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Effects of tobacco nicotine on maize yield and soil bacteria in Tabora western Tanzania

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NM-AIST

EFFECTS OF TOBACCO NICOTINE ON MAIZE YIELD AND SOIL BACTERIA IN TABORA WESTERN TANZANIA

Jacob Bulenga Lisuma

A Thesis Submitted in Partial Fulfilment of the Requirements for the Degree of Doctor of Philosophy in Life Sciences of the Nelson Mandela African Institution of Science and Technology

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ABSTRACT

Tobacco (*Nicotiana tabacum* L.) is globally known to be a primary source of nicotine worldwide. This study was conducted to determine the effects of nicotine on the rhizosphere and subsequent maize crop yield. Pot experiments were carried at the Nelson Mandela African Institution of Science and Technology (NM-AIST), using levels of root and leaf extracts drenching to maize seedlings. In the first year, the field experiment comprised of six treatments (a) fertilized tobacco, (b) fertilized maize, (c) fertilized tobacco incorporated with tobacco stalks, (d) unfertilized maize, (e) unfertilized tobacco and (f) fallow with a plot size of 6 m x 6 m, 1.2 m from the ridge to ridge and 0.50 m from plant to plant. In the second year, all plots with exception to fallow plots were planted with maize to observe the effects of tobacco on soil nutrients, bacteria diversity and maize yield. Soil samples were taken to measure nutrients, nicotine and study bacteria diversities. Results showed that fertilized tobacco, released higher nicotine into the soil (10.27 mg ha^{-1}) than unfertilized tobacco (3.07 mg ha^{-1}). High levels of nicotine released in soils 7.59 mg kg^{-1} were found at a depth of 30 - 50 cm and lowest level 5.50 mg kg^{-1} at a depth of 0 - 10 cm. Maximum adsorbed and desorbed nicotine were found to be 4.61 and 2.21 mg kg^{-1}, respectively. Maize absorbed nicotine but at a very low concentration (0.001%) in maize grain. Maize planted not after tobacco had the highest grain yields (3.86 t ha^{-1}), but maize planted as subsequent crop after tobacco had the lowest grain yields (3.53 t ha^{-1}). The low yields were due to the low absorption of P and K nutrients following extreme uptake of these nutrients by the tobacco plant. In tobacco rhizosphere, bacteria under *Proteobacteria,* influence solubilization of P, K, S, Cu^{2+}, Fe^{2+}, Zn^{2+}, Mn^{2+} hence increased uptake of macronutrients and reduced their levels in soils; and less uptake of micronutrients and increased their levels in soils. This study recommends further studies to re-calibrate new recommendations for P and K on maize crop planted after tobacco.

DECLARATION

I, **Jacob Bulenga Lisuma** do hereby declare to the Senate of the Nelson Mandela African Institution of Science and Technology that this Thesis is my own original work and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

Jacob Bulenga Lisuma

Name of candidate	**Signature**	**Date**

The above declaration is confirmed

Prof. Patrick A. Ndakidemi

Supervisor 1	**Signature**	**Date**

Dr. Ernest R. Mbega

Supervisor 2	**Signature**	**Date**

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CERTIFICATION

The undersigned certify that they have read Thesis titled "**Effects of Tobacco Nicotine on Maize Yield and Soil Bacteria in Tabora Western Tanzania**" and recommend for examination in fulfillment of the requirements for the degree of Doctor of Philosophy of Life Sciences (Sustainable Agriculture) of the Nelson Mandela African Institution of Science and Technology.

Prof. Patrick A. Ndakidemi

Supervisor 1	**Signature**	**Date**

Dr. Ernest R. Mbega

Supervisor 2	**Signature**	**Date**

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DEDICATION

This thesis is dedicated to my wife Asnath, my daughters Hellen, Mitchelle and my son Allen for their good company and encouraging words during my PhD studies.

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LIST OF ABBREVIATIONS AND SYMBOLS

ANOVA	Analysis of Variance
CEC	Cation Exchange Capacity
CRD	Completely Randomized Design
DNA	Deoxyribonucleic Acid
EC	Exchangeable Bases
ECP	Extract Concentration Parts
Fert NT	Fertilized Nicotiana Tabacum
Fert ZM	Fertilized Zea Mays
FOM	Fresh Organic Matter
HI	Harvest Index
MoA	Ministry of Agriculture
NIC	Nicotine
NM-AIST	Nelson Mandela African Institution of Science and Technology
OC	Organic Carbon
ODC	Ornithine Decarboxylase
OM	Organic Matter
PCR	Polymerase Chain Reaction
PP	Plant Parts
RCBD	Randomized Completely Block Design
RNA	Ribonucleic Acid
rRNA	Ribosomal Ribonucleic Acid
SI	Stalks Incorporation
SOM	Soil Organic Matter
SUA	Sokoine University of Agriculture
TORITA	Tobacco Research Institute of Tanzania
Unfert NT	Unfertilized Nicotiana Tabacum
Unfert ZM	Unfertilized Zea Mays

CHAPTER ONE

INTRODUCTION

1.1 Background of the problem

Tobacco (*Nicotiana tabacum* L.) is a herbaceous crop native to the Americans, Australia, South West Africa and the South Pacific (Marks, Newbigin & Ladiges, 2011; Voeks, 2009; Knapp, Chase & Clarkson, 2004; Charlton, 2004). The origin of the genus *Nicotiana* was identified after Jean Nicot (Charlton, 2004). It started being cultivated as a decorative plant, in the years 1530 -1604 by Jean Nicot from France who picked the tobacco plant from Portugal and introduced it to Europe due to its historical wondrous curative properties against worms, toothaches and mitigating obesity (Schäfer, 2008). With time, more uses of tobacco such as leaves for chewing, tea for drinking or smoke to inhale became famous. Today, tobacco is cultivated as a commercial crop and used for chewing, snuffing and smoking a cigarette (Anand & Sk, 2017).

Tobacco crop was introduced in Tanzania in the early 1950s by colonialists (Boesen & Mohele, 1979). The first records of tobacco cultivation in the country suggest Tabora region as the main port of first entry due to its favourable soils (Boesen & Mohele, 1979). Before the entry of tobacco in Tabora, farmers were growing millet, cassava, groundnuts and beans. However, millet was then replaced with maize and rice. To date, tobacco production has spread through the country and it is grown in Mbeya (Chunya), Kagera (Biharamulo), Shinyanga (Kahama), Singida (Manyoni), Iringa, Katavi (Mpanda), Ruvuma (Songea), Mara (Serengeti, Rorya and Tarime) (Tanzania Tobacco Board, 2018). Over fifty per cent of the country's tobacco production comes from the Tabora region only, indicating to be the hub region for tobacco production (Kidane & Ngeh, 2015). The region contributes significanttly to the country foreign exchange since tobacco is among the top three foreign exchange earner crop in Tanzania (Bank of Tanzania, 2016).

Tobacco cultivation in Tabora is done in rotation with maize, however, maize yield (but not tobacco) has continued to be low with average yield of 0.9 t ha^{-1} (National Bureau of Statistics, 2006) compared with the potential country yield of 5 t ha^{-1} (Mbwaga & Massawe, 2002; Barreiro-Hurle, 2012) at least for the past five years (Fig. 1).

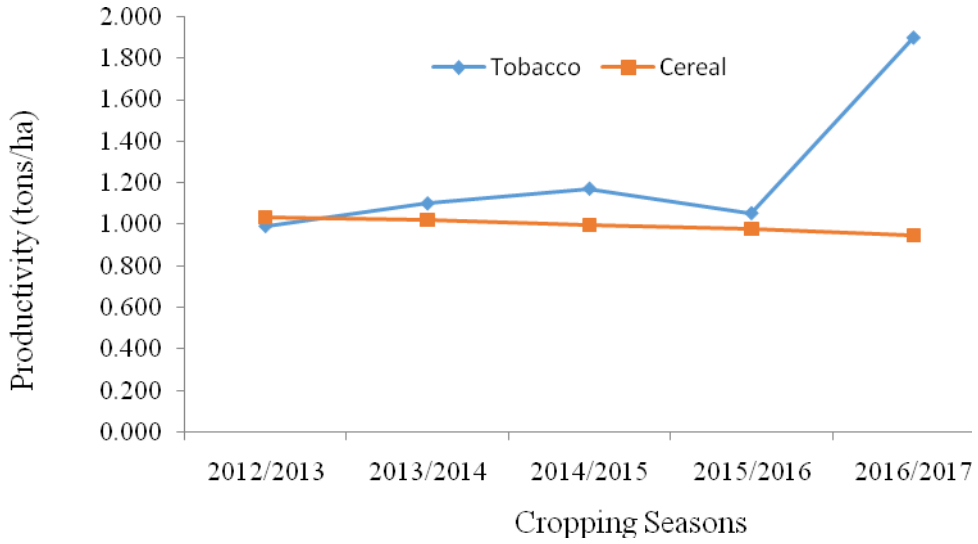


Figure 1: Tobacco and cereal (e.g. maize) production trend for Tabora region (Tabora Regional Agricultural Office [TRAO], 2018)

The low maize yield in Tabora could be due to the suppressive effects of tobacco nicotine to the soil bacteria population that play beneficial role in soil fertility managemenet in the rhizhosphere (Adediran, Mnkeni, Mafu & Muyima, 2004; Farooq, Hussain, Wakeel & Cheema, 2014), drought and/or depleting soil fertility based on the fact that tobacco is a heavy feeder of nutrients relative to maize, making the Tabora soil very low in soil fertility (Matata, Gama, Mbwaga, Mpanda & Byamungu, 2011). While the effects of succession of a light soil nutrient feeder crop after a heavy feeder crop is logically known (Kids, 2001), the effects of tobacco nicotine on maize growth, soil fertility and bacteria composition following tobacco cultivation, has not been studied.

1.2 Statement of the problem

Maize yields in Tabora region has been reported to be about 0.9 t ha^{-1} lower than the potential yield of 5 t ha^{-1} (as previously described) despite application of recommended fertilizer levels of 120 kg N ha^{-1}, 50 kg P ha^{-1} and 50 kg K ha^{-1} durimg the maize growing season and 83.75 kg N ha^{-1}, 90 kg P ha^{-1} and 120 kg K ha^{-1} applied in the field during the tobacco growing season (Mbwaga & Massawe, 2002; Barreiro-Hurle, 2012; Kuboja, Kazyoba, Lwezaura & Namwata, 2012).

A possible explanation for such trend is that tobacco is a heavy nutrient feeder crop; thus, any fertilizer application in maize crop possibly ends up in restoring soil nutrients equilibrium and only less becomes available for the maize crop (Prowse & Grassin, 2020). However, this is not always the case following a study by Yazdani and Bagheri (2011) and Baek *et al.*

(2017) who both reported that tobacco residues incorporated into the soils can affect seedlings emergence rate, chlorophyll contents and vigour index of the subsequent cereal crops such as maize which also was included in this study. Such reports raised a concern that tobacco residues might be associated with the low yields. Nevertheless, a contradicting report by some authors Rizvi, Mishra and Rizvi (1989), Rizvi, Tahir, Rizvi, Kohli and Ansari (1999), Farooq *et al*. (2014) and Zou *et al*. (2018) indicated a different trend where tobacco favoured the performance of cereals that were grown as subsequent crops.

Due to that controversey, it remained a riddle to the correct association of tobacco with either the low maize yields as recorded in the study locations or other associations. It has been established that the possible effects of tobacco to the cereal crops is controlled by the ability of tobacco roots to release nicotine as a metabolite to the soil (Darwent, Paterson, McDonald & Tomos, 2003; Dennis, Miller & Hirsch, 2010; Cheng & Cheng 2016). Besides, tobacco rotated with maize crop has been reported affecting the abundance of soil microbes that interact with soil fertility environments (Niu *et al*., 2016). However, there was no evidence of such claims in Tanzania, thus, it is from this background that formed the basis of investigating the effects of tobacco cultivation to the soil fertility, bacteria and subsequent maize crop yield in the country of study.

1.3 Rationale of the study

Farmers in Tabora region grow tobacco in rotation with cereals such as maize to avoid nematodes infestation on tobacco in situations where the crop is left continuously in the field. Maize occupies 232 860 ha out of 347 455 ha for cereals, and tobacco is grown in 32 490 ha out of 54 948 ha for cash crops (NBS, 2006). However, this practice seems not to favour cereal crops as productivity of the cereals such as maize has been stagnant from the year 2012 to 2016 (Fig. 1). In all these cropping seasons tobacco growers were and still supplied with two bags of fertilizer to support maize production as a food crop. Despite the fertilizer support for maize crop from the tobacco companies, maize yields continue to be low reaching 0.9 t ha^{-1}. The reasons for the low maize yield in Tabora is not known as no any research that has been carried out on the tobacco-maize farming system.

Some studies in other Asia and South America continents have been done in studying the effect of the tobacco crop to the growth of cereals such as maize. However, the results are not consistent as the first pillar of researchers indicates the growth of cereals crop and soil bacteria to be affected by tobacco crop (Adediran *et al*., 2004; Yazdani & Bagheri, 2011;

Baek *et al*., 2017). The second pillar of researchers indicates tobacco to favour the growth of maize (Rizvi *et al*., 1999; Farooq *et al*., 2014). From these findings, it indicates that the effect of tobacco on the cereals planted as a subsequent crop is soil texture dependent. Based on this background, the present study was undertaken to investigate the effects of tobacco nicotine on soil bacteria diversity and maize yield. The implication of this study relates to helping Tabora farmers to find solutions for increased maize yield and improve food security.

1.4 Objectives

1.4.1 General objective

The main objective of the research study was to investigate the effects of nicotine from tobacco on soil fertility, bacteria diversity and maize yield.

1.4.2 Specific objectives

(i) To investigate the levels of nicotine released by the tobacco plant within the rhizosphere under the fertilization and to assess the influence of soil depth on soil pH, OC, moisture and temperature.

(ii) To determine adsorption and desorption maximum levels of the released nicotine from tobacco plant by the soil using the best fitting Freundlich model.

(iii) To investigate the effects of tobacco nicotine on availability of soil nutrients under fertilization.

(iv) To determine the effects of nicotine on subsequent maize crop yield in different soil textures under fertilization.

(v) To determine the effect of nicotine on the diversity of bacteria in the soil and linking with their influence on soil fertility.

1.5 Hypothesis

This study was guided by the null hypothesis that tobacco does not affect the subsequent maize crop yield, soil fertility and soil bacteria. The alternative hypothesis was that, tobacco has effect on the subsequent maize crop yield, soil fertility and soil bacteria.

1.6 Significance of the study

The significance of this research will:

(i) Levels of nicotine released in the soils by the tobacco plant and their relationships will be known, and the problem of low maize yield would be solved through supplementation of P and K nutrients.

(ii) Policymakers will have quality information on the effect of tobacco crop in releasing nicotine in soils and would improve on regulatory and environmental policy.

(iii) Contribute to the agricultural sector through improving maize food security and sustainability of the soil environment to the Tabora western zone of Tanzania

1.7 Delineation of the study

The delineations of the present study are as follows:

(i) The problem of low maize yield in Tabora is revealed in this study. However, there are a series of research trials required to be conducted in future as time and resources for the current study was inadequate. Among the research trials required includes; research on quantifying the volume of K and P exhausted by tobacco plant from the soil to enable formulation of new fertilizer dosage for the maize crop.

(ii) The current research findings indicated that maize crop planted after tobacco absorbs the nicotine. The study did not establish the critical nicotine concentrations absorbed by the maize plant. Establishment of the critical nicotine concentrations absorbed by the maize plant would have given the extreme of the nicotine absorbed by the maize plant.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Tobacco (*Nicotiana tabacum* L.) is among the leading commercial crops and highly distributed worldwide. The cultivated land is about 3.9 million ha of which 60% is 'flue-cured' tobacco, 13% is burley tobacco, and 12% is oriental (Hoyos, Magnitskiy & Plaza, 2015). The top ten producers of tobacco are China, India, Brazil, USA, Indonesia, Zimbabwe, Zambia, Pakistan, Tanzania, and Argentina (Charlton, 2004; Voeks, 2009; Yang, 2010; Marks *et al*., 2011; FAOSTAT, 2016a). According to Hu and Lee (2015), Africa produced 650 000 tons (8.7%) of the world tobacco leaf in 2012 compared with 440 000 tons (7.3%) in 2003. The famous tobacco growing countries in Africa are Malawi, Tanzania, Zimbabwe, Zambia, and Mozambique (FAO, 2003; Sauer & Abdallah, 2007; Whittington, 2011).

Tobacco is cultivated in different scales depending on the country's target for economic reasons and/or a need to increase family income. Tobacco is mostly grown in rotation with cereals and/or leguminous crops, whereby maize (*Zea mays* L.) is the main food crop involved (FAOSTAT, 2016b). Tobacco production increase in African countries, could be associated with the increase of nicotine residual levels in the soils. Since maize is the main crop rotated with tobacco, there could be possibilities for the residual nicotine in soil being absorbed by the maize crop and cause an effect. Therefore, there is a need to study the effects of rotating tobacco with maize crop on yields, soil nutrients and bacteria. However, maize productivity in tobacco cultivated systems has been at a stagnant trend for the period of five consecutive years from 2012 to 2016 (Fig. 2). This is attributed to the depletion of soil fertility due to high nutrients uptake by tobacco plant, climatic change, and/or usage of unimproved maize varieties (Denning *et al*., 2009; MoAFS, 2011; Ngwira *et al*., 2012). In developing countries, the demand for food crops like maize is increasing due to high population pressure, which is reducing land under food production (MoAFS, 2011). Therefore, inclusion of maize in rotations with tobacco plant in smallholder settings would be one of the sustainable intensification options in crop productivity where tobacco cultivation is inevitable due to its demand as a cash crop.

