-0.320	0.752a	1			
TON	-0.225	-0.539	-0.530	-0.388	0.313	0.234	0.66a	0.796a	-0.316	0.052	0.379	-0.156	-0.045	1		
NH4-N	-0.766b	-0.836b	-0.528	0.136	0.347	0.616	0.365	0.649a	0.463	-0.198	0.469	-0.190	0.246	0.457	1	
O-N	0.496	0.487	0.678a	0.301	-0.139	-0.589	-0.888b	-0.689b	0.575	-0.066	-0.515	-0.111	0.129	-0.901b	-0.994b	1

The letters a and b represent significant positive and negative correlations respectively.

Table 4.3: Correlation coefficients for GHGs, P fractions, N fractions and other soil parameters in SPB. (n = 10, df = 8, critical value for Pearson, Crit. = 0.632, (P = 0.05)

	pH	*CH4*	*N2O*	*CO2*	*LP*	*MLP*	*NLP*	*LSP*	*AL-P*	*Fe-P*	*Ca-P*	*OC-P*	*O-N*	*NH4-N*	*TON*	*DOC*
pH	1															
CH4	0.494	1														
N2O	0.516	-0.294	1													
CO2	-0.656b	-0.399	0.083	1												
LP	-0.498	0.010	-0.535	-0.104	1											
MLP	-0.349	-0.089	-0.556	-0.066	-0.064	1										
NLP	0.097	0.620	-0.487	-0.310	0.178	0.158	1									
LSP	-0.965b	-0.454	-0.573	0.576	0.506	0.305	-0.050	1								
Al-P	-0.632b	-0.500	-0.433	0.231	0.018	0.873a	-0.111	0.558	1							
Fe-P	0.792a	0.494	0.191	-0.492	-0.671b	0.020	0.159	-0.738b	-0.318	1						
Ca-P	-0.917b	-0.421	-0.634b	0.457	0.467	0.434	0.102	0.965a	0.628	-0.638b	1					
OC-P	-0.910b	-0.418	-0.445	0.748a	0.298	0.399	-0.299	0.860a	0.622	-0.582	0.784a	1				
O-N	0.794a	0.407	0.619	-0.257	-0.390	-0.775b	-0.078	-0.716b	-0.901b	0.516	-0.764b	-0.699b	1			
NH4-N	-0.806b	-0.440	-0.590	0.289	0.385	0.751a	0.039	0.720a	0.898a	-0.530	0.759a	0.717a	-0.998b	1		
TON	0.318	0.601	-0.387	-0.569	-0.016	0.284	0.622	-0.169	-0.078	0.301	-0.035	-0.397	0.116	-0.176	1	
DOC	0.217	-0.133	0.321	0.103	-0.397	0.477	0.160	-0.497	0.503	-0.108	0.030	-0.177	-0.467	0.465	0.475	1

The letters a and b represent significant positive and negative correlations respectively.

Table 4.4: Correlation coefficients for GHGs, P fractions, N fractions and other soil parameters in SB. (n = 10, df = 8, critical value for Pearson, Crit. = 0.632, (P = 0.05)

	pH	*CH4*	*N2O*	*CO2*	*LP*	*MLP*	*NLP*	*LSP*	*AL-P*	*Fe-P*	*Ca-P*	*OCP*	*DOC*	*TON*	*NH4-N*	*O-N*
pH	1															
CH4	0.592	1														
N2O	-0.430	-0.08	1													
CO2	-0.610	-0.65b	-0.285	1												
LP	-0.450	-0.91b	0.156	0.433	1											
MLP	0.121	-0.32	-0.523	0.378	0.448	1										
NLP	0.304	0.175	-0.418	-0.22	-0.23	0.052	1									
LSP	-0.74b	-0.61	-0.193	0.904a	0.394	0.215	-0.17	1								
Al-P	0.006	-0.09	-0.105	0.09	0.172	0.398	-0.22	-0.19	1							
Fe-P	0.64a	0.62	-0.004	-0.51	-0.5	0.135	-0.14	-0.56	-0.06	1						
Ca-P	-0.23	-0.52	-0.479	0.463	0.464	0.61	0.228	0.613	-0.18	-0.23	1					
OC-P	0.145	0.37	-0.202	-0.28	-0.5	-0.21	0.89a	-0.19	-0.16	-0.1	0.007	1				
DOC	0.154	0.013	-0.01	-0.44	0.456	0.591	0.535	-0.45	-0.76b	0.097	0.445	0.324	1			
TON	0.716b	0.12	-0.557	-0.34	0.012	0.323	0.699a	-0.47	0.085	0.065	0.105	0.383	0.666a	1		
NH4-N	0.206	-0.31	-0.491	0.201	0.432	0.789a	0.154	-0.07	0.764a	-0.09	0.291	-0.071	0.692a	0.538	1	
O-N	-0.260	0.283	0.517	-0.16	-0.41	-0.78b	-0.21	0.107	-0.73b	0.076	-0.28	0.031	-0.71b	-0.604	-0.99b	1

The letters a and b represent significant positive and negative correlations respectively.

4.5.1 Correlation among P fractions

S and SPB had a significant negative correlation between iron bound P and loosely sorbed P. This shows that there is exchange of P between the two fractions. The reduction of Fe^{3+} to Fe^{2+} under anaerobic conditions could result into the release of iron bound P to the loosely sorbed P fraction. In SPB the Fe^{3+} could have been saturated by P from biochar and the mineral fertilizer allowing the excess to move to the loosely sorbed fraction. In SPB, a significant negative correlation was noted between iron bound P and labile organic P, as well as between calcium bound P and iron bound P. Calcium bound P is high in alkaline soils but as the pH reduces, more of it could be released to iron bound P. Biochar could also facilitate increase in iron bound P since it contains some iron which it adds to the soil. Some of the labile organic P could therefore be added to iron as it is involved in chelation with organic molecules sorbed on biochar surface (Deluca *et al., 2015*).

Significant positive correlations were noted between occluded P and loosely sorbed P, MLP and Fe-P, and between MLP and LP in S. The correlation between Fe-P and MLP indicates movement of P between the organic and inorganic pools while MLP and LP both come from decomposition of soil organic matter, hence the positive correlation. In SP, positive correlations were recorded between NLP and MLP, and between Oc-P and Ca-P. SPB showed significant positive correlations between Al-P and MLP, Ca-P and LSP, Oc-P and LSP as well as between Oc-P and Ca-P. A positive correlation was also noted between Oc-P and NLP in SB. This could be as a result of biochar providing more sorption sites for both organic and inorganic P (Deluca *et al.*, 2015) hence the positive correlation between occluded P and NLP.