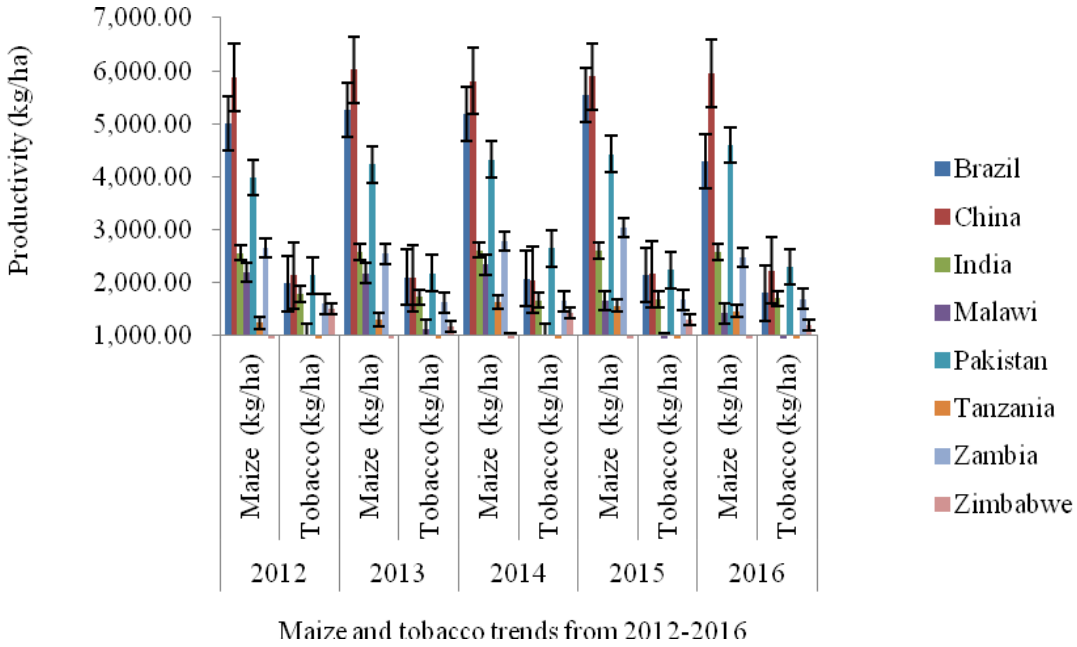


Figure 2: Tobacco and cereal (e.g. maize) production trend for Brazil, China, India, Malawi, Pakistan, Tanzania, Zambia and Zimbabwe (FAOSTAT, 2016ab)

Rotation is meant mainly for improving soil fertility and sustain its productivity (Butorac *et al*., 1999; Thierfelder *et al*., 2013; Shahzad *et al*., 2016). The benefits derived from rotations with tobacco are to be compatible with diverse crops such as maize, small grain cereals, grasses, rice, groundnuts, soybeans, cotton, and other legumes (Li *et al*., 2016). However, the positive and negative effects associated with tobacco nicotine allelopathy to the subsequent cereal and leguminous crops have not been widely explored (Baek *et al*., 2017). There are a few studies that have documented allelopathic effects of tobacco nicotine on growth of cereals such as maize (Rizvi *et al*., 1989; Karaman & Brohi, 2013; Farooq *et al*., 2014; Haq *et al*., 2018), rice (*Oryza sativa* L.) (Shakeel, 2014), and wheat (*Triticum aestivum* L.) (Shakeel, 2014; Baek *et al*., 2017).

Preliminary studies have indicated that cereal crops are favoured more than the legumes in terms of growth when rotated with tobacco crops (Rizvi *et al*., 1989; Rizvi *et al*., 1999). However, to the present, there are three clearly marked contradicting results of such effects to these crops. Firstly, some findings indicate that the growth performance of both crop species are hindered by the tobacco allelopathy (Yazdani & Bagheri, 2011; Baek *et al*., 2017). Secondly, the growth performance of these crops is equally favoured by the tobacco allelopathy (West & Post, 2002; Reed *et al*., 2012; Zou *et al*., 2018), and thirdly, other studies indicate that cereals growth are more favoured than legumes growth (Rizvi *et al*., 1989; Rizvi

et al., 1999; Farooq *et al*., 2014) due to tobacco allelopathy effects to these crops. Allelopathy constitutes secondary metabolites released by plants in their roots which in turn affects microorganisms such as viruses, bacteria, and fungi in soils (Narwal *et al*., 2005). There are very few studies in the tobacco sector addressing the allelopathy effects of nicotine on the growth of subsequent cereal crops such as maize. Therefore, there is a need of establishing studies on allelopathic effects of tobacco nicotine on the productivity of maize when cultivated as a subsequent crop. This will provide a basis for clearly identifying abiotic and biotic factors that affect the productivity of this crop when it is involved in tobacco cultivating systems.

This review focuses mainly on the allelopathic effects of tobacco nicotine on growth of subsequent cereal crop (maize) and the beneficial soil bacteria and fungi. The outcomes of this review would be pertinent to all stakeholders in this sector in understanding the practical implication of tobacco nicotine-crop, soil nutrients and bacteria/fungi interaction.

2.2 Chemical composition of tobacco plant

The constituents of tobacco are not individual compounds but classes of compounds such as alkaloids, proteins (soluble and insoluble fractions), nitrate-nitrogen, amino nitrogen, etc. (Talhout *et al*., 2011). Nicotine is indicated to be the most abundant of the volatile alkaloids in the tobacco leaf and the high levels of nutrient nitrogen increase nicotine and nitrate levels of the leaf (Leffingwell, 1999). Generally, tobacco plant is chemically composed of sugars, fats and amino acids which are also found in other plants. Other chemical constituents such as aromatic hydrocarbons, phenols, nitrosamines, aldehydes, alkanes, alkynes, toluene, benzene, nitrogen oxide, cadmium, and nicotine are also widely reported (Benowitz, Hukkanen & Jacob, 2009; Talhout *et al*., 2011; Rodgman & Perfetti, 2016). Table 1, summarizes the common chemical composition of tobacco plant (Down, 2014). It is widely documented that the biggest portion (96%) of the composition of tobacco metabolites is nicotine (Armstrong, Wang & Ercal, 1998; Jacob, Shulgin & Benowitz, 1999; Benowitz *et al*., 2009).

The physical and chemical composition of tobacco are influenced by the genetics, cropping practices, soil type and its nutrients, climatic conditions, diseases and pests, stalk position, harvesting and curing practices (Leffingwell, 1999). However, there is an important need of understanding the overriding constituents of tobacco nicotine as it has critical implication on both composition of soil bacteria, fungi and the subsequent crops. In tobacco leaves, various

post-harvest reactions during curing degrade nicotine into its nitrogen oxide as well as into cotinine and other alkaloids (Petterson *et al*., 1991; Wang *et al*., 2008). Tobacco residues are rich in essential nutrient elements such as Ca (3.7%), N (2.38%), K (0.4%) and P (0.5%) (Table 2); the contents of N and Ca are much higher than the rest nutrients and hence can improve soil fertility or growth of the subsequent crop (Adediran *et al*., 2004; Chaturvedi, Upretil, Tandon, Sharma & Dixi, 2008; Shakeel, 2014).

2.3 Properties of nicotine

Nicotine is an organic compound and the main alkaloid found throughout the tobacco plant particularly in leaves (Shoji, Ogawa & Hashimoto, 2008). Nicotine is a tertiary amine ($C_{10}H_{14}N_2$) consisting of a pyridine and a pyrrolidine ring (Benowitz, 2009) , and it forms 2 to 8% of the dry mass of the tobacco leaves (Armstrong *et al*., 1998). It is water soluble in its base form between 60 and 210° C, having molecular weight of 162.234, melting point of -79° C and boiling point of 247° C (Lide, 2007). Nicotine as a nitrogenous base forms salts with acids that are usually solid and water-soluble (O'Neil, 2006). Figure 3 presents the structure of nicotine.

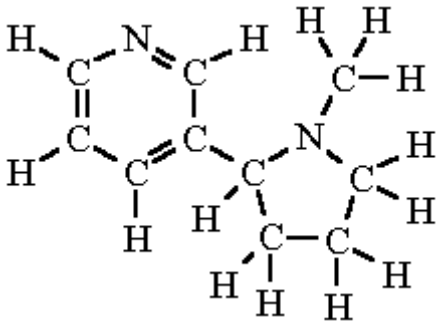


Figure 3: Nicotine ($C_{10}H_{14}N_2$) structural formula

2.4 Nicotine biosynthesis and its role in tobacco plant

Nicotine biosynthesis in tobacco plant starts from the prominent components, amino acids - aspartic acid, ornithine and methionine (Leete, 1992; Dewick, 2002). These amino acids together with a glucose degraded compound namely glyceraldehydes (Fig. 4) construct a pyridine and pyrrolidine which eventually combines them under the influence of the plant's jasmonic acid to produce nicotine in the tobacco plant roots with heterocyclic pyridine and pyrrolidine rings (Dewey & Xie, 2013).

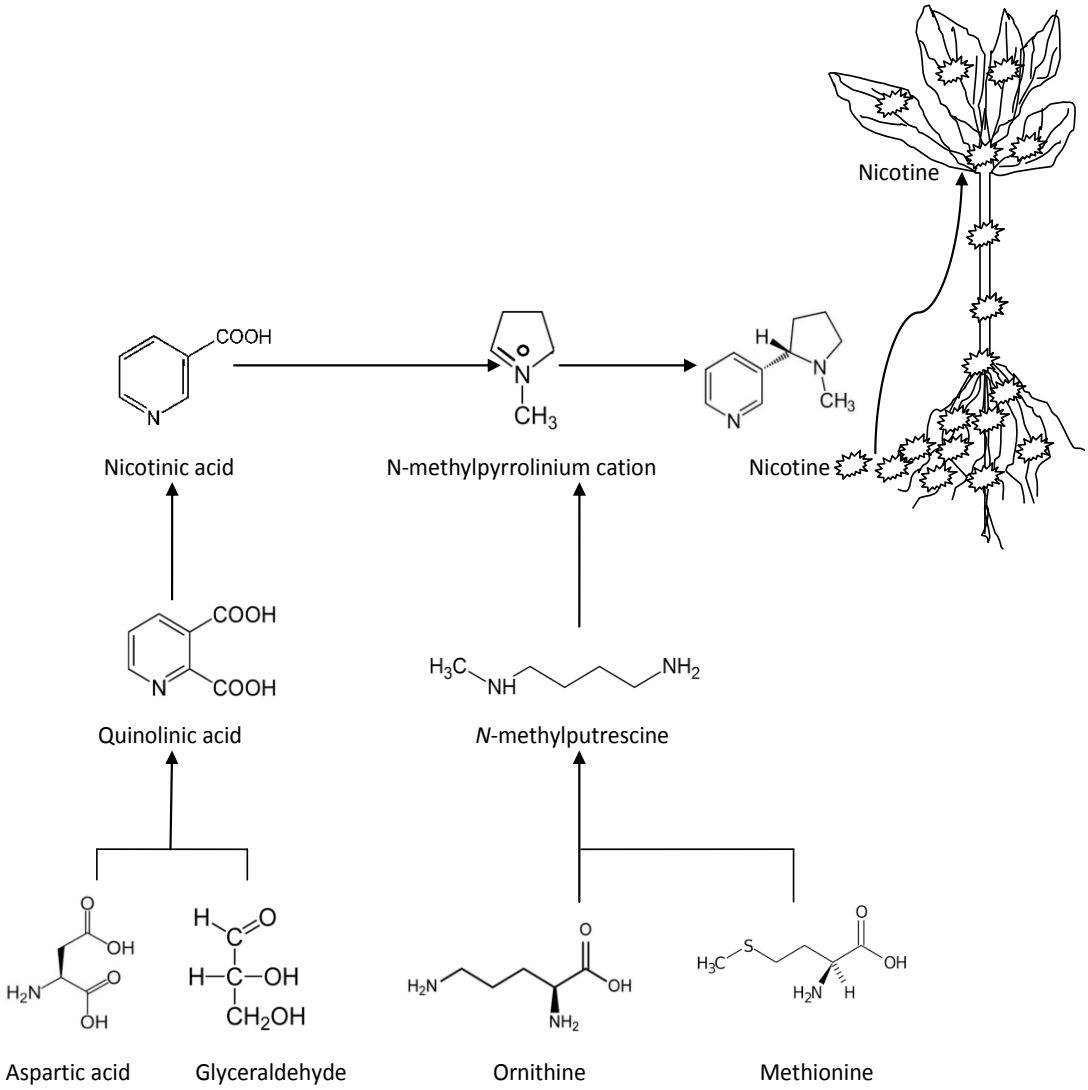


Figure 4: Model on steps for nicotine ($C_{10}H_{14}N_2$) synthesis

Jasmonic acid at the root zone has an influence in regulating expression of gene as well as in stimulating synthesis of the enzymes required for nicotine synthesis (Steppuhn, Gase, Krock, Halitschke & Baldwin, 2004; Katoh, Ohki, Inai & Hashimoto, 2005). The pyridine rings formed as a results of series reactions through ornithine decarboxylase (ODC), *putrescine N-methyltransferase (pmt)* and *N-methylputrescine oxidase (MPO)* that are responsible for nicotine synthesis in tobacco plant by over 95% (Steppuhn *et al*., 2004; Katoh *et al*., 2007; De Boer *et al*., 2011). Regulation of root growth and biosynthesis of nicotine is mediated by nicotine uptake permease 1 (NUP1), localized at the root plasma membranes (Katoh *et al*., 2015). Synthesized nicotine is then transported through xylem from the roots to the leaves, where it accumulates (Shoji *et al*., 2008) in the leaf vacuoles (Shitan, Morita & Yazaki, 2009). Genetically, the contents of nicotine in tobacco plants is thoroughly controlled by two

prominent distinct loci *NICOTINE 1* and *NICOTINE 2* (*NIC1* and *NIC 2*) (Hibi, Hagashiguchi, Hashimoto & Yamada, 1994).

The normal agronomic practice of topping flower parts prior to harvesting of ripened leaves has the desirable increase of leaf mass. However, this practice also has the influence of increasing nicotine in leaves (Xi *et al*., 2005; Shi *et al*., 2006; Wang *et al*., 2008). The increase in nicotine is linked to the lessening of auxins flowing from apex down to the roots where nicotine is synthesized. The effect of removing flower parts also results into an increase in jasmonic acid concentrations to the shoots and leaves within a short period (Shi *et al*., 2006). The produced nicotine has various functions to tobacco plant such as defence against predators and in triggering the formation of linolenic acid and jasmonic acids, the compounds that aid in plant growth processes (Ballaré, 2011).

2.5 Nicotine pathways to the soil environment as allelopathy and allelochemicals

The major nicotine pathway to the soil environment is through root exudation although the decomposing tobacco roots in the soils may also be accounted as the minor pathway Darwent *et al*., 2003). Following these pathways, tobacco plant can be considered to have both allelopathy and allelochemicals to the subsequent crops (Dennis *et al*., 2010; Cheng & Cheng, 2016). Therefore, tobacco plant has allelopathic effects to the subsequent crops because of its nicotine effects produced as a secondary metabolites towards the productivity of other plants and the composition of soil bacteria and fungi in natural communities and agricultural systems (Einhellig, 1995).

On the other hand, tobacco plant produces non-nutritive allelochemicals as secondary metabolites which are also active media of allelopathy. These allelochemicals released by tobacco plants includes amino acids and aspartic acids (Leete, 1992; Dewick, 2002), hydrocarbons, phenols, alkanes, alkynes (Benowitz *et al*., 2009; Talhout *et al*., 2011), flavonoids, alkaloids and isoprenoids (Nugroho & Verpoorte, 2002). All these chemicals could also have effects to the subsequent crops even though they exist in small concentrations compared with the nicotine.

2.6 Nicotine as defence agent against herbivores and soil nutrients competitors

Any wound caused by herbivores on part of the tobacco leaf stimulates synthesis of jasmonic acid, the hormone which is distributed throughout the tobacco plant (Ballaré, 2011). The same hormone is immediately transported through phloem to the roots which is the important

site for nicotine synthesis (Baldwin & Ohnmeiss, 1994; Hibi *et al*., 1994; Zhang & Baldwin, 1997). Jasmonic acid at the root zone is involved in the regulation of gene *pmt* for nicotine synthesis and nicotine is transported via xylem to the leaves where its content doubles in damaged leaf (Fig. 5; Steppuhn *et al*., 2004; Katoh *et al*., 2005; Shoji *et al*., 2008).

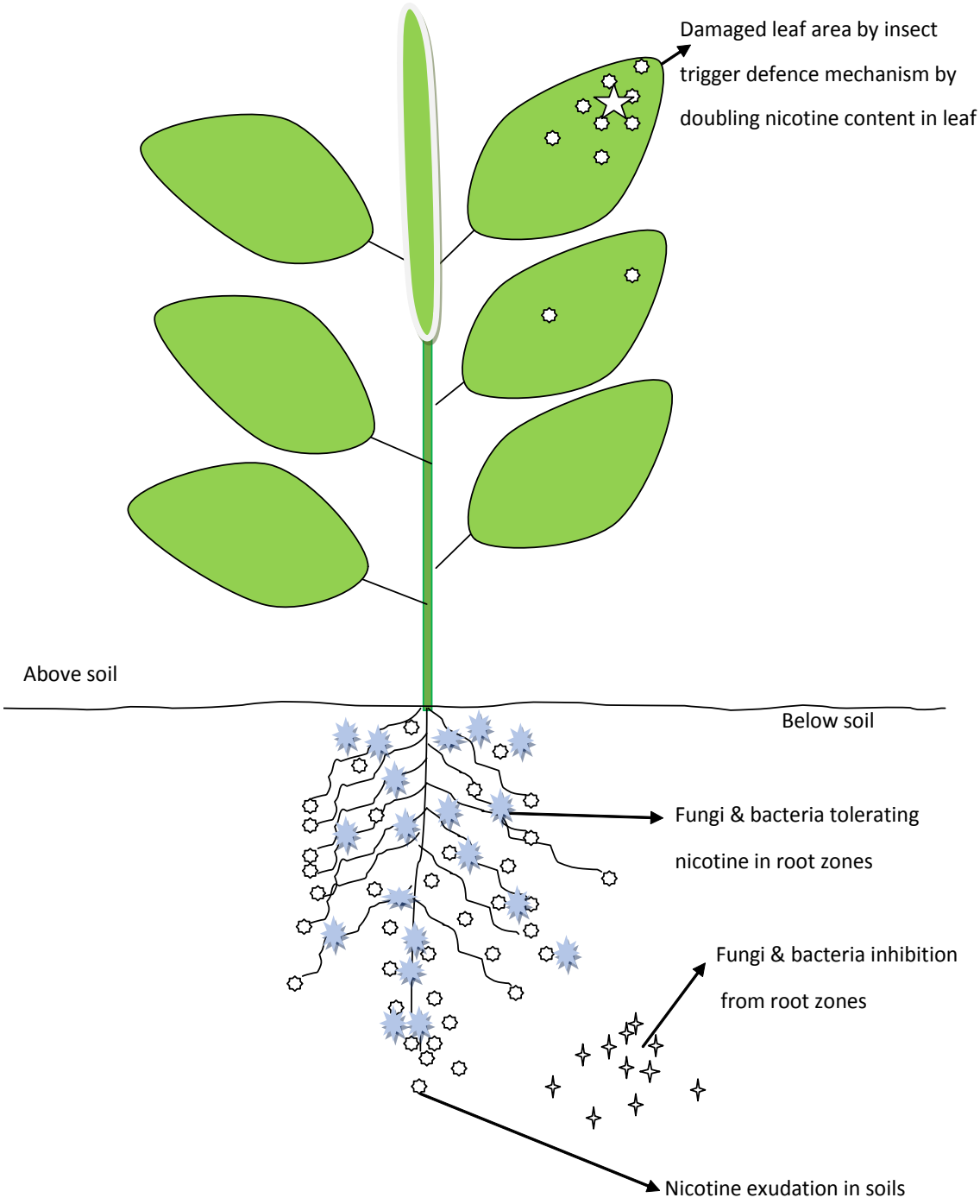


Figure 5: Nicotine acts in defence mechanism against predators and soil bacteria and fungi (Darwent, 2003; Ballaré, 2011; Niu *et al*., 2016)

The same defence characteristic of nicotine also happens in the root zones where it is released to the soil environment through root exudation and residual roots decomposition (Ndakidemi & Dakora, 2003). Nicotine released passively at meristematic root regions to the soil rhizosphere plays a key role in protecting the plant against major groups of soil bacteria and fungi hence reducing competition for soil nutrients which could have been metabolized by these pathogens (Darwent *et al*., 2003; Walker, Bais, Grotewold & Vivanco, 2003; Adediran *et al*., 2004; Niu *et al*., 2016). Based on these scenarios, tobacco seems to be a unique crop probably in the world for its defensive mechanisms against predators, biota above and below the soil surface, respectively (Fig. 5). Evaluating these mechanisms in field conditions where productivity of crops cultivated subsequent to tobacco and the composition characteristics of the soil bacteria/fungi is important under diverse agro-settings.