4.5.2 Correlations between P Fractions, pH and other Nutrients

DOC was significantly negatively correlated to Ca-P, and Oc-P in S, LSP in SP and Al-P in SB. This could be attributed to the idea that dissolution of organic C reduces sorption sites for P on the soil particles hence reduction in occluded P. Significant negative correlation was also recorded between pH and MLP in S, LSP, Al-P, Ca-P and Oc-P in SPB and in SB pH negatively correlated to LSP. Low pH facilitates P fixation on aluminium they are released from calcium (Ponnamperuma, 1972*)*. High pH on the other hand releases fixed forms of P to the LSP and MLP fractions in soils under anaerobic conditions (Ponnamperuma, 1972*)*. Acidification is also a key step in decomposition of soil organic matter under anaerobic conditions (Ponnamperuma, 1972). Organic nitrogen significantly correlated negatively to MLP in S, NLP in SP, MLP, LSP, Al-P, Ca-P, and Oc-P in SPB as well as MLP and Al-P in SB. This suggests that high P levels could enhance consumption of organic nitrogen fraction hence confirming the influence of P on soil nitrogen. The micro-organisms responsible for anaerobic decomposition and mineralization of soil organic matter utilize both P and N at specific ratios for metabolism (Bridgham *et al.*, 2013).

Significant positive correlations were registered between pH and LSP in S and Fe-P in SPB and SB. DOC positively correlated to LP in S and Fe-P in SP. TON, correlated positively to NLP in SP and SPB. MLP also showed a positive correlation with NH_4 –N in S, SPB and SB. This could be because they both originate from microbial mineralization of organic P and organic N that are both derived from soil organic matter. Ammoniacal N was also positively correlated to LSP, Ca-P, and Oc-P in SPB as well as Al-P in both SPB and SB.

4.5.3 Correlations among Other Nutrients and pH

Significant negative correlations were registered between NH_4-N and organic N in all the treatments. This is attributed to ammoniafication which converts organic N to NH4-N (Deluca *et al.*, 2015). DOC correlated negatively to O-N in SP and SB while TON negatively correlated to O-N only in SP. Mineralization of organic N could possibly release some soluble C as it is converted to inorganic N forms like TON. pH on the other hand negatively correlated to NH_4-N in S, SP, and SPB.

Positive correlations were recorded between DOC and TON as well as between DOC and NH_4-N in SP. DOC, TON and NH4-N are both products of mineralization of organic matter via microbial activity hence the positive correlation. In SB a significant positive correlation was noted between TON and NH_4-N, which are both products of mineralization of organic N. TON and pH also showed a significant positive correlation in SB.

4.5.4 GHG and P Fractions

Significant negative correlations were noted between CH_4 and MLP in S and SP, between Ca-P and N_2O in SPB and between CH_4 and LP in SB. This confirms the negative impact of P on CH4 and N_2O emission from paddy soils.

CO_2 showed significant positive correlation with NLP in S, Oc-P in SPB and LSP in SB. For anaerobic micro-organisms to respire CO_2, they need P and N which they immobilize from the soil *(*Bridgham *et al*., 2013*)* in case of inadequacy hence the positive correlation.

4.5.5 Correlations between GHGs, pH and Other Nutrients

Significant negative correlations were recorded between CH_4 and NH_4-N in S and SP, and between pH and CO_2 in SPB. The correlation between CH_4 and NH_4-N could be attributed to the inhibition property of N to methanogenesis (Dalal *et al*., 2008). The negative correlation between CH_4 and CO_2 shows the interdependence in their production from soil. Reduction of CO_2 using hydrogen generates methane while oxidation of methane gives carbon dioxide.

pH and CH_4 were positively correlated in S and SP. DOC and N_2O, O-N and CH_4 in S as well as O-N and N_2O in SP also showed significant positive correlations. Mineralization of organic N would provide the needed substrates for denitrification, as DOC provides the necessary energy to heterotrophic denitrifying bacteria, hence increasing the N_2O emissions (Dalal *et al*., 2008).

CHAPTER FIVE

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1 Summary

The soil pH is a parameter that greatly affects the concentration of water soluble minerals in soils and hence nutrient availability. Biochar and P treatment increased the initial soil pH. However, over the incubation period, biochar amendment had no effect on the soil pH while anaerobic conditions and P treatment increased the pH.

The study also established that biochar and addition of P increased soil total P while anaerobic conditions, biochar and P amendments had no effect on aluminium bound P, calcium bound P, occluded P, moderately labile P and non-labile P. Anaerobic conditions and addition of P increased loosely sorbed P while biochar reduced it and even minimized those added by P treatment. It was established that anaerobic conditions decreased iron bound P but biochar increased this fraction and to a greater extent in the presence of P treatment. Addition of P increased labile P but both biochar and anaerobic conditions had no effect on this fraction.
Biochar amendment, P treatment and anaerobic conditions had no effect on TOC, DOC, TON, and ammoniacal-N. Total N was reduced by P treatment but not affected by both biochar and anaerobic conditions. Organic N was increased by anaerobic conditions, reduced by addition of P but not affected by biochar amendment.

This study established that biochar amendment on flooded soils increased methane emission, although this influence was reduced by addition of P fertilizer. Biochar and addition of P also reduced carbon dioxide emission from the soil hence the alternative hypothesis holds. However, anaerobic conditions, P treatment and biochar addition had no effect on level of nitrous oxide emission although beyond 40 days of incubation additional P sharply increased cumulative N_2O. The CO_2/CH_4 ratio (>2) showed dominance of fermentation over methanogenesis with the S ratio being higher than those of other treatments.

5.2 Conclusions

This study established that:

1. Biochar amendment increased total P and iron bound P but reduced loosely sorbed P in anaerobic paddy soil. It however had no significant effect on Al-P, Ca-P, labile organic P,

occluded P, MLP and NLP. P treatment on the other hand increased total P, loosely sorbed P, and labile organic P.

2. Biochar amendment increased initial soil pH, and had no effect on organic N, ammoniacal N, TON, TOC, and DOC. P treatment significantly reduced total N while biochar amendment had no effect on total N.
3. Biochar amendment reduced cumulative carbon dioxide and nitrous oxide emissions but increased methane emissions although the methane levels were much lower compared to CO_2 and N_2O. P amendment on the other hand significantly increased cumulative N_2O emission, slightly increased methane emission and reduced cumulative CO_2emission.

5.3 Recommendation

It is thus commendable to introduce the use of biochar in farming as a way of stabilizing nutrients within the soil and limiting GHG emissions.

5.4 Suggestions for Future Research

1. A study should be conducted to establish the effect of biochar amendment on phosphorous, GHG emissions and nitrogen on different soils.
2. A study should be done to establish the effect of rate of biochar application on greenhouse gas emissions and nutrient availability both under aerobic and anaerobic conditions.

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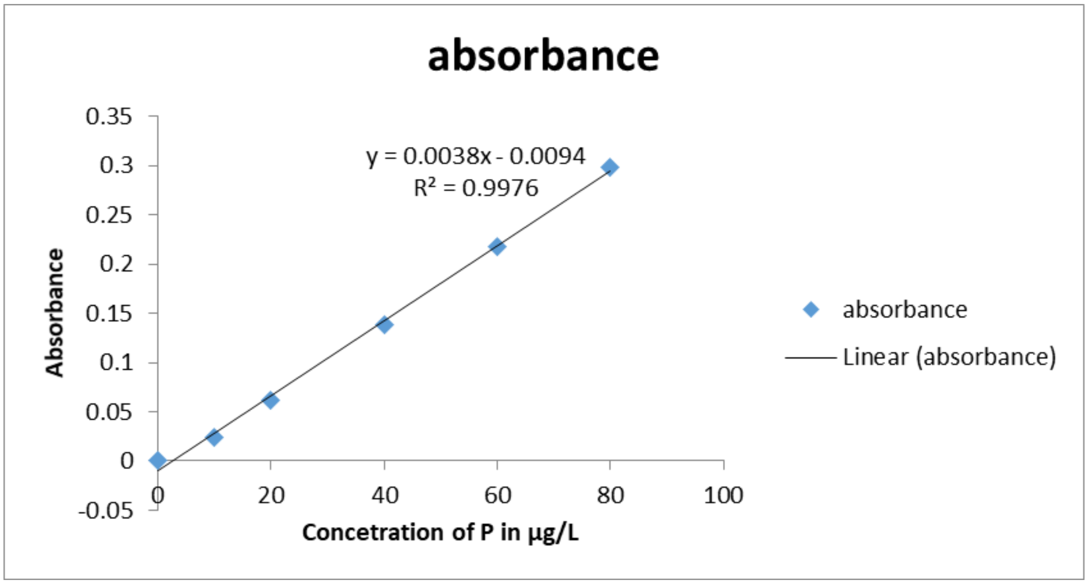
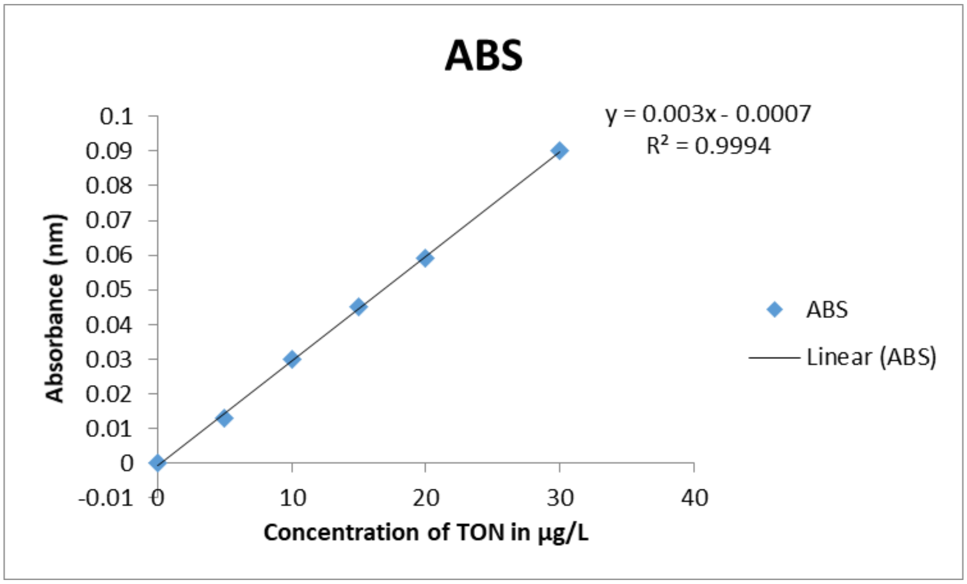
APPENDICES

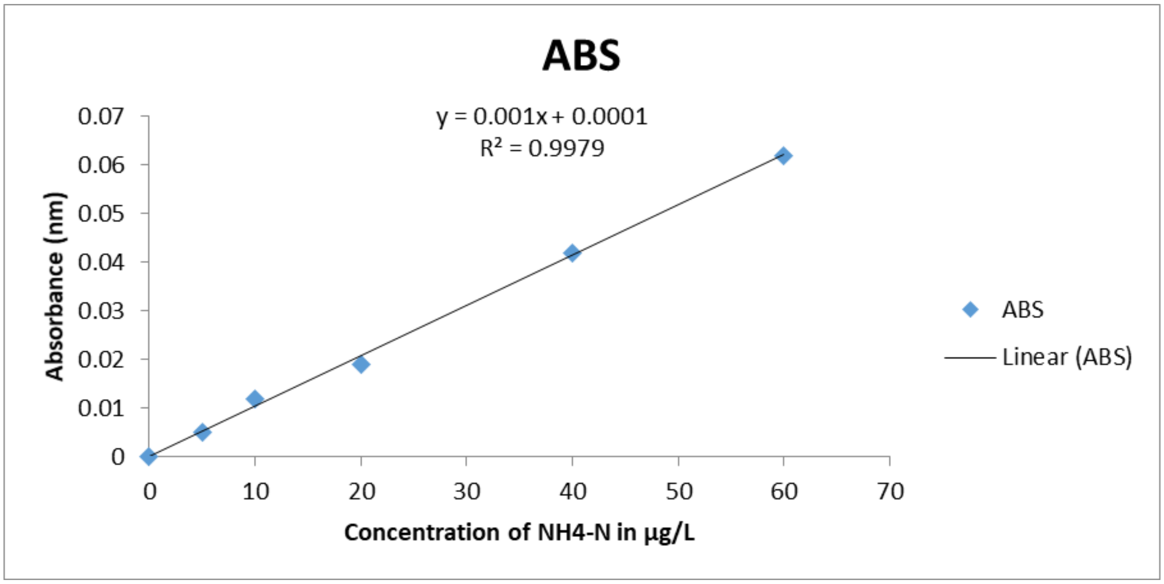
Appendix 1: Calibration curve for phosphorus



Appendix 2: Calibration curve for TON



Appendix 3: Calibration curve for ammoniacal nitrogen.