Regarding the damages caused by the excessive allelopathic effects of tobacco nicotine, other research paths are explored worldwide, such as the use of microorganism normally called Plant Growth Promoting Rhizobacteria (PGPR) (Saharan & Nehra, 2011; Gholami, Biyari, Gholipoor & Rahmani, 2012). PGPR are free-living soil-borne bacteria that colonize the rhizosphere and have great importance in governing the functional property of terrestrial ecosystems and have important role in plant health and soil fertility (Gholami *et al*., 2012). The famous known species of PGPR belong to the genus *Pseudomonas, Azospirillum, Azotobacter, Klebsiella, Enterobacter, Alcaligenes, Arthrobacte, Burkholderia, Bacillus* and *Serratia* (Yazdani, Bahmanyar, Pirdashti & Esmaili, 2009; Saharan & Nehra, 2011). Karnwal (2012) isolated *Pseudomonas, Bacillus, Azospirillum,* and *Azotobacter* and concluded asserted to be useful as crop-enhancer and bio-fertilizer for production of cereals like maize. Gholami *et al*. (2012) screening for PGPR properties showed significant difference between indole-3-acetic acid (IAA) and siderophores production and phosphosolubilization between *Pseudomonas* sp. and *Bacillus* sp. but *Pseudomonas* was a better producer of hydrogen cyanide (HCN) and siderophores. Therefore, understanding of the implications of nicotine as a defence agent against predating herbivores as well as favouring solubility and availability of essential nutrients in soils relative to other crops growing with/after tobacco is inevitably important.

2.7 Nicotine retention to acidic and alkaline soils

Nicotine an alkaloid having two N atoms, one in the pyridine and the second in the pyrrolidine ring released to the soil by the tobacco plant may form salts in acidic soils

(Leffingwell, 2001). However, the formed salts cannot be easily crystallized and are readily soluble in water (Talhout *et al*., 2011) or may be retained in the interlayer and inter-lattice positions of the soils due to its nature of donating electron through aliphatic N of its pyrrolidine ring (Singhal & Singh, 1974; Graton, van Mourik & Price, 2003). In clay soils, adsorption of nicotine at low concentrations is through formation of hydrogen bonds, while at higher nicotine concentrations is through electron-donor-acceptor interactions (Singhal & Singh, 1974; Graton *et al*., 2003).

Under acidic conditions, nicotine is adsorbed strongly through protonation of the pyrrolidine N atom by receiving a H^+ (proton) from carboxylic groups of the humic acid to form nicotine-humic acid salt (Khairy, Baghdadi & Ghabbour, 1990; Golia, Dimirkou & Mitsios, 2007; Xu, Wang & Xiao, 2008). In alkaline soils, nicotine is not strongly adsorbed due to the pair of electrons from pyrrolidine N atom of nicotine being quickly transferred to the humic particles and similarly to the electrons on the pyridine N atom (Khairy *et al*., 1990). Therefore, nicotine is adsorbed more in acidic than alkaline soils and does not require much temperature for its adsorption (Rakić *et al*., 2010).

The nicotine adsorbed in soil colloids has residual effect on growth of plants grown on such soils as well as the survival and proliferation of beneficial soil bacteria and fungi (Adediran *et al*., 2004). Residues of tobacco nicotine in soils also increase the total soluble phenolics, which may have both positive and negative effects to the subsequent crop and the beneficial soil bacteria and fungi (Weidner, Martins, Müller, Simon & Schmitz, 2005; Farooq *et al*., 2014). Many studies have documented the implication of soil reaction (acidity and/or alkalinity) on the adsorption of nicotine by soils. However, similar literature does not critically consider contribution of soil texture and nicotine-organic carbon, macronutrients and micronutrients interactions. There is still a gap of understanding these interactions and the period by which nicotine persists in soil colloids and its associated effects under alkaline and acidic conditions.

2.8 Allelopathic effects of tobacco nicotine on maize growth

Tobacco plant through its roots release nicotine to the soil environment is considered to be beneficial on its survival because it reduces nutrients competition against other plants, soil bacteria and fungi (Darwent *et al*., 2003; Walker *et al*., 2003; Batish, Singh, Kaur, Kohli & Yadav, 2008). Very few research have been conducted to study the allelopathic effects of

tobacco (nicotine) on established and growth of cereal crops in fields (Kruse, Strandberg & Strandberg, 2000; Farooq *et al*., 2014; Baek *et al*., 2017).

Tobacco allelopathy may reduce or increase growth of subsequent crops, because the soil still may contain remnants of nicotine as released to the soil (Wu, Pratley, Lemerle & Haig, 2001). Nicotine has been associated with the increasing chlorophyll content, leaf weight, seedling length and radicle length in cereal crops (Rizvi *et al*., 1989; Rizvi *et al*., 1999; Farooq *et al*., 2014). Other studies indicated that germination of grain legumes such as mug bean, soybean and cereals (red fife wheat) was hindered by the allelopathic chemicals released by the tobacco plants when sown in rotations (Yazdani & Bagheri, 2011; Baek *et al*., 2017). Some studies have shown rotation benefits of tobacco with cereals such as maize and legumes (West & Post, 2002; Reed *et al*., 2012; Li *et al*., 2016; Zou *et al*., 2018). However, majority of studies have indicated beneficial effects of maize growth when rotated with tobacco (Mamolos & Kalburtji, 2001; Yin, Yuan, Wang & Sun, 2009; Farooq *et al*., 2014; Zhou *et al*., 2014; Kim, Mark & Buck, 2017). Table 1 summarizes the allelopathic effects of tobacco nicotine on various components of the ecosystem, including maize crop among others.

Table 1: Overview on the effect of tobacco nicotine allelopathy on different crops, microorganisms and soil properties

Component of ecosystems	Effect of tobacco residues/allelopathy	References
Maize crop	Increase in: stand establishment; leaf emergence; growth; dry matter yields; total N concentrations; chlorophyll content	Rizvi *et al*. (1989), Karaman and Brohi (2013), Farooq *et al*. (2014), Haq, Qadir, Gill, Khaskheli and Lanjar (2018)
Rice crop	Protection against snail problem	Shakeel (2014)
Wheat crop	Increase in N; decrease in germination rate	Shakeel (2014), Baek *et al*. (2017)
Vegetables	Increase in N	Shakeel (2014)
Cowpea crop	Improvement in growth and yield	Agrawal, Rathore and Singh (2006)
Mungbean	Reduction in: emergence uniformity, seedling dry weight and chlorophyll contents	Farooq *et al*. (2014)
Microbial population in soils	Unfit for the insects breeding; reduce in the ants *Lasius niger* nest in the gardens; affects survival and/or proliferation of poor biodegradable microbes; may promote growth of plants' mutualistic fungi	Dakora and Phillips (2002), Lind *et al*. (2006), Shakeel (2014)
Soil	Improvement in: organic matter; electrical conductivity; water intake and its holding capacity; increase in N, Mg, Zn, Fe, nicotine, and total phenolics; increase in soil pH; total salts stability	Aggelides and Londra (2000), Bulluck, Brosius, Evanylo and Ristiano, (2002), Agrawal *et al*. (2006), Candemir, Dide, Yilmaz and Gulser (2012), Cercioglu, Okur, Delibacak and Ongum (2012), Farooq *et al*. (2014)

Nicotine released into the rhizosphere in sandy loam soils have been attributed to the substantial increase in growth rate, chlorophyll, number of leaves, plant height and dry matter yields in subsequent cereals (Farooq *et al*., 2014). However, in silty loam soils, Yazdani (2014) indicated that nicotine allelopathy on maize decreased seedlings emergency rate, seedling weight, vigour and chlorophyll content. Allelopathic effects of tobacco on growth of cereals are generally positive but there are some few cases of negativity. Allelopathic effects may differ with soil types and/or with varieties of crops used (Farooq *et al*., 2014; Yazdani, 2014). Studies about effects of tobacco nicotine on the subsequent crops under different soil types are limited. This prompts a need for execution of further studies in order to address effects of tobacco nicotine released into soils to such cropping systems.

Tobacco nicotine has strong allelopathic growth beneficial effects to the subsequent cereals compared with other crops such as grain legumes (Fig. 6; Farooq *et al*., 2014). This could be due to genetic variability between cereals and legumes and higher susceptibility of legumes to certain disorder in response to nicotine exposure. These genetic variability benefits for cereal crops such as maize could also be associated with increased uptake of total N, Fe, Zn and Ca (Lopez-Lefebre *et al*., 2001; Lopez-Lefebre *et al*., 2002; Farooq *et al*., 2014; Zou *et al*., 2018). The increased total N in soils could be due to the suppressing effect of nicotine on soil bacteria such as nitrosomonas, nitrococcus and nitrobacter involved in converting ammonia into nitrate (usable form by plants) and hence increase total N in soils (Farooq *et al*., 2014). This process also contributes to the minimization of soil N losses (Jabran, Farooq, Aziz & Siddique, 2012), that could be beneficial for both cereal and legume crops. Minimization of N loss in the soil causes an increase in total N, which has a great influence on boosting growth of maize crop.

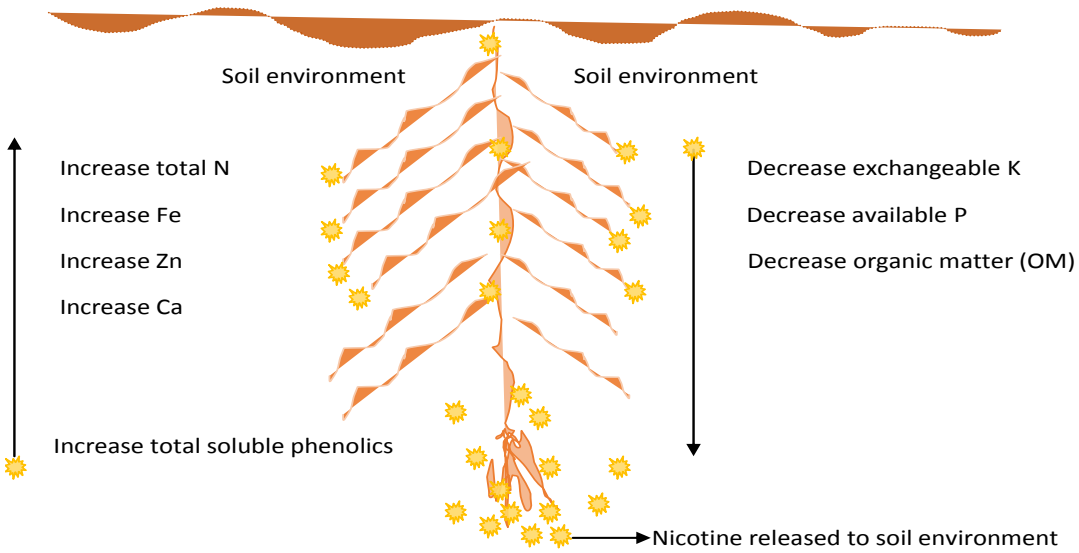


Figure 6: Effects of nicotine ($C_{10}H_{14}N_2$) released to the soil nutrients and total soluble phenolics

Nevertheless, other studies have revealed that nicotine allelopathy on its genetic nature influenced the tobacco plant. This effect is recognized when tobacco takes up more exchangeable K and available P. This situation leads into decrease in concentrations of P and K nutrients in soils (Xu, Wang & Xiao, 2008; Farooq *et al*., 2014; Moula, Hossain, Farazi, Ali & Mamun, 2018). Deficiencies of K and P in soils are inevitably likely to negatively affect performance of the subsequent cereal crops (Aziz *et al*., 2010; Annes *et al*., 2016; Pavuluri, Malley, Mzimbiri, Lewis & Meakin, 2017; Yue *et al*., 2018). Nutrient K plays a

positive role on transfer of N, starch, sugar, fat and protein synthesis (Rostami, 1997). On the other hand, P is responsible in growth of dense roots for nutrients absorption, seed and/or fruit formation and stem strength (Zhu & Lynch, 2004).

Very few researches on the effects of tobacco nicotine on soil nutrients have been conducted. Therefore, further research is required to study if cereals/legumes used as subsequent crops have abilities of taking up nicotine from the soils and trace associated effects to these crops.

2.9 Allelopathy effects of tobacco nicotine on soil bacteria and fungi

Plants deposit their photosynthetically fixed carbon into their direct surroundings such as spermosphere, phyllosphere, rhizosphere, and mycorrhizosphere while feeding the microbial community and influencing their composition and activities (Mendes, Garbeva & Raaijmakers, 2013). Some fungi and bacteria in soils cause a range of plant diseases and in some cases to devastate agricultural crops while others provide resistance to plant pathogens (Marschner, Crowley & Lieberei, 2001). The same soil organisms decompose plant residues, provide nutrients to plants, and stimulate plant growth (Jarosz & Davelos, 1995; Marschner *et al*., 2001; Smalla *et al*., 2001). Knowledge of the diversity and structure of bacterial and fungal communities in bulk and rhizosphere soils can lead to a better understanding of their roles in soil ecosystems. The rhizospheres of young maize plants are associated with Ascomycetes order Pleosporales, while different members of the Ascomycetes and basidiomycetic fungi are detected in the rhizospheres of senescent maize plants (Gomes *et al*., 2003). Maize growth stages influence density, diversity and community structure of some bacterial and fungal groups present in its rhizosphere (Cavaglieri, Orlandoa & Etcheverry, 2009).

Regardless of their composition in soils, other factors such as allelopathy may have significant effect on the bacteria population. Tobacco nicotine allelopathy has a depressing effect on composition of soil bacteria, fungi and their activities (Adediran *et al*., 2004). The population of soil bacteria and fungi decreased significantly when tobacco were planted continuously compared with when it was rotated with maize (Niu *et al*., 2016). This suggests that the decrease in soil bacteria and fungi could be due to the nicotine released to the soil environment by the tobacco plant roots.

Despite suppression effect of nicotine on soil bacteria and fungi population, still there are few bacteria in soils such as *Pseudomonas* which have great ability to withstand nicotine toxicity

(Table 2). These bacteria withstand high nicotine levels under the pH levels ranging from 6.5-7.0 (Wang *et al*., 2012). *Pseudomonas* (gram-negative) strain CS3, Nic22, ZUTSKD were found to tolerate high nicotine concentration up to 5 g L^{-1} in soil with high efficacy (over 85.4%) in degrading nicotine in soil at 30 - 34^{o} C and pH range of 6.0 - 7.0 (Chen, Li, Yang, Gong, Li & Zhang, 2008; Zhong *et al*., 2010; Wang *et al*., 2012). Strain HF-1 identified from the genus *Pseudomonas* (gram-negative) was found to have higher efficacy of nicotine-degrading by 99.6% at the soil pH range of 6.5-7.5 (Ruan *et al*., 2005).

At the soil pH of 4-10, gram-negative bacteria genera *Acinetobacter* sp. TW and *Sphingomonas* sp. TY were observed to have greater efficacy to degrade 1 g L^{-1} of nicotine by 94.7% and 98.7% within 12 – 18 hours, respectively at temperatures ranges of 15 - 45^{o} C (Wang *et al*., 2011). However, strain TW was found to have greater tolerance of high nicotine of up to 4.44 g L^{-1}. The strain S33 which was classified as *Agrobacterium tumefaciens* is among of the few bacteria identified to have higher tolerance ranges of nicotine (0.5 – 5 gL^{-1}) with 98.87% efficacy of degrading nicotine, but at only pH level of 7.0 and temperature of 30^{o}C (Wang, Liu & Xu, 2009). The only Gram-positive bacteria *Arthrobacter* sp. HF-2 was observed to have maximum degradation of soil nicotine by 100% at level of pH 7.0 and temperature of 30^{o} C but with very low nicotine tolerance level of up to 0.7 g L^{-1} (Ruan, Min & Zhu, 2006). Therefore, gram-positive bacteria seem to have very low tolerance degree to nicotine in soils than gram-negative bacteria.