Appendix 4: Initial total P in µg/g of soil

	TP	Stdev
S	500.11	34.38
SP	1001.98	30.34
SPB	1709.51	101.40
SB	978.90	47.20

Appendix 5: Loosely sorbed P in µg/g of soil

DAYS	S	STDEV	SP	STDEV	SPB	STDEV	SB	STDEV
1	34.33	0.00	185.92	2.47	254.50	0.47	109.67	9.19
7	31.67	0.47	115.83	2.12	109.75	15.20	40.50	0.94
14	31.08	0.12	109.92	24.63	137.08	23.69	39.25	1.30
21	40.42	2.00	51.25	0.12	56.67	4.95	37.25	0.59
28	43.17	9.43	56.67	0.24	50.50	2.83	34.83	0.24
35	38.25	4.83	44.83	1.89	47.83	3.54	39.17	1.89
50	145.42	9.07	148.17	8.72	81.50	3.06	56.33	4.24
60	86.08	6.95	104.00	14.61	60.58	9.07	41.75	0.82
74	92.92	6.72	117.67	0.71	45.17	0.71	47.17	2.59
90	90.67	9.90	118.83	1.18	45.83	1.18	47.33	2.83
100	93.17	4.71	120.08	2.47	44.75	0.35	46.83	0.71
mean	66.11	4.93	106.65	5.38	84.92	5.91	49.10	2.30
CV	7.46		5.04		6.96		4.69	
LSD P≤0.05	32.49		36.63		55.22		18.14	

Appendix 6: Aluminium bound P in µg/g of soil

DAYS	S	STDEV	SP	STDEV	SPB	STDEV	SB	STDEV
1	31.33	0.71	32.42	0.59	33.92	3.18	31.58	0.12
7	30.58	2.24	29.67	0.00	33.92	1.77	38.58	1.06
14	32.08	0.12	33.75	2.00	31.17	1.18	30.58	1.53
21	40.50	0.24	41.17	1.65	33.25	0.59	32.08	0.82
28	36.50	2.12	104.17	90.98	30.83	1.18	32.08	3.42
35	40.33	0.71	39.08	0.35	36.00	2.12	42.25	1.53
50	27.08	5.77	27.50	1.89	31.08	0.35	33.83	1.41
60	37.67	5.89	31.33	4.71	31.17	0.94	33.08	1.30
74	31.50	0.47	32.00	0.71	31.83	1.65	33.00	1.41
90	34.67	2.12	33.00	0.24	31.17	1.18	32.42	0.35
100	33.33	0.94	33.08	0.12	31.83	1.18	32.75	1.06
mean	34.14	1.94	39.74	9.39	32.38	1.39	33.84	1.27
CV	5.68		23.62		4.30		3.77	
LSD P≤0.05	3.66		18.78		1.44		2.99	

Appendix 7: Iron bound P in µg/g of soil

DAYS	S	STDEV	SP	STDEV	SPB	STDEV	SB	STDEV
1	156.33	4.71	187.33	16.03	47.42	3.89	31.75	1.53
7	147.67	0.47	153.58	6.95	117.58	6.25	96.42	5.07
14	142.50	10.61	149.33	13.20	101.50	2.12	99.42	4.60
21	156.08	12.61	160.17	4.95	295.67	9.66	266.75	16.85
28	150.25	33.12	192.67	111.02	203.50	17.91	118.17	3.77
35	78.08	2.71	118.50	2.12	161.17	11.31	127.17	22.39
50	62.25	4.36	70.17	8.49	186.83	0.47	115.75	34.77
60	48.42	1.53	59.08	2.24	288.75	16.62	236.58	9.31
74	91.75	13.32	96.58	10.96	181.67	12.73	108.67	46.67
90	97.42	25.57	140.50	8.01	156.42	16.15	114.25	44.19
100	94.67	20.74	135.92	8.60	181.75	10.49	117.75	32.17
mean	111.40	11.80	133.08	17.51	174.75	9.78	130.24	20.12
CV	10.59		13.16		5.60		15.45	
LSD P≤0.05	34.76		37.65		63.58		56.69	

Appendix 8: Calcium bound P in µg/g of soil

DAYS	S	STDEV	S+P	STDEV	S+P+B	STDEV	S+B	STDEV
1	34.75	0.82	36.67	0.94	43.33	4.24	40.75	3.65
7	33.75	3.89	34.75	2.47	34.92	0.59	35.83	0.94
14	35.50	1.65	38.00	2.36	36.08	0.12	35.25	2.71
21	43.17	5.89	44.83	1.18	34.08	1.06	37.33	0.24
28	47.08	9.55	37.00	0.94	32.92	0.12	35.33	1.18
35	36.83	0.47	35.83	3.54	30.67	2.36	34.17	1.18
50	46.58	5.30	37.08	2.00	32.08	1.06	32.67	0.94
60	40.08	7.42	59.17	3.30	32.50	0.24	34.00	1.89
74	32.83	0.00	34.17	3.30	32.00	0.47	35.58	0.59
90	33.08	1.06	32.58	2.00	32.00	1.41	36.17	0.47
100	31.25	1.77	34.50	1.65	30.75	1.30	32.00	1.89
mean	37.72	3.44	38.60	2.15	33.76	1.18	35.37	1.42
CV	9.12		5.58		3.49		4.03	
LSD P≤0.05	4.88		6.51		3.10		2.04	

Appendix 9: Occluded P in µg/g of soil

DAYS	S	STDEV	S+P	STDEV	S+P+B	STDEV	S+B	STDEV
1	35.83	1.89	40.50	0.24	36.50	0.24	37.33	3.77
7	25.42	0.82	39.17	1.18	32.67	1.18	33.75	2.95
14	31.83	0.94	35.08	2.00	30.75	0.35	34.50	2.59
21	32.33	2.59	35.92	1.53	30.67	0.94	32.83	1.18
28	34.17	0.24	38.17	0.24	28.58	1.06	100.50	94.99
35	34.25	0.12	38.33	0.71	30.33	0.47	30.67	0.94
50	41.58	4.36	39.92	2.71	33.33	1.18	34.42	0.59
60	36.25	4.36	48.08	10.49	30.25	0.82	33.67	1.18
74	36.17	5.66	32.83	1.41	29.17	0.71	33.50	1.18
90	34.58	6.25	33.42	1.77	30.17	0.24	33.58	0.82
100	29.75	1.30	30.50	1.65	30.00	0.94	34.17	1.65
mean	33.83	2.59	37.45	2.17	31.13	0.74	39.90	10.17
CV	7.66		5.81		2.37		25.48	
LSD $P \leq 0.05$	3.56		4.12		1.94		17.43	

Appendix 10: Labile organic P in µg/g of soil

DAYS	S	STDEV	SP	STDEV	SPB	STDEV	SB	STDEV
1	112.25	0.35	214.83	5.42	219.08	10.25	158.75	87.80
7	144.25	0.35	255.33	17.44	236.83	12.73	156.67	41.48
14	137.17	6.36	234.92	3.42	246.88	11.73	120.08	0.82
21	154.88	8.19	145.29	2.18	148.75	12.37	88.25	17.09
28	11.50	1.30	182.00	0.12	218.63	18.44	20.08	10.37
35	71.29	6.89	148.92	12.37	82.83	77.66		
50	26.08	2.00	95.17	0.82			34.67	22.63
60	76.38	5.60	321.50	10.14	180.08	109.25	28.50	1.18
74	35.83	6.60	32.92	6.95				
90	129.67	77.31	195.50	34.18	204.67	5.19	142.33	55.15
100	84.33	29.70	146.33	24.51	138.00	2.83	125.83	0.71
MEAN	89.42	13.15	179.34	10.69	186.19	28.94	97.24	26.36
CV	14.71		5.96		15.54		27.11	
LSD	43.40		68.25		46.53		48.69	

Appendix 11: Moderately labile organic P in µg/g of soil

DAYS	S	STDEV	SP	STDEV	SPB	STDEV	SB	STDEV
1	132.92	3.89	153.67	0.71	126.50	12.02	153.75	6.25
7	146.50	1.65	119.00	2.12	181.92	10.02	200.58	37.36
14	36.83	3.77	41.92	3.18	35.25	3.65	37.50	1.41
21	122.17	6.36	180.08	10.02	210.17	21.92	212.42	23.92
28	37.33	0.94	49.83	0.00	41.33	6.60	35.00	0.47
35	32.50	2.59	38.92	0.35	32.83	2.59	36.33	2.83
50	33.08	4.12	31.17	2.12	31.50	0.71	34.83	0.24
60	34.50	2.12	64.42	2.24	37.58	1.53	31.83	1.65
74	31.00	1.41	34.00	1.89	30.92	1.53	34.00	2.36
90	47.33	6.36	76.92	11.67	34.67	0.00	37.17	1.41
100	45.75	5.30	75.25	10.49	34.42	0.82	32.42	3.18
MEAN	63.63	3.50	78.65	4.07	72.46	5.58	76.89	7.37
CV	5.51		5.18		7.70		9.59	
LSD	39.53		43.90		58.19		63.38	

Appendix 12: Non-labile organic P in µg/g of soil

DAYS	S	STDEV	SP	STDEV	SPB	STDEV	SB	STDEV
1	42.42	0.59	46.50	0.24	57.83	3.54	50.17	15.08
7	42.00	1.89	78.92	2.47	46.42	3.89	48.17	9.43
14	56.83	0.24	58.58	2.24	75.50	6.60	61.00	16.50
21	43.83	2.59	106.75	0.12	99.92	1.53	49.92	9.78
28	120.08	4.83	37.67	1.18	178.25	14.50	102.75	11.90
35	36.17	3.54	34.50	0.24	34.00	1.41	32.92	4.12
50	32.58	2.00	32.17	1.41	32.42	1.77	30.83	0.24
60	33.75	1.77	32.42	0.12	35.92	1.53	30.75	0.59
74	33.42	1.53	30.58	0.12	33.50	2.12	32.00	1.89
90	36.92	2.71	38.58	0.59	34.75	4.12	37.42	1.53
100	38.33	3.77	40.83	3.54	32.83	1.18	36.25	0.35
MEAN	46.94	2.31	48.86	1.11	60.12	3.84	46.56	6.49
CV	4.93		2.28		6.38		13.94	
LSD	21.80		20.72		38.77		18.30	

Appendix 13: Methane emission in µg/g of soil in µg/g of soil

DAYS	S	STDEV	SP	STDEV	SPB	STDEV	SB	STDEV
1	4.97E-06	6.93E-07	4.47E-06	2.63E-07	4.63E-06	7.47E-08	4.99E-06	2.47E-07
2	5.69E-06	1.15E-07	4.92E-06	6.61E-08	4.81E-06	1.36E-07	6.14E-06	3.25E-07
4	6.55E-06	2.45E-07	6.23E-06	2.95E-07	6.29E-06	1.72E-07	9.72E-06	7.97E-07
8	6.50E-06	8.38E-07	9.17E-06	1.89E-06	1.03E-05	7.35E-07	1.51E-05	2.04E-06
12	8.03E-06	1.38E-06	9.80E-06	3.54E-06	1.12E-05	6.82E-07	1.45E-05	2.84E-06
14	8.06E-06	1.03E-06	1.07E-05	3.69E-06	1.53E-05	1.52E-06	2.13E-05	2.74E-06
18	8.09E-06	2.40E-06	1.14E-05	4.37E-06	1.52E-05	2.24E-06	2.67E-05	5.66E-06
21	7.91E-06	2.21E-06	1.06E-05	3.45E-06	1.75E-05	3.15E-06	3.24E-05	7.15E-06
25	8.13E-06	2.23E-06	1.11E-05	4.32E-06	2.08E-05	5.05E-06	3.62E-05	4.20E-06
40	8.76E-06	1.40E-06	1.57E-05	6.83E-06	2.19E-05	5.21E-06	4.16E-05	2.65E-06
46	8.01E-06	1.56E-06	1.27E-05	5.28E-06	1.90E-05	5.08E-06	3.38E-05	1.25E-05
50	8.41E-06	1.04E-06	1.55E-05	1.96E-06	1.89E-05	5.05E-06	3.86E-05	1.74E-05
54	7.75E-06	9.31E-07	1.78E-05	7.91E-06	1.50E-05	3.87E-06	3.07E-05	2.72E-06
60	7.84E-06	1.06E-06	1.36E-05	6.26E-06	1.17E-05	3.04E-06	3.19E-05	4.63E-06
65	7.12E-06	2.39E-07	1.41E-05	2.39E-06	1.21E-05	1.45E-06	2.92E-05	1.82E-06
67	6.67E-06	5.94E-07	1.25E-05	1.08E-06	1.08E-05	3.23E-06	2.33E-05	7.41E-07
70	7.13E-06	6.05E-07	1.29E-05	4.55E-07	1.05E-05	3.08E-06	2.34E-05	8.56E-07
73	7.53E-06	5.75E-07	1.40E-05	6.14E-07	9.99E-06	3.35E-06	2.18E-05	5.04E-08
76	7.21E-06	5.50E-07	1.27E-05	9.24E-07	1.07E-05	2.62E-06	2.12E-05	4.95E-07
81	6.88E-06	8.75E-07	1.02E-05	9.48E-07	9.40E-06	2.61E-06	1.99E-05	2.95E-07
83	7.09E-06	8.77E-07	1.25E-05	5.30E-06	9.51E-06	3.15E-06	2.05E-05	3.26E-07
87	7.30E-06	1.04E-06	1.10E-05	1.03E-06	9.68E-06	2.31E-06	1.96E-05	1.54E-08
90	9.03E-06	6.31E-07	1.06E-05	4.84E-06	1.03E-05	1.93E-06	2.13E-05	5.69E-06
95	8.79E-06	1.74E-06	1.06E-05	6.13E-06	8.95E-06	2.72E-06	2.07E-05	9.82E-07
100	8.20E-06	1.51E-06	1.05E-05	5.36E-07	9.17E-06	2.73E-06	1.76E-05	7.73E-06
MEAN	7.52E-06	1.05E-06	1.14E-05	2.97E-06	1.21E-05	2.61E-06	2.33E-05	3.40E-06