Table 2: Selected nicotine-degrading microorganisms

Microorganism	Time (h or d)	Required conditions (pH & Temp)	Nicotine degradation efficacy (%)	Reference
Gram-negative bacteria				
Acinetobacter sp. TW	12 h	7.0 & 30°C	94.70	Wang *et al*. (2011)
Ochrobactrum sp. 4-40	12 h	7.0 & 28°C	51.50	Ma *et al*. (2012)
Pseudoxanthomonas sp. 5-52	12 h	7.0 & 28°C	47.20	Ma *et al*. (2012)
Sinorhizobium sp. 5-28	12 h	7.0 & 28°C	72.50	Ma *et al*. (2012)
Agrobacterium tumefaciens S33	18 h	7.0 & 30°C	98.87	Wang, Liu and Xu (2009)
Sphingomonas sp. TY	18 h	7.0 & 30°C	98.70	Wang *et al*. (2011)
Pseudomonas sp. CS3	24 h	7.0 & 30°C	98.6	Wang *et al*. (2012)
Pseudomonas sp. HF-1	25 h	6.5-7.5 & 30°C	99.6	Ruan, Min, Peng and Huang (2005)
Ochrobactrumintermedium DN2	36 h	7.0 & 30°C	97.60	Yuan *et al*. (2006)
Fungi				
*Aspergillus oryzae*112822	40 h	6.5 & 28°C	60.80	Meng, Lu, Gu and Xiao (2010)
Gram-positive bacteria				
Arthrobacter sp. HF-2	48 h	7.0 & 30°C	94.20	Ruan *et al*. (2006)
Gram-negative bacteria				
Pseudomonas sp. Nic22	48 h	6.5&30-34°C	96.50	Chen *et al*. (2008)
Rhodococcus sp. Y22	52 h	7.0 & 28°C	96.00	Gong *et al*. (2009)
Fungi				
Cunninghamella echinulata IFO-4444	13 d	5.5 & 28°C	72.00	Uchida, Maeda and Kisaki (1983)
Pellicularia filamentosa JTS-208	20 d	5.5 & 28°C	09.00	Uchida *et al*. (1983)

Fungi groups generally have low efficacy in degrading nicotine compared with the bacteria. For instance, Basidiomycetes and Saprophytes such as *Pellicularia filamentosa* JTS-208 and *Cunningham ellaechinulata* IFO-4444, respectively, have less abilities to degrade (S)-nicotine (Uchida *et al*., 1983). *Pellicularia filamentosa* was observed to degrade nicotine by 9% into nornicotine in 20 days, whereby *C. echinulata* degrade nicotine by 72% into nornicotine and N-methylmyosmine within 13 days (Uchida *et al*., 1983). Fungus *Aspergillus oryzae* designated as strain 112822 was observed to bio-degrade nicotine by 60.8% in 40 h at pH level of 6.5 and temperature of 28° C (Meng *et al*., 2010). In general, fungi are considered to have poor abilities in tolerating and degrading high nicotine levels in the ecosystems.

In summary, the most of the isolated bacteria that degrade-nicotine have been largely explored in China and partly in India. Studies on nicotine degrading bacteria are limited in most of countries producing tobacco. Majority of tobacco producing countries need also to engage more on research pertaining to the isolation of nicotine degrading bacteria and fungi in soils since share of tobacco produced increased from 57% in 1961 to 90% in the year 2006 (Geist, Chang, Etges & Abdallah, 2009). The strains tolerating high efficacy levels of nicotine in soils with good abilities of degrading nicotine can eventually be used for bioremediation of nicotine contaminated soils among the main tobacco production and industrial areas.

2.10 Management options for residual effects of nicotine in tobacco production areas

The protection mechanisms possessed by tobacco plants through its nicotine against predators and in gaining competitive advantage on nutrients over other plants and/or microorganisms retain a good trait for tobacco survival (Ballaré, 2011). Nicotine synthesized in tobacco plant also potentially threatens the performance of subsequent crops by inhibiting the rhizospheric acquisition as well as uptake of some macronutrients such as exchangeable K and available P (Yue *et al*., 2018). With this in mind, tobacco crop may be grown in rotation with screened plants/crops that have abilities to withstand the residual effects of nicotine or are able to restore soil fertility such as sunhemp (*Crotalaria juncea* L.) plant (Márton, 2010). Sunn hemp is a fiber inedible leguminous crop characterized by low N requirements due to its ability to fix atmospheric N, grows in marginal soils, drought resistance and resistance to root-knot nematodes (Cook & White, 1996). The fastest growing species of the *C. juncea* plants may be used also as part of a cropping system for integrated pest management (Tavares *et al*., 2011).

Crotalaria plant may be used to intercede the tobacco's and food crops' main seasons and it is expected to create conducive environments for the subsequent food crop in the same piece of land after tobacco plant has been harvested. A staple cereal crop such as maize can then be grown in the next main season in order take advantage of replenished soil fertility, probably with also abundance of beneficial soil bacteria. However, in situations where land is scarce, there is also a need of ensuring that the supply of macronutrients such as K and P does not confront the growth and/or productivity of subsequent food crops. Optimization and sustainable productivity of cereal crops and improvement of food security in tobacco producing areas could be met by continuously use of nutrients K and P among other essential nutrients as well as maintaining optimal levels of other soil properties.

The mechanisms in creating competitive advantage of tobacco plant against soil bacteria and fungi for nutrients in soils have also remained poorly understood. Therefore, use of both molecular/genetic approaches and ecological/environmental techniques such as allelopathy may be important in evaluating the most appropriate options in management of nicotine discharged and adsorbed into soils in tobacco producing areas. This aims at optimizing growth and productivity of food crops cultivated in rotations with tobacco but along with enhancing diversity of soil bacteria and fungi.

2.11 Conclusion

This review demonstrated that, tobacco is a unique crop for its defensive mechanisms against predators, bacteria and fungi above and below the soil ground, respectively. Tobacco nicotine allelopathy favours growth of subsequent food cereal crops such as maize as it enhances availability of essential nutrients such as total N, Ca, Fe and Zn in soils. However, the same nicotine decreases availability of K and P, which may have adverse effects on the overall growth and productivity of subsequent crops if these nutrients are not supplemented in soils. Therefore, in future there is a need for extending research on allelopathic effects of tobacco towards productivity of cereals standing crop such as maize. Tobacco nicotine allelopathy also decreases significantly the population of bacteria and fungi in soils when tobacco is continuously cultivated instead of being rotated with crops of different species such as food cereal crops. In addition, our suggestion is that inedible leguminous plants such as *Crotalaria* may be planted in same field immediately after tobacco harvest. In this way, the subsequent food cereal like maize will benefit from the replenished soil fertility and improved structure as well as the restored abundance of beneficial bacteria.

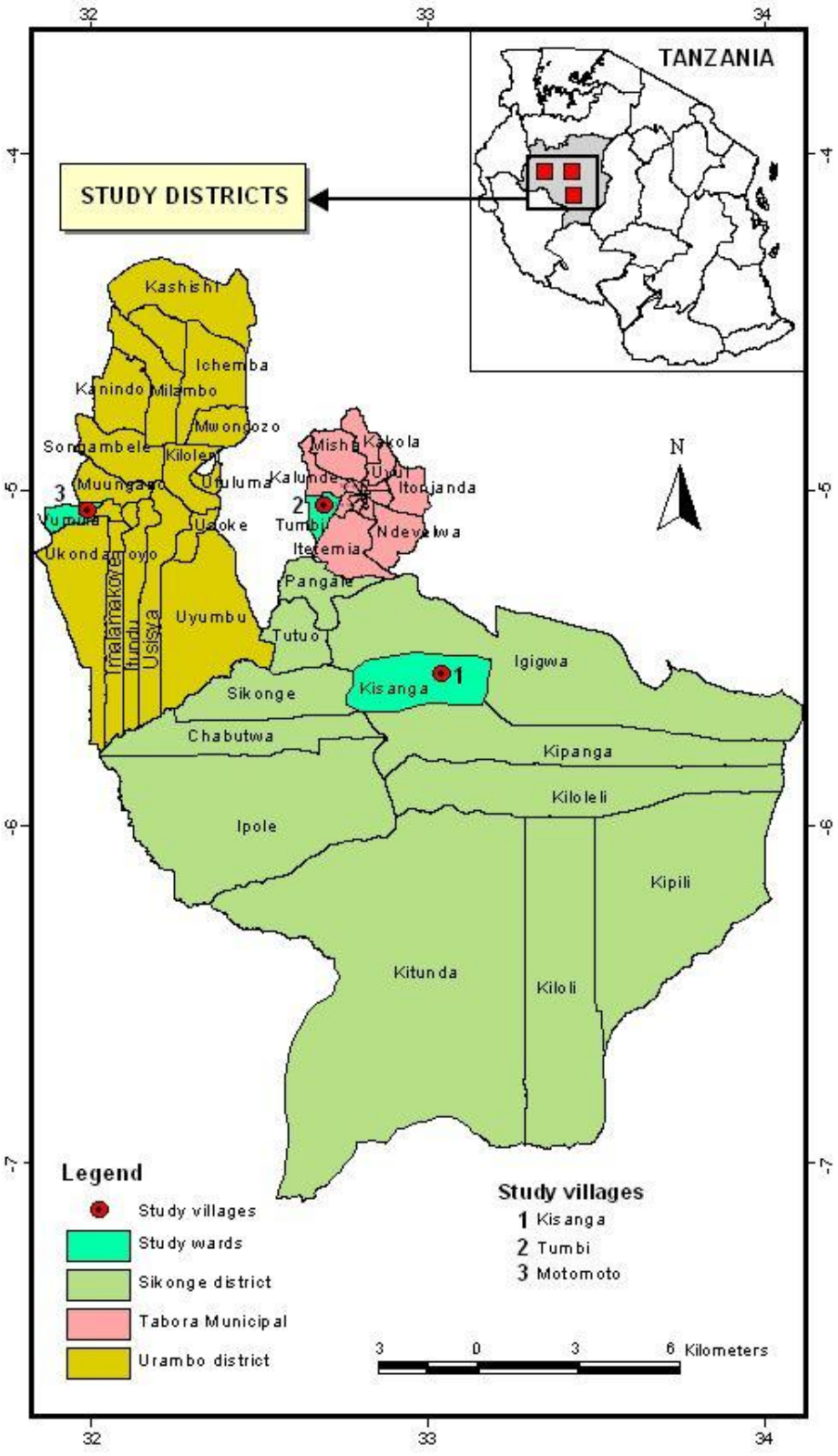
CHAPTER THREE

MATERIALS AND METHODS

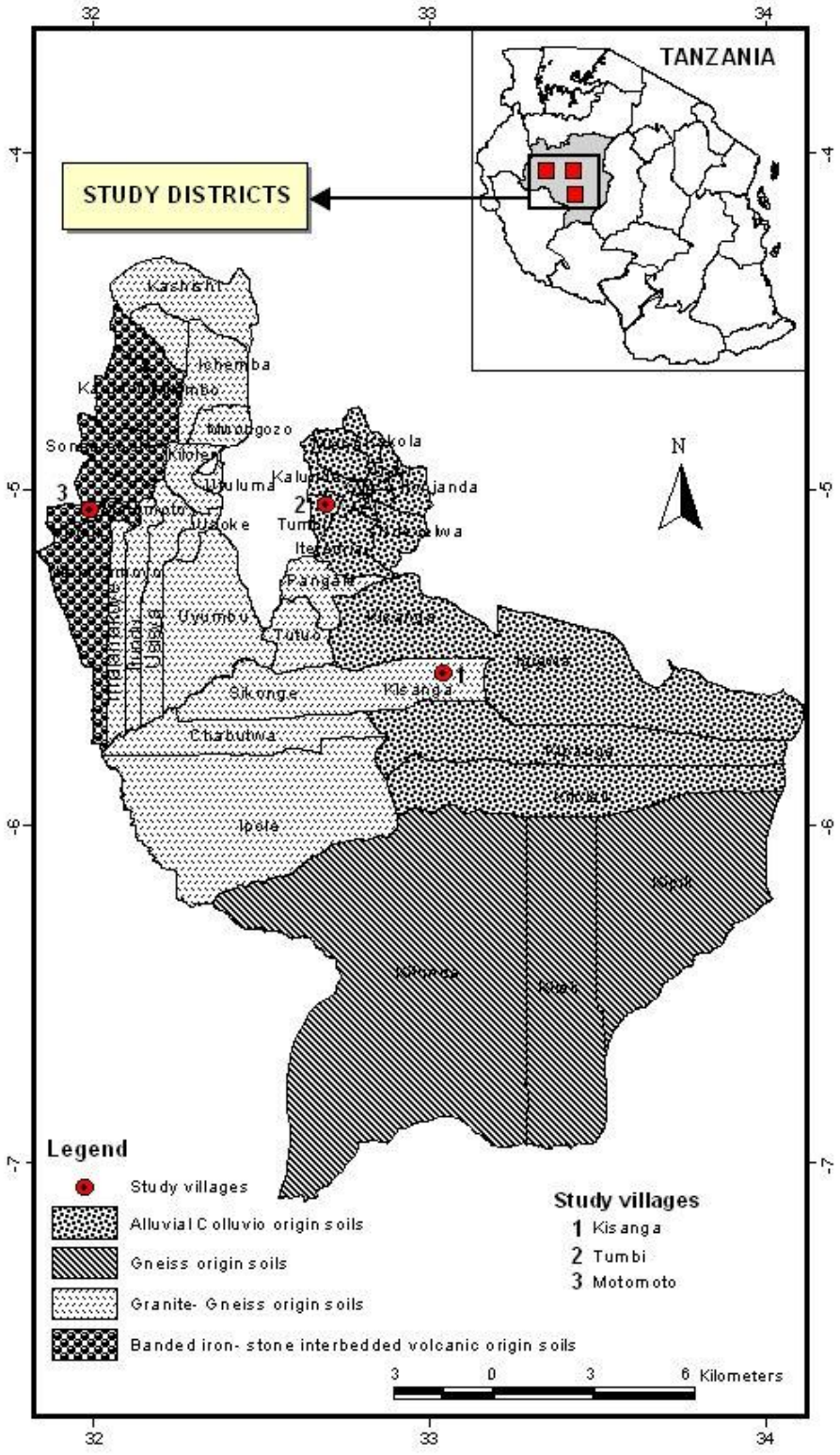
3.1 Description of the study area

Experiments were conducted from 2017/18 to 2018/19 cropping seasons in three sites namely Sikonge, Tabora, and Urambo, all in Tabora region (Map 1). The Sikonge site was located at S 05^0 31' 47.4'' and E 032^0 50' 03.2'' at an elevation of 1191 m.a.s.l. with annual mean rainfall and air temperature of 1050 mm and 29 °C, respectively. Tabora site is located at S 05^0 03' 44.4'' and E 032^0 40' 07.4'' at an elevation of 1160 m.a.s.l. with annual mean rainfall and air temperature of 950 mm and 27 °C, respectively. Urambo site is located at S 05^0 04' 33.5'' and E 032^0 00' 09.8'' at an elevation of 1108 m.a.s.l. with annual mean rainfall and air temperature of 890 mm and 25 °C, respectively.

The sites were characterized by unreliable transitional bimodal rainfall pattern with short and long rain seasons. The mean annual rainfall for the five years ranged between 850 mm and 1060 mm. Soils original rocks for the study sites are shown in Map 2. The soil textures for Sikonge was categorized as loamy sand soil. The soils developed from granite-gneiss origin soils. Tabora soil texture was sandy soils which were formed due to deposition of sediments brought by rivers or floods that consisted of largest contents of sand and small portions of silt and clay. Urambo site had sandy loam soil, originally developed from banded iron-stone interbedded volcanic origin soils.



Map 1: Field trial sites: Sikonge (Kisanga), Tabora (Tumbi) and Urambo (Vumilia)



Map 2: Soils original rocks from the study sites of Sikonge (Kisanga), Tabora (Tumbi) and Urambo (Vumilia)

3.1.1 Screen house experiments

Screen house experiment was carried out at the Nelson Mandela African Institution of Science and Technology (NM-AIST) Arusha, Tanzania at the screen house located at S 03^0 23' 56.6'' E 036^0 47' 40.2''. Screen house pot experiment involved testing of soils collected from Tabora only and tobacco roots and leave extracts, each at 5 levels (0, 25, 50 75 and 100%). The experiment was arranged using a Completely Randomized Design (CRD) and replicated three times.

Materials used for the research collected from Tobacco Research Institute of Tanzania (TORITA) includes seed varieties *Nicotiana tabacum* (K326), *Zea mays* (DKC8053); Fertilizers for tobacco $N_{10}P_{18}K_{24}$ and CAN 27%; fertilizers for maize Yaramila cereal. Other materials/equipment's include, measuring tape, hoe, spade, field knife, sample bags, labels, marker pens, buckets, sisal twigs, stationary, soil auger, soil moisture meter, soil thermometer, digital caliper, Global Positioning System (GPS) and a digital camera.

The screen house pot experiments tested two exracts (roots and leaves). This was arranged using a Completely Randomized Design (CRD). The treatments were replicated three times. Each plant extract (roots and leaves) had five concentrations of 0, 25, 50, 75 and 100%. Ten kilogram soils sieved through a 6 mm sieve were weighed into ten-litre plastic pots. The treatment combinations were assigned as shown in Table 3.

Table 3: Treatments allocation to the NM-AIST screen house experiments

Crop	Tobacco root extract concentration (%)	Crop	Tobacco leaf extract concentration (%)
Z. mays	0 NT	*Z. mays*	0 NT
Z. mays	25 NT	*Z. mays*	25 NT
Z. mays	50 NT	*Z. mays*	50 NT
Z. mays	75 NT	*Z. mays*	75 NT
Z. mays	100 NT	*Z. mays*	100 NT

KEY: NT = *Nicotiana tabacum*

3.1.2 Field experiments

A total of fifteen soil samples from depth of 0-20 cm were collected from each site of the three locations to make a composite for each site. Samples were analysed for pH (in water 1:2.5), particle size determination (PSD), OC, Total N, extractable P, K, Ca, Na, Mg, B, Cu, Zn, Mn, Fe, CEC and nicotine.

A randomised complete block design (RCBD) was used in this fallow experiment. The experiment consisted of three replications each with 6 treatments alternating with crops for year 1 and 2 as shown in Table 4. The dimensions of each plot were 6.0 m x 6.0 m in the fallow field, with interblock and interplot spacing of 2 m and 1 m, respectively. A 2 m pathway was maintained around the entire experimental area. The K 326 tobacco seedlings were transplanted at a spacing of 1.2 m (ridge to ridge) and 0.50 m from plant to plant. Maize seeds were sown using similar spacing as used in tobacco in order to study the effects of nicotine on maize.