Appendix 14: Organic N in µg/g of soil

DAYS	S	STDEV	SP	STDEV	SPB	STDEV	SB	STDEV
1	1042.53	125.55	98.13	3.61	12.87	2.67	1479.95	162.05
7	1058.86	121.31	23.80	10.37	10.03	1.02	883.62	199.84
14	1807.78	116.32	271.37	4.32	748.45	94.08	1491.03	143.86
21	1735.78	118.05	252.53	1.73	523.95	118.99	1369.03	194.22
28	1849.36	117.38	359.70	2.28	709.95	94.16	1505.86	147.79
35	1729.78	118.44	242.53	2.99	790.78	98.96	1599.45	151.67
50	1891.45	119.38	389.28	3.34	854.78	93.69	1621.12	145.78
60	1887.20	117.85	388.62	3.18	848.53	91.30	1632.37	142.52
74	1882.45	117.18	386.53	5.66	842.28	92.67	1631.62	143.74
90	1880.20	116.12	381.37	5.74	842.20	90.67	1633.12	142.64
100	1890.20	116.59	391.53	4.09	840.62	91.57	1631.45	143.27
MEAN	1695.96		203.78		637.68		1498.05	

Appendix 15: Total N in µg/g of soil

treatment	T.N	stdev
S	1987.83	345.30
SP	502.00	2.36
SPB	952.83	269.88
SB	1736.17	425.44

Appendix 16: TON in µg/g of soil

DAYS	S	STDEV	S+P	STDEV	S+P+B	STDEV	S+B	STDEV
1	32.70	2.36	29.03	1.41	22.87	7.54	29.12	4.12
7	39.87	1.65	82.70	0.47	32.70	0.94	70.45	8.60
14	64.95	2.24	61.03	1.41	74.78	6.01	81.53	2.59
21	81.95	1.77	49.37	0.00	81.78	12.14	61.03	15.79
28	35.37	1.18	43.70	2.36	57.28	2.00	70.70	5.89
35	61.95	0.12	49.37	0.94	51.95	17.09	40.62	1.30
50	26.28	1.53	27.62	3.42	27.45	2.00	28.95	2.00
60	28.03	0.47	28.78	2.24	30.70	0.47	30.20	1.41
74	28.78	1.30	33.87	2.59	33.95	4.60	29.45	1.53
90	27.53	0.24	31.53	2.12	34.03	1.41	29.45	0.35
100	27.03	0.94	29.87	2.12	32.12	2.00	29.62	0.12
MEAN	41.31	1.25	42.44	1.74	43.60	5.11	45.56	3.97
CV	3.03		4.09		11.72		8.73	
LSD	17.34		7.63		17.25		18.07	

Appendix 17: Ammoniacal N in µg/g of soil

		NH4-N						
DAYS	S	STDEV	S+P	STDEV	S+P+B	STDEV	S+B	STDEV
1	912.60	28.99	871.10	7.07	917.10	39.60	227.10	56.57
7	889.10	16.97	843.10	28.28	920.10	62.23	782.10	165.46
14	115.10	1.41	169.60	9.19	129.60	6.36	163.60	3.54
21	170.10	7.07	200.10	2.83	347.10	74.95	306.10	141.42
28	103.10	5.66	98.60	2.12	185.60	10.61	159.60	12.02
35	196.10	9.90	210.10	5.66	110.10	9.90	96.10	28.28
50	70.10	11.31	85.10	4.24	70.60	9.19	86.10	9.90
60	72.60	7.78	84.60	4.95	73.60	3.54	73.60	0.71
74	76.60	4.95	81.60	12.02	76.60	3.54	75.10	4.24
90	80.10	2.83	89.10	12.73	76.60	0.71	73.60	2.12
100	70.60	3.54	80.60	7.78	80.10	2.83	75.10	4.24
MEAN	250.55	9.13	255.78	8.81	271.55	20.31	192.55	38.96
CV	3.64		3.44		7.48		20.23	
LSD	280.41		260.59		285.35		181.41	

Appendix 18: TOC (%)

SAMPLE	TOC%	STDEV
S	6.50	0.37
SP	6.86	0.45
SPB	6.89	0.18
SB	6.61	0.35

Appendix 19: pH

DAYS	S	Dev	SP	Dev	SPB	Dev	SB	Dev
1	3.96	0.15	4.98	0.05	5.00	0.12	4.84	0.06
7	4.32	0.06	5.06	0.22	5.66	0.05	5.66	0.10
14	4.64	0.11	5.16	0.10	5.84	0.09	6.04	0.04
21	5.48	0.07	6.04	0.12	6.17	0.10	6.09	0.02
28	5.51	0.08	6.02	0.11	6.12	0.13	5.75	0.12
35	5.46	0.02	6.04	0.06	6.08	0.05	5.73	0.05
50	5.53	0.04	6.04	0.02	5.89	0.02	5.48	0.21
60	5.66	0.12	5.94	0.07	6.20	0.05	5.77	0.05
74	5.44	0.17	5.98	0.13	6.16	0.06	5.48	0.07
90	5.78	0.21	5.88	0.08	6.12	0.04	5.13	0.11
100	5.60	0.05	5.94	0.20	6.12	0.02	5.20	0.10
mean	5.22	0.10	5.73	0.11	5.94	0.07	5.56	0.08

Appendix 20: Nitrous Oxide Emission in µg/g of soil

DAYS	S	STDEV1	SP	STDEV2	SPB	STDEV3	SB	STDEV4
1	5.24	0.24	4.63	0.07	5.89	0.18	7.03	0.55
2	4.91	0.08	4.37	0.22	5.22	0.14	6.59	0.29
4	5.07	0.17	4.30	0.17	5.81	0.90	5.74	0.34
8	3.96	0.22	4.20	0.92	6.60	1.54	6.82	0.38
12	4.14	0.19	3.76	0.91	6.10	1.72	6.96	0.32
14	3.41	0.28	2.86	0.29	5.05	1.60	7.54	0.70
18	3.37	0.24	2.56	0.04	3.99	1.22	6.51	0.28
21	3.74	0.45	2.84	0.09	4.45	1.73	6.35	0.88
25	8.25	1.01	11.89	2.31	21.30	4.60	12.83	1.97
40	8.64	0.68	12.37	3.21	22.73	4.18	8.37	0.92
46	20.08	6.13	31.47	11.19	21.20	1.84	3.58	0.40
50	8.67	0.47	38.09	4.44	32.41	0.45	19.20	0.14
54	8.29	1.03	62.63	4.70	38.72	1.92	12.99	2.64
60	13.10	1.16	56.22	11.26	27.03	4.94	17.35	4.38
65	16.85	1.90	50.38	13.02	17.05	0.23	23.97	0.29
67	16.11	1.82	25.39	1.65	17.38	1.74	20.86	0.84
70	16.22	0.18	33.71	1.68	19.67	1.25	30.46	3.03
73	21.90	1.83	30.88	1.26	9.00	0.35	21.43	2.43
76	27.37	5.03	38.64	3.56	20.66	1.93	24.79	0.11
81	47.30	3.69	19.25	2.33	38.49	7.30	34.99	3.35
83	14.83	1.76	13.99	4.12	31.00	3.97	17.62	0.34
87	66.76	10.53	9.70	2.37	35.48	1.98	16.39	0.47
90	68.10	11.07	10.13	2.39	35.24	5.94	41.94	0.81
95	65.06	0.01	10.49	0.23	9.10	0.56	8.50	1.05
100	61.33	11.05	14.61	1.06	25.20	0.19	42.83	0.33
MEAN	20.91	2.45	19.97	2.94	18.59	2.10	16.47	1.09

Appendix 21: Carbon Dioxide Emission µg/g of soil

DAYS	S	STDEV	SP	STDEV	SPB	STDEV	SB	STDEV
1	11.99	4.87	12.64	5.29	12.57	1.20	17.96	6.75
2	10.18	4.91	8.46	3.57	4.03	3.59	11.06	5.00
4	4.18	0.80	4.40	2.96	7.29	5.07	7.08	2.23
8	7.44	2.59	5.28	3.41	2.91	2.24	7.87	1.07
12	9.08	4.11	2.73	1.00	19.42	7.15	1.13	0.40
14	5.10	1.92	3.31	0.86	2.57	0.45	3.81	1.82
18	3.58	2.83	1.87	0.84	1.07	0.65	2.96	0.98
21	5.23	0.99	6.17	2.59	2.24	1.60	3.62	0.74
25	24.82	6.17	3.01	1.40	2.78	1.39	1.65	0.39
40	6.43	2.06	6.96	2.99	3.83	1.35	1.32	0.94
46	9.70	7.38	1.50	0.64	1.81	0.09	4.68	1.01
50	16.64	9.34	18.65	3.80	9.47	2.49	7.57	3.25
54	6.89	2.46	11.77	1.17	16.44	6.51	10.39	4.00
60	2.96	0.70	2.59	1.58	2.65	0.13	4.95	3.24
65	15.77	6.20	18.34	5.87	30.35	7.39	12.66	4.10
67	2.02	0.07	4.55	1.31	2.50	0.76	2.86	2.23
70	6.63	1.47	10.98	4.44	6.13	3.13	7.89	5.25
73	9.50	2.98	11.22	4.84	2.11	0.07	4.68	2.74
76	10.50	2.64	3.89	0.91	7.01	5.97	5.78	2.83
81	15.44	8.97	6.50	2.63	10.27	6.80	8.29	3.43
83	7.15	3.59	4.12	0.55	4.08	0.11	1.82	0.31
87	22.01	4.24	4.32	0.51	3.75	1.92	1.21	0.12
90	11.27	2.48	3.45	0.47	5.05	0.52	1.59	0.07
95	6.21	2.37	10.49	0.23	9.10	0.56	8.50	1.04
100	8.04	2.42	7.10	1.28	8.35	0.80	7.67	0.31
MEAN	9.55	3.54	6.97	2.21	7.11	2.48	5.96	2.17

Appendix 22: Cumulative Carbon Dioxide Emission µg/g of soil

DAYS	S	STDEV	SP	STDEV	SPB	STDEV	SB	STDEV
1	11.99	7.87	12.64	5.29	12.57	1.20	17.96	6.75
2	22.17	7.41	21.09	4.36	16.60	2.40	29.02	12.06
4	26.35	8.18	25.50	4.15	23.89	7.46	36.10	6.44
8	33.79	13.71	30.78	3.63	26.80	6.29	43.97	8.78
12	42.86	17.62	33.51	2.16	46.22	24.12	45.10	8.39
14	47.97	19.38	36.82	3.01	48.78	24.51	48.90	8.00
18	51.55	22.03	38.69	3.98	49.86	24.45	51.86	5.04
21	56.77	22.82	44.85	2.15	52.10	23.68	55.48	5.25
25	81.59	37.31	47.87	1.66	54.88	24.55	57.14	5.57
40	88.01	40.99	54.83	1.97	58.71	23.58	58.46	4.69
46	97.72	34.90	56.33	1.38	60.52	23.51	63.14	5.09
50	114.35	41.03	74.98	14.82	69.99	23.09	70.71	4.54
54	121.24	48.25	86.75	13.94	86.43	19.83	81.10	9.78
60	124.20	48.93	89.34	15.36	89.08	19.85	86.04	6.95
65	139.97	62.75	107.68	13.58	119.42	27.23	98.70	9.20
67	141.98	62.81	112.23	14.66	121.93	26.55	101.56	7.13
70	148.61	64.05	123.21	20.99	128.05	23.42	109.45	1.88
73	158.11	66.78	134.43	14.59	130.16	23.39	114.13	0.87
76	168.61	69.14	138.32	14.61	137.17	27.23	119.91	3.00
81	184.05	77.28	144.82	16.97	147.44	29.90	128.21	4.74
83	191.20	82.42	148.93	17.38	151.52	29.80	130.03	4.97
87	213.20	85.60	153.26	17.69	155.27	29.12	131.24	4.94
90	224.47	85.78	156.71	17.22	160.32	29.23	132.83	4.91
95	230.68	84.00	167.20	17.38	169.42	29.03	141.33	5.62
100	238.72	81.76	174.30	18.39	177.76	29.14	149.00	5.34
MEAN	118.41	47.71	88.60	10.45	91.79	22.10	84.05	6.00