Table 4: Treatments allocation to the field trials

Treatment no.	1st year treatments	2nd year treatments
1.	*N. tabacum*	*Z. mays*
2.	*Z. mays*	*Z. mays*
3.	*N. tabacum* (SI)	*Z. mays*
4.	*N. tabacum* (no fertilizer)	*Z. mays* (no fertilizer)
5.	*Z. mays* (no fertilizer)	*Z. mays* (no fertilizer)
6.	Absolute Control	Absolute Control

Key: SI = Stalks incorporated in soils after harvesting in order to gauge its effects

3.2 Methods

3.2.1 Screen house experiments

Tobacco leaves and roots were taken from K326 variety planted in previous season at Tumbi, Tabora. The leaves and roots were washed using distilled water to eliminate unwanted particles and air dried for two weeks under room temperature. After two weeks of drying leaves and roots separately, they were then grinded into powder form. Grounded portion, 20 g each from leaves and roots were collected for analyzing selected nutrients N, P, K, Ca, Mn and Zn as per proceedures given.

About 200 g of each ground powder of leaves and roots for each crop were soaked in distilled water separately in a closed container of 2 L for three days. After three days of soaking, the extracts were filtered through Whatman filter paper No. 1 separately. The extracts were diluted using distilled water to make the percentage concentrations of 0 (100 mL of distilled water); 25 (25 mL of extract + 75 mL of distilled water); 50 (50 mL of extract + 50 mL of distilled water); 75 (75 mL of extract + 25 mL of distilled water) and 100 (100 mL of

extract). Six maize seeds were soaked to each concentrations levels of leaf and root extracts for 8 h before sowing in pots.

In maize pots, first split of N fertilizer plus P and K fertilizers were mixed thoroughly with the ten kg of soil before filling the pots and seeding with maize. Two seeds per pot were sown using hybrid variety DKC 8053 and thinned to one seedling ten days after seedling emergence. About 100 mls of extracts of tobacco leaf leaf and root extracts were drenched to each concentration levels for three days prior application of fertilizer. The rates of NPK applied in maize pots were 240 mg N kg^{-1}, 100 mg P kg^{-1} and 100 mg K kg^{-1} to make sure that these nutrients did not limit plant growth. Before sowing maize seeds in the pots, all pots were watered using 2 975 mL distilled water per pot, equivalent to 90% of the field capacity, and allowed to equilibrate for one day. Potted soils throughout the experiment were maintained at approximately field capacity by watering using distilled water.

Three weeks after sowing maize, the second N split dose was applied at the rate of 120 mg N kg^{-1} to each maize pot. Maize plant height and stem thickness were measured using tape measure and digital caliper respectively 42 days after planting. Plant shoots for maize were harvested at 42 days after planting. Stems were cut to about 1 cm above the soil roots zone and weighed. The plants shoots were dried at 65 °C to a constant weight followed by determining dry matter yields. Nutrient uptake values were calculated by multiplying concentrations values by its dry matter yield. The plant samples were ground to pass through 0.5 mm sieve for mineral analysis (N, P, K, Ca, Mn and Zn). Soil samples were taken from each pot for determination of soil pH, nicotine, and some indicative nutrients residuals levels such as N, P, K, Ca, Mg, K and Cu after harvesting plant shoots using the procedures outlined in section 3.3.

3.2.2 Field experiments

Seedbed nursery was established for each of the three sites of Tabora, Urambo and Sikonge. To each nursery, 3 g of tobacco seeds K326 variety were sown in a standard tobacco seedbed of 1.5 m width and 20 m length with a boost of 5 kg of $N_{10}P_{18}K_{24}$. The seedlings were taken care of for a period of one month followed by resetting seedlings to another seedbed of similar size. Seedlings were well managed for another thirty (30) days with confidor and deltamethrine for controlling pests and diseases. Seedlings were clipped and hardened off just for two weeks prior transplanting in experimental fields.

To all three sites for each year, planting day was the same for both tobacco seedlings (in the first year) and maize seeds (in the second year). For tobacco, K326 variety which is commonly used to all tobacco growing areas was used whereby mature and healthy seedlings raised from the nursery were selected and transplanted in fields (one seedling per plant hill). Basal NPK fertilizer for tobacco crop was manually applied as per recommended rates of 50 kg N ha^{-1}, 90 kg P ha^{-1} and 120 kg K ha^{-1} seven days after transplanting seedlings in field. Two weeks after NPK basal application, 33.75 kg N ha^{-1} of CAN (27%) per seedling was manually applied as per recommended practice.

The maize variety used was DKC 8053 which is commonly preferred by majority growers. Two maize seeds were sown and then thinned to one plant per hill two weeks after seedling emergence. Fertilizer for maize crop was manually applied as per recommended of 120 kg N ha^{-1}, 50 kg P ha^{-1} and 50 kg K ha^{-1} in three splits after seedling emergence (5-10 cm), at knee height (40-45 cm) and two weeks prior flowering. Frequent weeding was done so that the experimental plots were almost free of weeds for most of the plant growth period.

Tobacco plant leaf sampling was done at 15 weeks after transplanting in the field. One matured middle leaf each from 3 plants per row from each inner 3 rows out of 5 rows, giving a total of 9 leaves per plot, were sampled. These tobacco plant leaves were sampled when almost 90% of tobacco plants had been topped. Maize plant leaf sampling was done at 15 weeks after sowing by taking three ear leaves per row from each of inner 3 rows out of 5 rows, giving a total of 9 leaves per plot. These plants were sampled when almost 90% of maize plants had tasselled. Maize and tobacco leaf samples were separately oven dried at 65°C to constant weight and cut to small pieces and ground to pass through 0.5 mm sieve. Determination of nicotine ($C_{10}H_{14}N_2$), K, P, N, S, Ca, Mg, Mn, Fe, B, Cu and Zn in the plant materials was done using the procedures outlined in section 3.4.

Maize grain was harvested at 120 days after planting. A guard row was left around each plot so that only the inner 3 rows were harvested. Cobs were dried to 12.5% moisture, shelled, grain per cob counted and weighed. The grain yields were reported in tonnes ha^{-1} at 12.5% moisture content. About 0.25 kg of dry maize grain from each plot was ground to pass through 0.5 mm sieve for determination of nicotine as per methods outlined in section 3.3.10. Soil samples were also collected for the determination of N, P, K, Ca, S, Mg, Fe, B, Cu and Zn as per methods outlined in section 3.3.

3.3 Analysis of chemical-physical properties of soils and released nicotine in the soil

3.3.1 Total N

Total N was determined by the Kjedahl method (Bremner & Mulvaney, 1982). One gram (1 g) of soil was introduced in digestion tube followed by 10 ml of 98% H_2SO_4, scoop of mixed catalyst having 100 g K_2SO_4, 10 g CU_2SO_4 and 1.55 g selenium powder. The mixture of these ingredients was digested in a digestion block at 360°C for 1 hour. The digest was distilled after adding 25 mL of 40% NaOH, then distillate collected over 4% boric acid, followed by titration with 0.05 N H_2SO_4. The titre value was used to compute total N.

$$N\% = \frac{\text{mls } H_2SO_4 \text{ x Normality of acid x } 0.014 \text{ x } 100}{\text{Oven dry weight}}$$

3.3.2 Extractable P

Extractable P was determined using Bray 1 method (Moberg, 2001). Five grams (5 g) of soil was introduced into 50 mL plastic bottle followed by 25 mL of extraction solution. The mixture was shaken manually (by hand) for 1 min and then filtered. About 5 mL of the filtrate was transferred into a 50 mL volumetric flask. Distilled water (30 mL) was added preceded by 10 mL of phosphor-molybdate reagent. The mixture in the volumetric flask was then filled to the mark using distilled water. The mixture was allowed to settle for about 30 mins purposely for colour development. The absorbance was measured by spectrophotometer at 884 nm wavelength.

3.3.3 Cation Exchange Capacity (CEC) and Exchangeable Bases (EB)

Cation exchange capacity (CEC) and EB in soils were determined by ammonium acetate saturation at pH 7.0. About 5 g of soil was introduced in 100 mL plastic bottle, followed by 35 mL of 1 M ammonium acetate buffered at pH 7 (Moberg, 2001). The mixture was shaken for 30 mins and finally left to settle over night. The suspension was then filtered into 100 mL volumetric flask ready for determination of exchangeable K, Ca, Mg and Na. The exchangeable Ca and Mg was determined using atomic absorption spectrophotometer while exchangeable K and Na were determined through the use of flame photometer.

Remnant of soil was washed using 80% ethanol and leached with 1 M KCL and filled into 100 mL volumetric flask. The leachate was then transferred into a Kjeldtex distillation tube, whereby 10 mL of 40% NaOH was added. The distillate was collected over 4% of boric acid

and eventually titred using 0.1 N H_2SO_4. The titre value obtained from titration, was used to calculate CEC.

$$CEC = \frac{\text{mls } H_2SO_4 \text{ x NOA x } 100}{\text{ODW (g)}}$$

Where: CEC = Cation exchange capacity; NOA = Normality of acid; ODW = Oven dry weight (g)

3.3.4 Extractable B, Cu, Fe, Mn and Zn

Boron (B) was determined using boron in non-ashed extracts through digestion at 150^{o}C. About 7.50 g soil was placed into digestion tubes in the digestion rack except two tubes served as blanks. About 15 mL of 0.01 M $CaCl_2$ was added to each tube including the blank, then tubes were placed in the heater digester for boiling at 150^{o}C. After boiling, the temperature was reduced to 110^{o}C and boiled for about 5 mins and the digester turned off and the tubes were cooled in cold water for 15 mins. The suspensions were filtered into dry plastic bottles. About 2 mL of the soil filtrate was transferred into another dry plastic bottle, 4 mL of buffer soulution was added and mixed. Then after 4 mL of aromethine-H reagent was added and mixed and settled for 30 mins. After 30 mins, the samples were measured through absorbance at 420 nm on a colour spectrophotometer. Then calculation was done by using a formular as follows:

{Reading-Blank} x 10 = mg B kg^{-1}.

Moberg (2001) method was used for the determination of extractable Cu, Fe, Mn and Zn. About 15 g of soil was placed in 100 mL plastic bottles, followed by 40 mL of Diethylene Triamine Pentaacetic Acid (DTPA) extractant. The mixture was shaken for 2 h in a shaker and then after filtered into 50 mL plastic bottles. Then finally the filtrate was used to determine Cu, Fe, Mn and Zn at respective wavelengths using atomic spectrophotometer.

3.3.5 Extractable S

About 5 g of soil was weighed on analytical balance and placed into 100 mL plastic bottle followed by addition of 25 mL of sulfur (S) extraction solution. The mixtures were shaken for 30 mins and filtered into a dry 100 mL flask. About 10 mL of the soil extract were pipetted into a 50 mL bottle, followed by addition of 10 mL acid solution and 5 mL turbidimetric reagent and mixed thoroughly for 20 mins. The absorbance of the mixtures and standard

solutions were measured through the spectrophotometer at 535 nm using normal cells. Results were presented in mg S kg^{-1} soil.

3.3.6 Organic carbon (OC)

The Walkley Black method as modified by Moberg (2001) was used for the determination of soil OC. In this method 1 g of finely ground soil was placed in a conical flask. About 10 mL of $K_2Cr_2O_7$ solution, 10 mL of 85% phosphoric acid (H_3PO_4) solution and 20 mL of 98% H_2SO_4 were added. The mixture was swirled to mix, left for 30 mins to cool before being titrated. An indicator Diphylemine was added and mixture titrated using ferrous sulfate ($FeSO_4$). The organic carbon was finally computed using amount of dichromate as used in the oxidation as per formular below.

$$\%OC = \frac{\text{Meq } K_2Cr_2O_7 - \text{Meq } FeSO_4 \times 0.003 \times 100 \times 1.3}{\text{ODW (g)}}$$

Where: OC = Organic carbon; ODW = Oven dry weight (g); Meq = Millirquivalent; $K_2Cr_2O_7$ = Potassium dichromate; $FeSO_4$ = Iron sulphate

3.3.7 Soil pH

Soil pH was determined in water using the soil water ratio of 1:2.5 extractant (Moberg, 2001). About 10 g of soil was transferred into 100 mL plastic bottle followed by additional of 25 mL of the water extractant. The mixture was shaken for 30 mins and then allowed to settle for 5 mins and the supernatant solution was read using an electrode pH meter.

3.3.8 Soil Moisture

Soil moisture was determined by using soil moisture probe series 2900F that reads the moisture of soil at the desired depth. The moisture probe was calibrated before it was used by pressing the vent pin located at the top of the gauge followed by turning null knob clockwise while the porous ceramic sensing tip was inserted in water until a red ring was seen. The pointer dropped to zero from 45 bars. Then the knob was turned slowly counterclockwise until it was loose and removed. Water was filled slowly to avoid trapping air bubbles. The null knob was screwed back to the hand while water oozed out through the porous ceramic tip, until the null knob reached its fitting size. The removed ceramic porous tip from water was then dried using absorbent tissue and the gauge pointer raised to nearly 25 bars as the tip dried. The null knob was turned counterclockwise until a red ring was seen and the gause

rose to 80 bars. The ceramic porous tip was then immersed in water and the pointer dropped to zero. Similar process was repeated and finally the carrying case tube was filled with water and the moisture probe fitted to its case and allowed to stand for about three minutes prior measuring the soil moisture. Prior measuring of the soil moisture, the steel coring tool was pushed vertically into the desired depth of the soil, then removed and the moisture probe inserted and allowed some few mins to pass for reading the moisture. Three readings were taken for each plot and for each reading multiplied by 1.5 factor as described in a manual, then an average reading calculated in percentage (%). For each tobacco plot, moisture readings were taken from the soil depths of 0-10 cm, 10-30 cm and 30-50 cm.

3.3.9 Soil temperature

Soil temperature from each of three depths (0-10 cm, 10-30 cm and 30-50 cm) was measured through a soil thermometer. A coring tool with same thickness as thermometer was pushed first to the desired depth and the thermometer inserted and allowed three mins to pass before taking the reading. Three readings were taken for each depth and an average reading calculated to represent the soil temperature.

3.3.10 Soil nicotine

About 0.3 g of powdered air-dried composite soil samples were sieved through a set of 2 mm, 1 mm, and 0.5 mm in order to remove fine root tips (Guo, Mitchell & Hendricks, 2004) soil was weighed and immersed in 10 mL of methanol. The mixture was stirred by a shaker for 30 min at 200 g, then 25 mL of distilled water and 1 mL of 2 N NaOH were added and mixed thoroughly for 30 min, and then the solution was heated in a boiling water basin for 10 min to evaporate the methanol.

The cooled mixture was filtered using Whatman filter paper no. P41 with 20 μm pore size. About 1 mL of zinc acetate and K hexacyanoferrate (II) was added to the filtered extract and then transferred into a 50-mL volumetric flask, and distilled water was added to the mark. The mixture was shaken and centrifuged at 4000 g for 5 min. The supernatant was collected in a beaker and the residue discarded. Then, 1 mg of animal charcoal was added, thoroughly mixed, and allowed to settle for 2 min at room temperature before adding 0.01 N NaOH to increase pH and filtering was done through 2.5 μm pore size. The solution was made up to 50 mL with distilled water and introduced to the UV-visible single beam spectrophotometer fixed at 602 nm and 1 cm quartz cell for determination of nicotine (Figueiredo, Oliveira, de

Siqueira & Arruda, 2009). Total nicotine content was determined using a calibration curve concentration of 0.06 – 3 mgL^{-1}. For nicotine analysis, the nicotine standards generated an accurate nicotine concentration plot with R^2 = 0.98743. Furthermore, our trial sites did not have any history of tobacco growing and we followed clean procedures with our equipment and instruments to avoid contamination were added.

3.4 Plant sampling and analysis of plant Mn, Fe, Cu, Ca, Mg, K, P, N and nicotine

Plants targeted for the plant leaf sampling in the fields were randomly selected within the plot net area at the innermost three rows and marked. Sampled leaves were placed in bags and labeled. In the laboratory, leaf samples were washed thoroughly to remove dirt/debris using distilled water. The samples were put in the oven to dry at 65°C to constant weight. The dried samples were ground to a very fine texture using a plant grinder. The ground leaf samples were subjected to the dry ashing and wet digestion.

For dry ashing, 0.5 g of the leaf samples was weighed in crucibles and placed in a muffle furnace and heated for 3 h at 600°C. Then after 10 mL of 6 N HCL and 10 mL of distilled water, were added into the crucibles to dissolve the ash. Solution was filtered using Whatman filter paper number 42. The filtrate collected was introduced into 25 mL volumetric flask and then topped up to the desired mark using distilled water. The extract was used for determining plant Mn, Fe, Zn and Cu with the use of Atomic Adsorption Spectrophotometer (AAS) at the respective wavelength of each element. One mL of extract was diluted and used for determining Ca and Mg in AAS and for K using a flame photometer.

Total N in the plant samples was determined by the Kjedahl method as described in section 3.3.1. The amount of P in the extract was determined using the ascorbic acid molybdate blue method. Nicotine was determine by the method described by Figueiredo *et al.* (2009) by using spectrophotometric analysis whereby Ultraviolet-visible single beam spectrophotometer fixed at 602 nm and 1 cm quartz cell was used as described in section 3.3.10.

3.5 Soil sample processing for bacteria DNA extraction

Three soil samples for each treatment were immediately collected after harvesting tobacco and maize. Soil samples were collected in each plot each using a soil core in a zig zag manner. Each soil sample (single core) weighted nearly 400 g. The three soil samples per each treatment were mixed to make one composite sample.

3.5.1 Bacteria DNA extraction from the soil samples

Bacteria DNA extraction from three site soils were collected from tobacco, maize and fallow plots. For bacterial DNA extraction, 0.25 g of each soil sample was used for DNA extraction. DNA was extracted using DNeasy® PowerSoil® Kit (Qiagen, Hilden, Germany) as per manufacturer's instructions. Extracted DNA was quantified using Qubit™ 3.0 Fluorometer (Thermo Fisher Scientific, Grand Island, NY). To ensure purified DNA was of high-quality, DNA was also visualized through 1.0 % agarose gel electrophoresis.