Appendix 23: Cumulative Nitrous Oxide Emission µg/g of soil

DAYS	S	STDEV1	SP	STDEV2	SPB	STDEV3	SB	STDEV4
1	5.24	0.24	4.64	0.07	5.89	0.18	7.03	0.55
2	10.16	0.31	9.01	0.25	11.11	0.31	13.62	0.81
4	15.22	0.44	13.30	0.37	16.92	0.97	19.36	1.14
8	19.19	0.62	17.50	1.29	23.52	2.29	26.19	1.49
12	23.33	0.80	21.26	2.20	29.62	3.88	33.15	1.72
14	26.74	1.08	24.13	2.48	34.67	5.44	40.69	2.23
18	30.12	1.31	26.68	2.49	38.66	6.65	47.20	2.50
21	33.85	1.65	29.52	2.48	43.11	8.30	53.55	3.37
25	42.11	1.95	41.41	2.11	64.40	11.99	66.37	5.10
40	50.75	2.12	53.78	4.32	87.13	16.09	74.75	5.92
46	70.83	6.18	85.24	14.93	108.33	16.61	78.33	6.24
50	79.51	5.84	123.34	11.10	140.74	17.06	97.53	6.36
54	87.80	4.95	185.97	6.43	179.46	15.14	110.52	7.50
60	100.90	4.69	242.19	4.85	206.49	19.57	127.87	10.29
65	117.75	4.53	292.57	17.87	223.53	19.44	151.85	10.17
67	133.86	4.88	317.96	19.19	240.91	20.20	172.70	10.90
70	150.07	5.02	351.67	20.86	260.58	18.96	203.16	7.96
73	171.97	4.24	382.55	19.63	269.58	19.26	224.59	6.31
76	199.34	7.80	421.18	17.56	290.24	17.46	249.38	6.25
81	246.64	11.42	440.44	19.85	328.73	24.76	284.37	7.35
83	261.47	12.14	454.42	23.92	359.73	27.99	301.98	7.67
87	328.23	17.51	464.13	26.27	395.22	26.23	318.37	7.95
90	396.33	26.12	474.26	28.66	430.46	30.60	360.31	8.50
95	461.40	26.10	484.75	28.55	439.55	30.90	368.81	8.16
100	522.73	34.18	499.36	29.61	464.76	31.05	411.64	7.88
MEAN	143.42	7.45	218.45	12.29	187.73	15.65	153.73	5.77

Appendix 24: Cumulative Methane Emission in µg/g of soil

DAYS	S	STDEV1	SP	STDEV2	SPB	STDEV3	SB	STDEV4
1	4.97E-06	1.60E-07	4.47E-06	1.09E-07	4.63E-06	1.05E-07	4.99E-06	2.53E-07
2	1.07E-05	5.03E-08	9.39E-06	1.20E-07	9.44E-06	2.40E-10	1.11E-05	3.64E-07
4	1.72E-05	1.74E-07	1.56E-05	4.15E-07	1.57E-05	2.43E-07	2.09E-05	1.83E-07
8	2.37E-05	1.36E-06	2.48E-05	1.51E-06	2.61E-05	7.86E-07	3.59E-05	6.05E-07
12	3.17E-05	6.06E-07	3.46E-05	4.13E-06	3.73E-05	1.50E-07	5.05E-05	3.41E-06
14	3.98E-05	8.42E-08	4.53E-05	7.32E-06	5.26E-05	2.00E-06	7.18E-05	3.12E-06
18	4.79E-05	2.71E-06	5.67E-05	1.05E-05	6.79E-05	4.88E-06	9.85E-05	1.74E-06
21	5.58E-05	5.45E-06	6.73E-05	1.22E-05	8.53E-05	8.37E-06	1.31E-04	8.33E-06
25	6.39E-05	8.27E-06	7.84E-05	1.37E-05	1.06E-04	1.30E-05	1.67E-04	2.52E-05
40	7.27E-05	8.66E-06	9.41E-05	1.25E-05	1.28E-04	1.52E-05	2.09E-04	3.49E-05
46	8.07E-05	1.07E-05	1.07E-04	1.05E-05	1.47E-04	1.53E-05	2.43E-04	4.51E-05
50	8.91E-05	1.15E-05	1.22E-04	8.56E-06	1.66E-04	1.61E-05	2.81E-04	5.97E-05
54	9.69E-05	1.25E-05	1.40E-04	4.36E-06	1.81E-04	1.41E-05	3.12E-04	7.45E-05
60	1.05E-04	1.10E-05	1.54E-04	1.70E-06	1.93E-04	1.12E-05	3.44E-04	9.56E-05
65	1.12E-04	1.07E-05	1.68E-04	3.71E-06	2.05E-04	9.48E-06	3.73E-04	1.12E-04
67	1.19E-04	1.01E-05	1.80E-04	2.63E-06	2.16E-04	6.65E-06	3.96E-04	1.19E-04
70	1.26E-04	9.21E-06	1.93E-04	2.18E-06	2.26E-04	9.07E-06	4.20E-04	1.25E-04
73	1.33E-04	8.50E-06	2.07E-04	7.93E-06	2.36E-04	6.54E-06	4.41E-04	1.33E-04
76	1.40E-04	7.72E-06	2.20E-04	8.85E-06	2.47E-04	5.73E-06	4.63E-04	1.39E-04
81	1.47E-04	6.50E-06	2.30E-04	9.80E-06	2.56E-04	3.96E-06	4.82E-04	1.45E-04
83	1.54E-04	5.27E-06	2.43E-04	6.04E-06	2.66E-04	1.51E-06	5.03E-04	1.51E-04
87	1.62E-04	3.80E-06	2.54E-04	7.07E-06	2.75E-04	1.65E-08	5.23E-04	1.58E-04
90	1.71E-04	3.74E-06	2.64E-04	7.42E-06	2.86E-04	3.17E-09	5.44E-04	1.61E-04
95	1.79E-04	1.35E-06	2.75E-04	8.08E-06	2.95E-04	1.16E-06	5.65E-04	1.70E-04
100	1.88E-04	6.73E-07	2.85E-04	8.61E-06	3.04E-04	3.09E-06	5.82E-04	1.75E-04
MEAN	9.48E-05	5.63E-06	1.39E-04	6.40E-06	1.61E-04	5.94E-06	2.91E-04	7.76E-05

Appendix 25: DOC in mg/L

DAYS	S	DEVA	SP	DEVB	SPB	DEVC	SB	DEVD
21	0.82	0.01	0.79	0.02	0.80	0.01	0.80	0.07
28	0.76	0.02	0.79	0.02	0.78	0.02	0.78	0.01
35	0.76	0.02	0.92	0.11	0.84	0.05	0.88	0.01
50	0.77	0.02	0.74	0.02	0.76	0.01	0.75	0.03
60	0.80	0.00	0.70	0.05	0.75	0.01	0.72	0.01
74	0.81	0.00	0.71	0.04	0.76	0.01	0.73	0.01
90	0.84	0.02	0.76	0.01	0.80	0.00	0.78	0.00
100	0.87	0.03	0.74	0.02	0.80	0.00	0.77	0.04
Mean	0.80	0.02	0.77	0.04	0.79	0.01	0.78	0.03