Bacteria DNA extraction based on DNeasy® PowerSoil® Kit used the following steps;

(i) 0.25 g of soil sample introduced to the PowerBed Tube and subjected to vortex gently in order to mixed thoroughly

(ii) 60 µL of solution of C1 were added and vortexed briefly

(iii) PowerBead Tubes were secured horizontally using a vortex adapter tube holder (13000-V1-24) to a maximum speed for 10 mins

(iv) Tubes were then subjected to centrifuged at 10,000 x g for 30 s

(v) Supernatant transferred to a clean 2 mL collection tube

(vi) 250 µL of solution C2 were added to the 2 mL collection tube and vortexed for 5 s, and then incubated at 4°C for 5 min

(vii) Tubes were centrifuge for 1 min at 10,000 x g

(viii) 600 µL of supernatant were transferred to a clean 2 mL collection tube

(ix) 200 µL of solution C3 were added, vortexed briefly and incubated at 4°C for 5 mins.

(x) The tubes were centrifuged for 1 min at 10,000 x g

(xi) Pellet were avoided by transferring 750 µL of supernatant to a clean 2 mL collection tube

(xii) Solution C4 shaken and 1200 µL pipette and added to collection tube with 250 µL of supernatant and vortexed for 5 s

(xiii) 675 µL of supernatant loaded into MB Spin column (having high affinity for DNA) and centrifuge at 10,000 x g for 1 min and discard flow through. This step was repeated twice until all the sample processed

(xiv) 500 µL of solution C5 added and centrifuged for 30 S at 10,000 x g

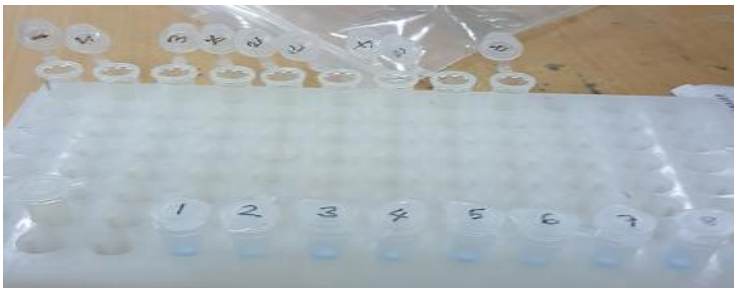
(xv) The flow through were discarded, centrifuged again for 1 min at 10,000 x g

(xvi) Carefully while avoiding splashing of any solution C5 onto the column, the MB Spin Column placed into 2 mL collection tube.

(xvii) 100 µL of solution C6 added to the centre of the white filter membrane (C6 reagent removed binding affinity for DNA ready for collection and also C6 reagent is used for DNA storage in longer period). Alternatively, sterile DNA-Free PCR Grade Water could have been used for this step

(xviii) The mixture was subjected to the centrifuge at room temperature for 30 s at 10,000 x g. The MB Spin Column were discarded, at this stage the DNA were ready for downstream applications

Digital scale

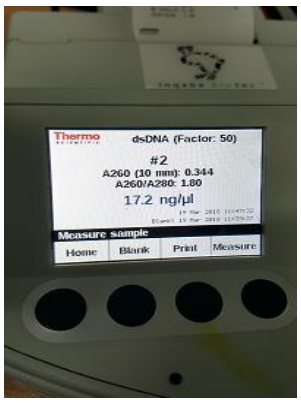


PowerBed Tubes

Discarded tube

Vortex M/C for mixing and breaking down cells

Centrifuge M/C version 8 (Megafuse 8) for separation of cells



Nanodrop LITE quantity & quality-DNA

Image 1: Equipment and machines used for soil bacteria DNA extraction

Since solution C6 contained 10 mM Tris-HCL, pH 8.5. DNA storage were at frozen (-20 to -80°C) as solution C 6 did not contain EDTA. Before storage of DNA extracted, all tubes were tested for DNA through nanodrop spectrophotometer to know the quality and quantity of DNA. The quantity results obtained are indicated in a Table 5 below, and the quality of DNA ranged between 1.7 and 1.9.

Table 5: Quantities of soil bacteria DNA extracted from three sites

Field label no	Field label details	Lab no	Quantity of DNA (ng/µL)
S1	Tobacco plot	7	7.1
S2	Maize plot	8	3.2
S6	Absolute control plot	9	1.8
T1	Tobacco plot	1	6.7
T2	Maize plot	2	17.2
T6	Absolute control plot	3	5.8
U1	Tobacco plot	4	6.1
U2	Maize plot	5	8.5
U6	Absolute control plot	6	7.3

Key: S = Sikonge; T = Tabora; U = Urambo

3.5.2 Microbiome 16S rRNA sequencing

The purified DNA was transported on dry ice to Inqaba Biotec™, a commercial sequencing service provider located in Pretoria, South Africa for the microbiome analysis. The V3-V4 hyper-variable regions of the 16S rRNA gene were amplified from the DNA extracts during the first PCR step using the universal primer pair 341F forward primer (5'-CCTACGGGNGGCWGCAG-3') and uniquely barcoded 785R reverse primer (5'-GACTACHVGGGTATCTA ATCC-3') for each sample. Resulting amplicons were gel purified, end-repaired and Illumina TrueSeq adapters were ligated to each amplicon. Then samples were individually indexed, and another bead-based purification step was performed. Following quantification and equimolar pooling, amplicons were then sequenced on Illumina's MiSeq platform, using a MiSeq v3 600 cycles kit. 20Mb of data (2x300bp long paired-end reads) were produced for each sample. The length of the obtained sequences averaged 231 bp.

3.5.3 Bioinformatics for microbiota composition

Due to very low-quality scores of the reverse-end reads, microbiome analyses were performed using only forward-end reads. Analysis of demultiplexed forward-end 16S rRNA gene reads was performed based on DADA2 (ver. 1.14.0) (Callahan *et al*., 2016) in R software (ver. 3.6.2) (R Core Team, 2019). The DADA2 pipeline includes trimming and filtering of the quality reads, dereplicates sequences, learns error rates, generates amplicon sequence variants (ASV) abundance table, removes chimeric sequences using "bimera *denovo*" method, taxonomic assignment and classification of the ASVs using the SILVA reference (ver. 132) database (Quast *et al*., 2013). About 427 218 forward-end FASTQ reads generated from 9 samples were pre-processed in DADA2 pipeline by removing low-quality reads using the truncated length set at 220 bp and adapters trimmed at less than 10 bp. Reads were further filtered to remove reads with ambiguous base by setting maxN=0 and maximum expected errors greater than two were discarded by setting the quality filtering measure (maxEE=2). The DADA2 pipeline detected 5.8% of the relative abundance in all reads as chimeric and removed from the datasets. The resulting ASV abundance table contained 375 429 high quality non-chimeric reads from 9 samples.

3.6 Data analysis

The Statistica 8.0 software package version 7 was used for statistical analysis. Nicotine levels were evaluated based on the interactions among sites and fertilizers, as well as each factor individually. Two-way ANOVA statistical analyses were performed through split plot design with treatments being agro-ecological zones (main plots) and fertilizers (sub plots). In evaluating effect of soil properties, three-way ANOVA statistical analyses were performed through split-split-plot design with treatments being agro-ecological zones (main plots), fertilizers (sub plots) and soil depths (sub-sub plots). To isolate interaction and/or individual effects of agro-ecological zones (Sikonge, Tabora and Urambo), fertilizers (NPK + CAN) and sampling depths (0-10 cm, 10-20 cm and 30-50cm), a post-hoc Tukey's-HSD multiple comparison test was used due to a higher degree of freedom (three sites x fertilizer levels = nine for the tobacco plant measured variables, and three sites x three fertilizer levels x three soil depths = 27 for the soil measured variables). The significance threshold was set at p = 0.05 and p = 0.001 for highly significance. The treatment means were compared by the standard error of difference of the mean.

Statistical analyses (two factors: sites; Sikonge, Tabora, Urambo and fertilization) were done using STATISTICA 8th Edition and ANOVA. The significant means were compared using Fisher Least Significance difference at p = 0.05. A multiple linear regression analysis was performed such that nicotine was regressed as a response variate (Y) and the fitted terms such as soil nutrients or other soil properties such as soil moisture, soil temperature, organic carbon and soil pH in order to measure its effects. The correlation and multiple regression analyses at $p < 0.05$ among soil biochemical properties, and bacterial diversity in tobacco plots were performed in STATISTICA 8th Edition.

For the microbiota phyla composition, downstream analyses included data inspection, normalization, abundance visualization, alpha and beta-diversity (observed and Shannon) analyses, and heatmaps were generated in R software (ver. 3.6.2) (R Core Team, 2019). After filtering and normalization of the sequence reads, 90 % rarefaction depth of the minimum sample depth in the dataset were used to simulate even number of reads per sample. Results show that 68 OTUs were removed because they were not present in any sample after random sub-sampling. The alpha-diversity indexes (species richness) for the study sites and different experimental treatments (fallow/control, maize and tobacco plots) at phylum level were calculated using the Observed and Shannon Diversity Indexes in *phloseq* (Wagner *et al*.,

2018) package in R. Moreover, the beta-diversity indexes for the study sites and experimental treatments of the samples, PCoA with weighted Unifrac at phylum level was performed using *phyloseq* (McMurdie & Holmes, 2013) package in R software (ver. 3.6.2). Statistical analyses between the groups for the alpha-diversity, and beta-diversity indexes were performed using the pairwise-wilcoxon test and the permutational ANOVA (PERMANOVA) analysis using *vegan* (Oksanen, 2011) package in R, respectively.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Screen house experiments

4.1.1 Chemical properties of tobacco leave and roots used to make extracts for screen house experiments

Nicotine extracted from *N. tabacum* leaves and roots separately, were used for screen house pot studies to test the effect of *N. tabacum* in *Z. mays* as shown in Table 6. The P content for both leaves and roots parts (0.08%) were very low probably due to its usage during the growth stage. Furthermore, P could have been utilized extensively for root development and growth of the plants during its active development.

Table 6: Chemical properties of tobacco leaves and roots extracts for pot study

K 326 tobacco part extracts	% Tobacco leaf and root nutrients						Nicotine (%)
	P	N	Zn	Mn	Ca^{2+}	K^{+}	$C_{10}H_{14}N_2$
Leaf	0.08	1.68	9.55	343.44	3.90	0.78	1.18
Root	0.08	1.96	12.73	507.38	2.84	0.44	0.99

Nitrogen concentrations were 1.68 and 1.96% for the leaves and roots, respectively (Table 6). These concentrations were generally closely to the adequate range of 2-5% for N content in tobacco leaves (Haghighi, Daliri, Mobaser & Moosavi, 2011). The high N content in roots indicates that, nitrogen is stored for nicotine synthesis in roots and it is one of the dominant elements in the structure of nicotine. The N content in nicotine has been associated with nicotine in influencing plant growth (Kena, 1990). Zinc and Ca concentrations in leaves were 9.55 and 3.90% while in roots it was 12.73 and 2.84%, respectively (Table 6). These nutrients appear to be nearly stable due to their involvement in plant growth, chlorophyll component, formation of cell wall and plasma membrane (Marshner, 1995; Leffingwell, 1999; Lopez-Lefebre *et al*., 2001).

The K concentrations for both leaves and roots were 0.78 and 0.48%, respectively (Table 6) and were very low (Bryson & Mills, 2014) indicating that this element could have been utilized efficiently during the active growth of the plant for producing hard and strong stems and increased performance and transfer of starch, sugar and fat (Rostami, 1997). The low

concentrations of K could also be as a result of the tobacco plant itself reducing its content to enable substantial amount for nicotine synthesis. It seems that when N and nicotine are at high levels, K is always at low level meaning that K is utilized efficiently in producing reducing sugars and nicotine in tobacco plant (Yang *et al*., 2007). Manganese concentrations was 343.44% in leaves and very high in roots reaching 507.38% (Table 6). This indicate that Mn is stored more in roots due to it's role in diseases resistant (Livorness & Smith, 1982; Huber & Wilhelm, 1988). Nicotine in roots was 0.99% lower than in leaves 1.18%, indicating that nicotine synthesized in roots is transported to the leaves for storage (Shitan *et al*., 2009). The content of nicotine in leaves was low than expected because the outgrowth leaves was sampled from the lower part of the plant. It could be that, the low nicotine concentration in roots might have triggered more concentration of Mn in roots to allow plant survival in resisting the attack from pests and diseases (Huber & Wilhelm, 1988).

4.1.2 Effects of tobacco extracts on the levels of soil pH and nicotine absorbed by maize seedlings

Tobacco leaf and root extracts drenched on maize seedlings resulted into lowering soil pH, but tobacco leaves extract lowered soil pH more than tobacco roots extracts. The lowering of soil pH was related with the levels of nicotine in leaves and roots extracts (Table 7). Tobacco leaves extracts resulted into significant (P <0.001) level of nicotine (0.18 mg kg^{-1}) than the tobacco roots extracts (0.13 mg kg^{-1}) in maize seedlings. The lowering of soil pH for both leaf and root extracts showed a similar trend of increased concentrations from 0-100%. However, the tobacco leaf extracts had more pronounced effects in lowering soil pH than tobacco root extracts.

Table 7: Effect of tobacco leaf and root extracts on soil pH and contents of nicotine in maize seedlings

Description of the plant parts/levels	Soil pH	Nicotine (mg kg^{-1})
Plant Parts (PP) of Tobacco		
Tobacco leaves	5.63±0.05b	0.18±0.04a
Tobaccp roots	5.73±0.03a	0.13±0.03b
Extract Concentration Parts (ECP)		
Tobacco leave extracts drenched on maize		
0%	5.94±0.01a	0.01±0.01f
25%	5.68±0.00cd	0.08±0.01e
50%	5.66±0.01d	0.13±0.01d
75%	5.58±0.01f	0.31±0.03b
100%	5.31±0.01g	0.39±0.01a
Tobacco root extracts drenched on maize		
0%	5.95±0.01a	0.02±0.01f
25%	5.72±0.01ab	0.02±0.01f
50%	5.70±0.01bc	0.11±0.02de
75%	5.68±0.01cd	0.20±0.01c
100%	5.61±0.01e	0.28±0.02b
2-Way ANOVA F-statistics		
PP	426***	37.6302***
ECP	1085***	170.971***
PP X ECP	128***	7.331***

Values presented are means ± SE (Standard Error); *** significant at $P \leq 0.001$ *respectively*; ns non-significant; Means in the same category of evaluated interface sharing similar letter(s) do not differ significantly based on their respective Least Significance Difference (LSD) value at 5% error rate

There were significant interaction effects between tobacco extracts from leaf and root and their concentrations levels on soil pH and nicotine absorbed by maize seedlings (Figs. 7 & 8). The highest soil pH for the soil drenched with leaf extract recorded at 0% with soil pH of 5.94, while the lowest soil pH recorded at 100% with 5.31. For the soil drenched with root extract, high soil pH was 5.95 recorded at 0% and the lowest soil pH of 5.61 recorded at 100% (Fig. 7).

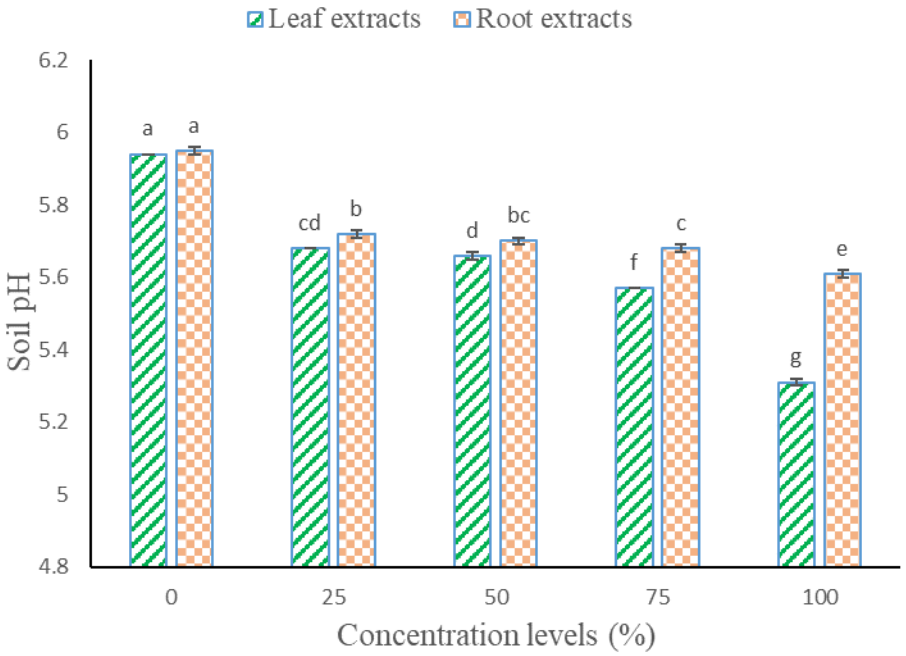


Figure 7: Interaction effects of tobacco root and leaf extracts and soil pH

The highest amount of nicotine (0.39 mg kg^{-1}) nicotine was absorbed in maize seedlings drenched at 100% of leaf extracts and the lowest amount of nicotine (0.01 mg kg^{-1}) absorbed by maize seedlings was from the control treatment which received 0% of leaf extract. For the root extract drenching to maize seedlings at 100%, the highest 0.28 mg kg^{-1} of nicotine was absorbed in maize seedlings and the lowest recorded at control treatment (0%) with 0.02 mg kg^{-1} of nicotine (Fig. 8). The recorded low levels of nicotine in the absolute control (0%), indicating that in the analysis of nicotine, levels of N which is one component of nicotine is captured as nicotine by an average of 25% (Table 9).

Thus, the tobacco leaf left overs under the ridges in maize fields as most farmers do practice for the purpose of improving maize growth, apart from improving the growth, increases also levels of nicotine absorved by the maize seedlings.

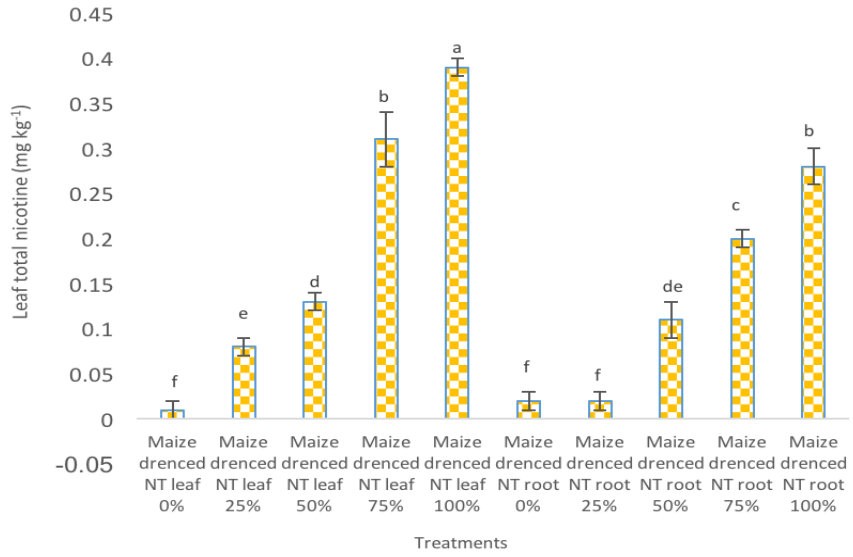


Figure 8: Interaction effects of tobacco leaf and root extracts and their concentrations on maize seedlings absorption levels on nicotine

4.1.3 Influence of tobacco extracts on the maize growth

Table 8 shows the influence of leaf and root extracts on maize growth above and below the ground. Tobacco leaf extracts had significantly effect in increasing maize plant height and root length than the root extracts ($P < 0.001$). Despite the tobacco leaf extract to have an effect of increasing maize plant height and root length, but did not increase significantly ($P < 0.001$) the stem thickness and the shoot weight. This could be due to the short duration of only 42 d to cause any effects as the plant still required more time to grow and develop its vegetative parts. These results suggest that, extract levels drenched in maize seedlings as increased from 0% to 100%, improved growth of maize seedlings on both shoots and roots. However, tobacco leaf extract had more impact in increasing root length at early stage of growth (Image 2) and plant height (Table 8 & Image 2). There were no any interaction effects between the tobacco leaf and root extracts and their concentrations levels on maize stem thickness, shoot weight, plant height and root length (Table 8).

Table 8: Effect of tobacco leaf and root extracts on maize height, shoot weight and root length

Treatments	Stem Thickness (mm)	Shoot Green Weight (g)	Shoot Dry Weight (g)	Plant Height (cm)	Root Length (mm)
Plant Parts (PP)					
Tobacco leaves	3.24±0.09a	139.00±5.73a	23.60±0.74a	77.40±2.08a	44.57±1.92a
Tobaccp roots	3.33±0.09a	154.46±5.54a	26.33±1.07a	69.96±1.37b	39.63±2.07b
Extract Concentration Parts (ECP)					
Tobacco leaf					
0%	3.27±0.23a	143.33±9.13ab	23.66±1.33ab	74.67±2.40ab	32.70±2.65c
25%	3.13±0.35a	133.00±22.60ab	23.66±2.96ab	73.33±6.66ab	45.70±1.96ab
50%	3.07±0.23a	136.33±8.41ab	24.00±1.15ab	78.33±2.33ab	44.40±3.78ab
75%	3.27±0.09a	121.33±4.97b	21.00±0.58b	76.66±6.35ab	51.50±0.28a
100%	3.47±0.09a	161.00±5.77a	25.67±1.20ab	84.00±4.58a	48.53±1.29ab
Tobacco root					
0%	3.30±0.35a	165.33±9.61a	28.33±1.76a	66.67±4.25b	28.36±0.95c
25%	3.43±0.23a	166.00±19.07a	29.00±3.60a	72.77±2.82ab	34.00±0.58c
50%	3.30±0.10a	154.67±5.92ab	26.33±2.18ab	68.33±2.40b	43.00±2.08b
75%	3.20±0.15a	141.66±8.95ab	23.66±1.76ab	70.40±1.70b	44.13±2.06b
100%	3.40±0.21a	144.66±15.71ab	24.33±2.33ab	71.67±4.33b	48.63±2.36ab
2-Way ANOVA F-statistics					
PP	0.38ns	3.88ns	4.33ns	8.09*	14.38***
ECP	0.35ns	1.09ns	1.15**	0.79ns	25.48***
PP X ECP	0.29ns	1.13ns	0.79ns	0.585ns	2.65ns

Values presented are means ± SE (Standard Error); *, **, significant at $P \leq 0.05$, $P \leq 0.01$, *respectively*; ns non significant; Means in the same category of evaluated interface sharing similar letter(s) do not differ significantly based on their respective Least Significance Difference (LSD) value at 5% error rate

Image 2: Maize drenched with tobacco leaf extract

Image 3: Maize drenched with tobacco root extract

4.1.4 Influence of tobacco on soil N, P, K, Ca, Mg and Cu

Tobacco leaf extracts (Table 9) significantly (P <0.001) increased soil Cu^{2+}, Ca^{2+} and total N when compared with the Tabora soil initial status of these nutrients (Table 11). The increase of soil Cu^{2+}, Ca^{2+} and total N influenced the increase of maize plant height and root length following drenching of leaf extracts (Table 9). In this aspect, the increase of Cu^{2+} and total N is synergistic as observed by Tandon, (1995). Tobacco root extracts, on the other hand, decreased the soil Cu^{2+} significantly, but kept on increasing Ca^{2+} and soil total N. Therefore, low levels of extracts concentration resulting into reducing levels of Cu^{2+} in the soil. The increase of extract concentration also increased acidic and solubility of Cu^{2+} in soil.

Table 9: Influence of leaf and root extracts of tobacco to soil N, P, K, Cu, Ca and Mg

Treatments	Soil Cu^{2-}(mg kg^{-1})	Soil Total N%	Soil P (mg kg^{-1})	Exchangable Bases (Cmol (+) kg^{-1})		
				Soil Ca^{2+}	Soil Mg^{2+}	Soil K^{+}
Plant Parts of tobacco (PP)						
Tobacco leaves	0.21±0.01a	0.06±0.00a	43.17±0.71a	0.47±0.0a	0.03±0.00b	0.04±0.00a
Tobacco roots	0.07±0.02b	0.04±0.00b	42.00±0.56b	0.38±0.0b	0.059±0.00a	0.03±0.00bc
Extract Concentration Parts (ECP)						
Tobacco leaves						
0%	0.18±0.01b	0.04±0.01bc	44.55±0.01b	0.52±0.00a	0.05±0.01b	0.04±0.01ab
25%	0.18±0.01b	0.08±0.01a	41.32±0.01d	0.52±0.00a	0.03±0.00de	0.04±0.00b
50%	0.18±0.01b	0.07±0.01a	41.46±0.01d	0.40±0.00b	0.04±0.01cd	0.04±0.01bc
75%	0.26±0.01a	0.04±0.01bc	40.76±0.01de	0.52±0.00a	0.02±0.01e	0.04±0.01ab
100%	0.26±0.01a	0.05±0.01c	47.77±0.01a	0.40±0.00b	0.035±0.01cd	0.06±0.01a
Tobacco roots						
0%	0.02±0.00d	0.04±0.01bc	45.25±0.58b	0.29±0.01c	0.06±0.00ab	0.03±0.01bc
25%	0.02±0.00d	0.04±0.01bc	42.30±0.01c	0.40±0.00b	0.07±0.01a	0.02±0.01c
50%	0.02±0.00d	0.05±0.01c	42.72±0.58c	0.29±0.01c	0.07±0.01a	0.03±0.01bc
75%	0.10±0.01c	0.04±0.01bc	39.50±0.00f	0.52±0.00a	0.05±0.00bc	0.03±0.00bc
100%	0.18±0.01b	0.03±0.01c	40.23±0.03de	0.40±0.00b	0.05±0.01b	0.04±0.01ab
2-Way ANOVA F-statistics						
PP	2001.689***	19.2000***	51.309***	1162.318***	55.377***	13.749***
ECP	250.075***	7.2000***	106.377***	481.192***	5.304**	3.626*
PP X ECP	23.834***	4.2000**	102.220***	254.466***	2.457ns	0.168ns

Values presented are means ± SE (Standard Error); *, **, *** significant at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$ *respectively*; ns non significant; Means in the same category of evaluated interface sharing similar letter(s) do not differ significantly based on their respective Least Significance Difference (LSD) value at 5% error rate.

Total N and Ca^{2+} increased in soils following drenching of tobacco leaf than root extracts. Increase of soil N could be due to the more increase of extract levels as N is one of its major components. Calcium levels in the soil increased sharply, probably due to the increased levels of nicotine that may have hastened the decomposition of soil organic matter and hence increased Ca^{2+} in the soil.

Tobacco leaf and root extracts drenched on maize seedlings, significantly (P <0.001) decreased soil P, Mg and K. The decrease of these nutrients could be due to the maize seedlings higher needs for improving its root and shoot growth.

There was an interaction effect of tobacco leaf, root extracts and their concentrations on soil N, P, Cu^{2+} and Ca^{2+} (Fig. 9). Tobacco leaf extract concentration increased soil total N higher than root extract but decreased soil P levels. The decrease of soil P could be related to the potential need for P in improving roots development. Copper and Ca^{2+} increased in the soil as leaf and root extracts drenched to the maize seedlings. The increase of Ca^{2+} levels in the soil was higher than Cu^{2+} indicating that Ca^{2+} requirement is essential to the increase of tobacco biomass. Additional of tobacco extracts to the potting soil increased the soil acidity and hasten decomposition of organic matter and parent materials and hence increased soil Ca^{2+} levels.

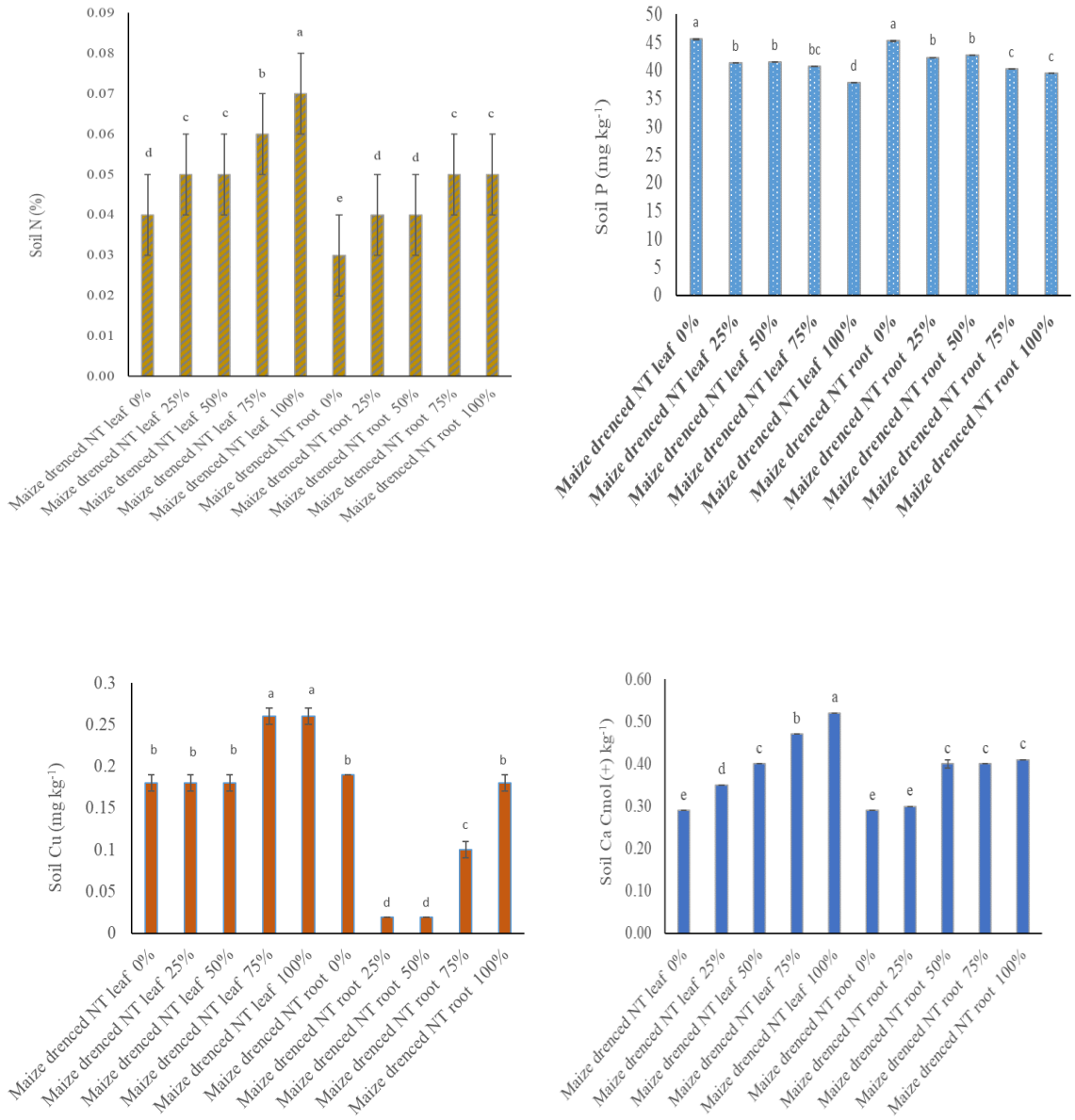


Figure 9: Interaction effects of tobacco leaf and root extracts with levels of soil N, P, Cu and Ca in the soil

4.1.5 Influence of tobacco leaf and root extracts on maize plant nutrients uptake

The effect of tobacco extracts from leaf and root on nutrients maize seedlings uptake is shown in Table 10. Tobacco extracts slightly decreased the uptake of plant P and K^+ while uptake of N, Mn, Ca^{2+} and Zn^{2+} were generally at a constant rate. Uptake of these nutrients could be required by the maize seedlings at the lower levels as seedlings were still young. However, the interaction effects of extract concentrations and nutrients uptake were observed in maize plant Mn and Ca^{2+} (Fig. 10). The uptake of these nutrients seemed to be at the initial stage as their uptakes did not vary widely.

Table 10: Effect of tobacco leaf and root extracts on maize plant nutrients uptake

Treatments	Plant Uptake Total (%)					
	P	N	Zn	Mn	Ca^{2+}	K^{+}
Plant Parts of tobacco (PP)						
Tobacco leaves	0.25±0.01a	2.28±0.03b	6.73±0.01b	116.23±0.02a	0.52±0.01a	0.970.03a
Tobacco roots	0.24±0.01a	2.38±0.03a	8.72±0.03a	115.46±0.39b	0.52±0.02a	0.85±0.03b
Extract Concentration Parts (ECP)						
Tobacco leaves						
0%	0.27±0.01ab	2.33±0.02b-d	6.71±0.01c	116.29±0.02a	0.55±0.03ab	1.08±0.11a
25%	0.25±0.01a-c	2.27±0.05b-d	6.71±0.01c	116.09±0..02b	0.50±0.01c	0.91±0.01b-d
50%	0.23±0.01bc	2.24±0.10cd	6.73±0.00c	116.24±0.07a	0.49±0.02c	0.92±0.00a-d
75%	0.25±0.01a-c	2.37±0.01bc	6.73±0.00c	116.24±0.01a	0.53±0.00bc	0.94±0.05a-d
100%	0.26±0.02a-c	2.20±0.04d	6.77±0.01c	116.30±0.00a	0.52±0.00bc	0.99±0.10a-c
Tobacco roots						
0%	0.24±0.01bc	2.28±0.02b-d	8.58±0.16b	113.10±1.13c	0.43±0.03d	1.01±0.03ab
25%	0.29±0.03a	2.40±0.08bc	8.71±0.01ab	115.15±0.03b	0.50±0.01c	0.84±0.03c-e
50%	0.23±0.01c	2.43±0.08a	8.75±0.01a	116.25±0.04a	0.53±0.01bc	0.82±0.02de
75%	0.24±0.01bc	2.43±0.03a	8.76±0.01a	116.42±0.01a	0.57±0.01ab	0.82±0.04de
100%	0.23±0.01c	2.35±0.04b-d	8.81±0.01a	116.38±0.01a	0.58±0.01a	0.74±0.02e
2-Way ANOVA F-statistics						
PP	0.818ns	7.385**	4011.939***	11.727**	0.136ns	12.761**
ECP	1.923ns	1.310ns	2.293ns	7.860***	5.663**	3.937*
PP X ECP	2.289ns	1.443ns	1.015ns	7.896**	10.504***	1.007ns

Values presented are means ± SE (Standard Error); *, **, *** significant at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$ *respectively*; ns non significant; Means in the same category of evaluated interface sharing similar letter(s) do not differ significantly based on their respective Least Significance Difference (LSD) value at 5% error rate

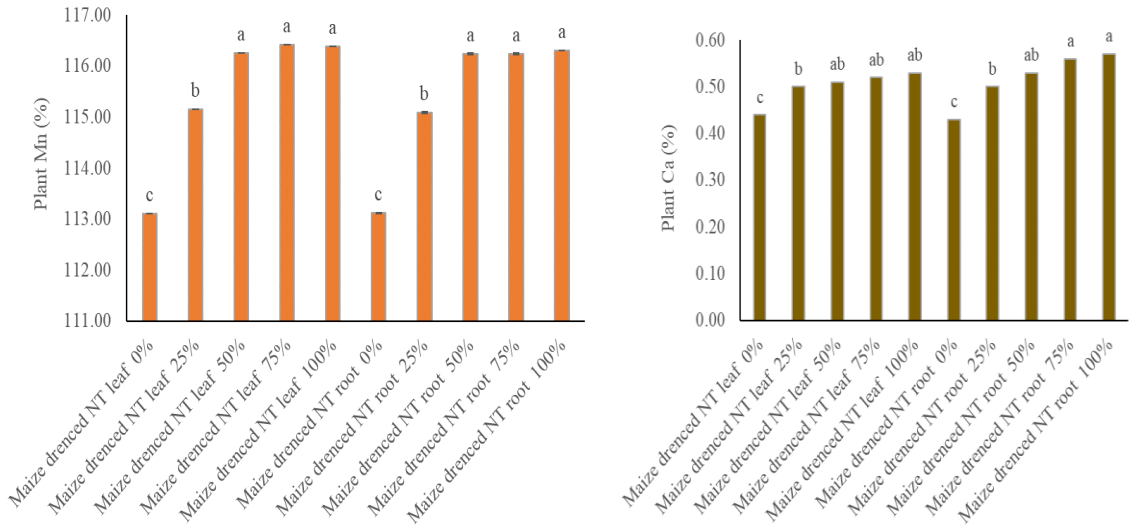


Figure 10: Interaction effects of tobacco leaf and root extracts on the uptake for Mn^{2+} and Ca^{2+}

4.2 Field experiments

4.2.1 Chemical and physical properties of the field soils

Some of the physical and chemical properties of Tabora Tumbi soil which was used for field experiments in Tabora, Urambo and Sikonge are shown in Table 11.

Table 11: Some physical-chemical characteristics of field experimental soils

Descriptive parameter	Unit	Experimental Field Sites (0 – 30 cm depth)		
		Tabora	Urambo	Sikonge
Soil pH	pH (1:2.5) in H_2O	5.49	5.87	5.89
	% Clay	6.96	12.12	11.5
Particle size determination	% Silt	4.64	2.92	3.48
(P.S.D)	% Sand	88.4	84.96	85.04
Texture Class	Texture Class	Sand	Sandy loam	Loamy sand
Nicotine ($mgkg^{-1}$)	Nic	0.01	0.02	0.01
	Ca^{2+}	0.10	0.40	1.29
Exchangeable Bases	Mg^{2+}	0.24	0.26	0.29
($Cmolkg^{-1}$)	K^{+}	0.29	0.25	0.53
	Na^{+}	0.10	0.01	0.02
Cation Exchangeable Capacity	CEC ($Cmol\ kg^{-1}$)	2.60	3.20	4.40
Micronutrients	B ($mgkg^{-1}$)	0.3	0.32	0.34
	Cu ($mgkg^{-1}$)	0.14	0.26	0.21
	Fe ($mgkg^{-1}$)	12.95	13.32	14.54
	Mn ($mgkg^{-1}$)	11.90	24.07	24.32
	Zn ($mgkg^{-1}$)	0.11	0.37	0.47
Macronutrients	P ($mgkg^{-1}$)	53.39	44.41	43.48
	S ($mgkg^{-1}$)	8.09	8.19	9.12
	Total N (%)	0.04	0.04	0.05
Organic carbon	OC (%)	0.16	0.25	0.36

Results indicated that textures for soils from Sikonge, Tabora and Urambo are loamy sand, sand, and sandy loam, respectively. Ratings of the studied parameters in these soils were based on the descriptions compiled by Landon (1991). The soil pH in Tabora soil was strongly acidic (5.1–5.5) and medium acidic (5.6–6.0) in soils from Sikonge and Urambo. Both organic carbon (< 0.6%) and total nitrogen (< 0.1%) were very low in soils from all three sites. Extractable sulphur was medium (7–11 mg kg^{-1}) while available phosphorus was high (> 25 mg kg^{-1}) in all soils. Exchangeable calcium was low in Sikonge (0.5–2.0 cmol (+) kg^{-1}) and in Tabora (0.2–0.5 cmol (+) kg^{-1}) soils; and very low (< 0.2 cmol (+) kg^{-1}) in Urambo soils. Soil exchangeable magnesium was low (0.25–0.75 cmol (+) kg^{-1}) in Sikonge soils as well as in Tabora and Urambo soils (0.2–0.5 cmol (+) kg^{-1}). Exchangeable potassium was medium (0.26–0.80 cmol (+) kg^{-1}) in Sikonge soils as well as in soils from Tabora and Urambo sites (0.11–0.4 cmol (+) kg^{-1}). Results indicated that extractable B was very low (0–0.4 mg kg^{-1}) in all soils. Extractable Cu was low (deficient) (0–0.4 mg kg^{-1}) while Fe (>4.5 mg kg^{-1}), and Mn and Zn (>1.0 mg kg^{-1}) were high in all soils.

4.3 To investigate the levels of nicotine released by tobacco plant within the rhizosphere under the fertilization and to assess the influence of soil depth on soil pH, OC, moisture and temperature

The key information on nicotine produced by the tobacco roots has long been known, with the assumption that 100% of its concentration is distributed among soil ecosystems, tobacco leaves, and part of it is retained in roots. However, the mechanism behind these differential distributions is not clearly known although some literature state that, xylem transportations as well as exudation of some nicotine into the rhizosphere occurs during the course of tobacco plant growth (Bais, Park, Weir, Callaway & Vivanco, 2004). There are also evidences of residual remnants of nicotine in soils as tobacco roots die, decay, and decompose into the soil (Hsiao & Xu, 2000). It is evident that most of the nicotine produced in tobacco roots is transferred via xylem and stored in vacuole of tobacco leaves (Shitan *et al*., 2009). Our study elucidates that a lot of tobacco nicotine is beyond reasonable doubt that it is stored in tobacco leaves, little in the roots, and part is released into soils. Higher tobacco nicotine contents transferred from roots to leaves present one of the preferred qualities of the flue-cured tobacco leaves (Benowitz, Jacob & Herrera, 2006).

Table 12 shows the effect of sites, fertilizer application on nicotine concentration in plant-soil interfaces. The present study revealed that inclusion of fertilizer in tobacco cultivated soils,

$N_{10}P_{18}K_{24}$ as basal application followed by CAN 27%N as top dressing, was significantly superior in inducing higher contents of nicotine transferred to tobacco leaves as well as that released into the soils by the tobacco roots.

Table 12: Nicotine concentrations in soils and different tobacco parts as affected by heterogeneity in three site

Descriptions	Interfaces evaluated for tobacco nicotine			
	Leaves	Roots	Soils	Total (leaves, roots and soils)
	(mg kg^{-1})			
Site				
Sikonge	30.98 ± 2.38 a	8.98 ± 0.21 a	9.55 ± 2.12 a	49.51 ± 4.30 a
Tabora	23.53 ± 2.26 b	6.92 ± 0.07 b	6.04 ± 1.52 b	36.48 ± 3.82 b
Urambo	20.91 ± 2.33 c	5.72 ± 0.21 c	4.42 ± 1.20 c	31.04 ± 3.54 c
Fertilizer				
Fertilized	29.99 ± 1.45 a	7.29 ± 0.49 a	10.27 ± 1.06 a	47.54 ± 2.98 a
Unfertilized	20.29 ± 1.82 b	7.12 ± 0.49 a	3.07 ± 0.47 b	30.48 ± 2.64 b
2-Way ANOVA F-statistics				
Site (S)	31.94***	72.28***	223.98***	124.16***
Fertilizer (F)	82.46***	0.55ns	1265.84***	300.92***
S×F	0.15ns	0.09ns	34.73***	0.73ns

Values presented are means ± $SE_{\bar{x}}$ (Standard error of means); *** = significant at $P < 0.001$; ns = non-significant ($P \geq 0.05$). Means in the same category of evaluated interface sharing similar letter(s) do not differ significantly based on their respective Standard error (SE) at 5% error rate

The trends of tobacco nicotine concentrations in tobacco leaves was in the decreasing order of Sikonge, Tabora, and Urambo sites and the similar observation was made in tobacco roots. However, there was inconsistent trend with respect to the tobacco nicotine retained in soils whereby 9.55 mg kg^{-1} was found in loamy sand (Sikonge soil), which is 19.29% of the total nicotine produced. This was higher compared with that recorded in sandy loam (Urambo soil) whereby the nicotine released into the soil was 4.42 mg kg^{-1} equivalent to 14.24% of the total nicotine produced. The nicotine in tobacco roots for both unfertilized and fertilized tobacco plants did not differ significantly while it differed significantly in unfertilized and fertilized tobacco plants.

The present study revealed that regardless of the agro-ecological differences, the fertilized tobacco plants released twice (21.60%) as much of the total tobacco nicotine into the soils relative to the unfertilized tobacco plants, which was 10.07% of the total nicotine produced. This depicts that tobacco plant had the minimum required nicotine level to the leaves and thus the unfertilized tobacco released less nicotine to the soils and maintain 20.29 mg kg^{-1}

equivalent to 2% of nicotine in leaves. For fertilized tobacco plants, the amount of nicotine increased beyond 2% in leaves but the amount of nicotine released to the soils increased in order exceeding not harmful levels. Nicotine levels in tobacco plant can reach 4% and beyond depending on the varieties of tobacco (Moldoveanu, Scott & Lawson, 2016). However, Nagarajan and Prasadrao (2004) insisted that the nicotine concentration in tobacco leaves should be limited to 1.75–2.00%, although Xie, He, Xu and Tu (2017) depicted that this concentration is influenced by agronomic traits, climate conditions, pests and diseases.

The quantities of N applied, time and frequencies of its applications are closely correlated with nicotine concentration as N is involved in the production of jasmonic acid (JA), which regulates nicotine synthesis in tobacco roots and in tobacco leaves (Xie *et al*., 2017). The higher amounts of nicotine recorded in cases of fertilized soils from all three sites involved in the present study would also be associated with timely transplanting the seedlings and harvesting of green tobacco leaves. For instance, Xie *et al*. (2017) indicated that delaying transplanting time promoted dry matter and N accumulation but significantly decreased the nicotine concentration. The finding of the present study suggests that if farmers do not use fertilizers in tobacco cultivating systems, the concentrations of nicotine in soils are likely to be reduced and have less impact to the subsequent crop. However, the quality of tobacco in terms of nicotine in leaves as harvestable and valuable part becomes highly hampered, signaling a need for investigation of mechanisms that will favour transfer of more nicotine into leaves and retain low quantities in soils.

4.3.1 Effects of sites, fertilizer and soil depths on tobacco nicotine in different soil properties

Considering the soils from three contrasting sites involved in the present study, the order of decrease in tobacco nicotine concentrations was Sikonge > Tabora > Urambo, but the former outperformed others by over 20% suggesting that loamy sand soils from Sikonge retained more nicotine (Table 13).

Table 13: Effects of the sites, fertilizer and soil depths on released tobacco nicotine

Descriptions	Measured variables in soils				
	pH	OC (%)	Nicotine (mg kg^{-1})	Temperature (^oC)	Moisture (cB)
Site					
Sikonge	5.33 ± 0.05 c	0.27 ± 0.01 a	9.55 ± 1.16 a	29.11 ± 1.04 a	13.37 ± 1.75 a
Tabora	5.50 ± 0.02 b	0.15 ± 0.00 c	6.04 ± 0.84 b	27.66 ± 1.13 b	11.51 ± 0.84 b
Urambo	5.69 ± 0.02 a	0.21 ± 0.01 b	4.42 ± 0.82 c	28.27 ± 0.63 ab	9.66 ± 1.27 c
Fertilizer					
Fertilized	5.49 ± 0.04 a	0.23 ± 0.01 a	10.27 ± 0.69 a	28.31 ± 0.78 a	12.62 ± 1.19 a
Unfertilized	5.42 ± 0.03 a	0.19 ±0.01 b	3.07 ± 0.27 b	28.39 ± 0.78 a	10.39 ± 1.16 b
Depth (cm)					
0–10	5.59 ± 0.05 a	0.22 ± 0.01 a	5.50 ± 1.00 c	33.14 ± 0.47 a	4.95 ± 0.65 c
10–30	5.48 ± 0.05 ab	0.23 ± 0.02 a	6.92 ± 1.05 b	27.39 ± 0.39 b	12.59 ± 0.91 b
30–50	5.44 ± 0.04 b	0.19 ± 0.01 b	7.59 ± 1.13 a	24.53 ± 0.43 c	16.98 ± 1.01 a
3-Way ANOVA F-statistics					
Site (S)	27.45***	104.51***	497.42***	7.15**	19.84***
Fertilizer (F)	0.91ns	37.46***	2812.55***	0.06ns	21.45***
Depth (D)	5.06**	10.92***	82.51***	261.47***	213.09***
S×F	2.34ns	20.62***	77.19***	5.43**	34.23***
S×D	0.66ns	7.82***	15.76***	14.24***	13.25***
F×D	0.72ns	0.02ns	39.82***	1.95ns	0.91ns
S×F×D	0.17ns	0.864ns	10.85***	2.32ns	0.53ns

Values presented are means ± $SE_{\bar{x}}$ (Standard error of means); *** = significant at $P < 0.001$; ** = significant at $0.001 \leq P < 0.01$; ns = non-significant ($P \geq 0.05$). Means in the same category of evaluated interface sharing similar letter(s) do not differ significantly based on their respective Standard error (SE) at 5% error rate

The concentrations of nicotine in soils decreased in unfertilized tobacco cultivated soils and this decrease almost coincided with the nicotine stored in tobacco leaves but not in roots. Interaction of sites and fertilizer treatments have showed that the soil organic carbon and soil moisture increased more in fertilized soils than unfertilized soils indicating that these measured variables have positive interaction in influencing tobacco nicotine released into the soils. The soil OC, moisture, and temperature showed positive interaction in influencing tobacco nicotine released into the soils. There is a clear implication of OC that its microbial decomposition in form of soil organic matter is favoured by temperature and moisture where mineral N is also released (Xie *et al.* (2017). In a different study, it was indicated that the depletion of soil moisture for tobacco plant productivity should be approximately between 50 to 55% (Biglouei, Assimi & Akbarzadeh, 2010). It is also reported that low temperatures (<18 °C) and rainfall (<80 mm) in the early growth stages of tobacco plant are also likely to stagnate the growth of soil microbes and lower inorganic N released and its availability (Xie

et al. (2017). However, this study revealed that soil OC and moisture are more pronounced than soil temperature in influencing the amounts of nicotine released into the soils by the tobacco plant.

Literature has shown that soil temperature and soil moisture are the meteorological data which have been found singly and/or in interaction to significantly affect N mineralization rate in tobacco cultivated soils. According to Hu, Tian, Di, Liu and Zhang (2018), the highest N mineralization in tobacco cultivated fields appeared at 35°C and the lowest was at 10°C but the effect of soil moisture on N mineralization rate was a single peak curve at 40%. Consequently, favourable soil temperature and moisture conditions stimulated the growth and development of tobacco roots as well as N uptake and accumulation in tobacco leaves (Thomsen, Laegdsmand & Olesen, 2010; Rowe, Emmett, Frogbrook, Robinson & Hughes, 2012). However, with little or no application of N-containing fertilizers the exogenous mineral N is likely to decrease rapidly mainly due to plant acquisition, microbial immobilization, leaching, and denitrification (Xie *et al*., 2017). This study also indicated that there was a positive relationship between nicotine concentration and soil OC, which is one of the components of soil organic matter (SOM). Referring to the importance of mineral N in nicotine production, this finding depicts that the nutrients derived from SOM, including mineral N are increasingly important throughout the tobacco crop cycle as SOM is the main source of N for plants (Xi *et al*., 2005).

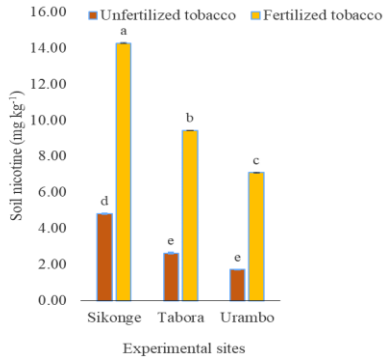
The present study indicates that at the shallow depths (0-10 cm) the soils contain more fresh materials (i.e. fresh organic matter – FOM), which are not completely finished into soil organic matter (SOM) enough to have implications on soil characteristics. The concentration of SOM in soils generally ranges from 1 to 6% of the total topsoil (5.1 to 20 cm deep) and is where most of the earth's biological soil activity occurs (Marsh, 2010). The three macronutrients contained in SOM are nitrogen (N), phosphorus (P), and sulphur (S) along with micronutrients, which are slowly released upon SOM mineralization. The SOM is also typically estimated to contain 58% of carbon (C) (Bianchi, Miyazawa, de Oliveira & Pavan, 2008). Due to this, there was less OC in all soils at a depth of 0-10 cm and was higher at a depth of 10-30 cm, with the lowest being at a depth of 30-50 cm. Therefore, the highest OC was recorded at the depth of 10-30 cm in all soils but the loamy sand Sikonge soil outperformed others. The OC correlated positively with nicotine released into the fertilized soils. Xu, Wang, Wang and Xiao (2006) indicated that the relationships between tobacco

nicotine in leaf and the soil organic matter could be described by linear-flat model. The transfer of N in form of nitrate (NO_3^-) and nitrite (NO_2^-) into tobacco leaf increased with increasing soil organic matter content (Xu *et al*., 2006), which therefore, has increasing effect on nicotine production.

4.3.2 Interaction effects of site and fertilizer on soil nicotine, OC, moisture and temperature

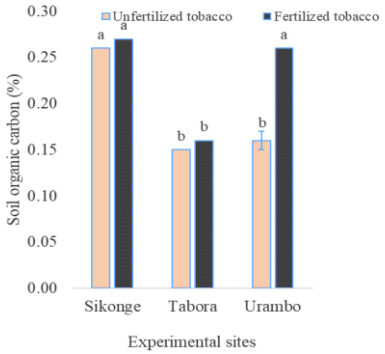
Soil OC, pH, T, SM, and nicotine content were assessed under different levels of fertilizer application and at different soil depths (Table 13). Nicotine contents at 0–10 cm, 10–30 cm, and 30–50 cm were 5.50 mg kg^{-1}, 6.92 mg kg^{-1}, and 7.59 mg kg^{-1}, respectively. Site and fertilizer interaction significantly ($P \leq 0.001$) increased soil OC content (Fig. 11b) and SM (Fig. 11c). The highest OC was recorded in the loamy sand of Sikonge (0.27%), while the lowest was in the sandy loam of Urambo (0.15%).

[a]



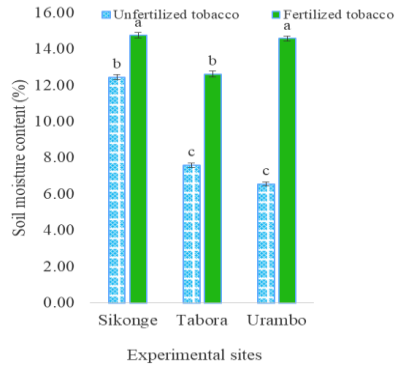
Interaction of sites and fertilizer on soil nicotine

[b]



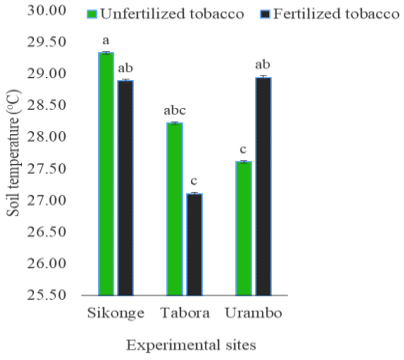
Interaction of sites and fertilizer on organic carbon

[c]



Interaction of sites and fertilizer on soil moisture

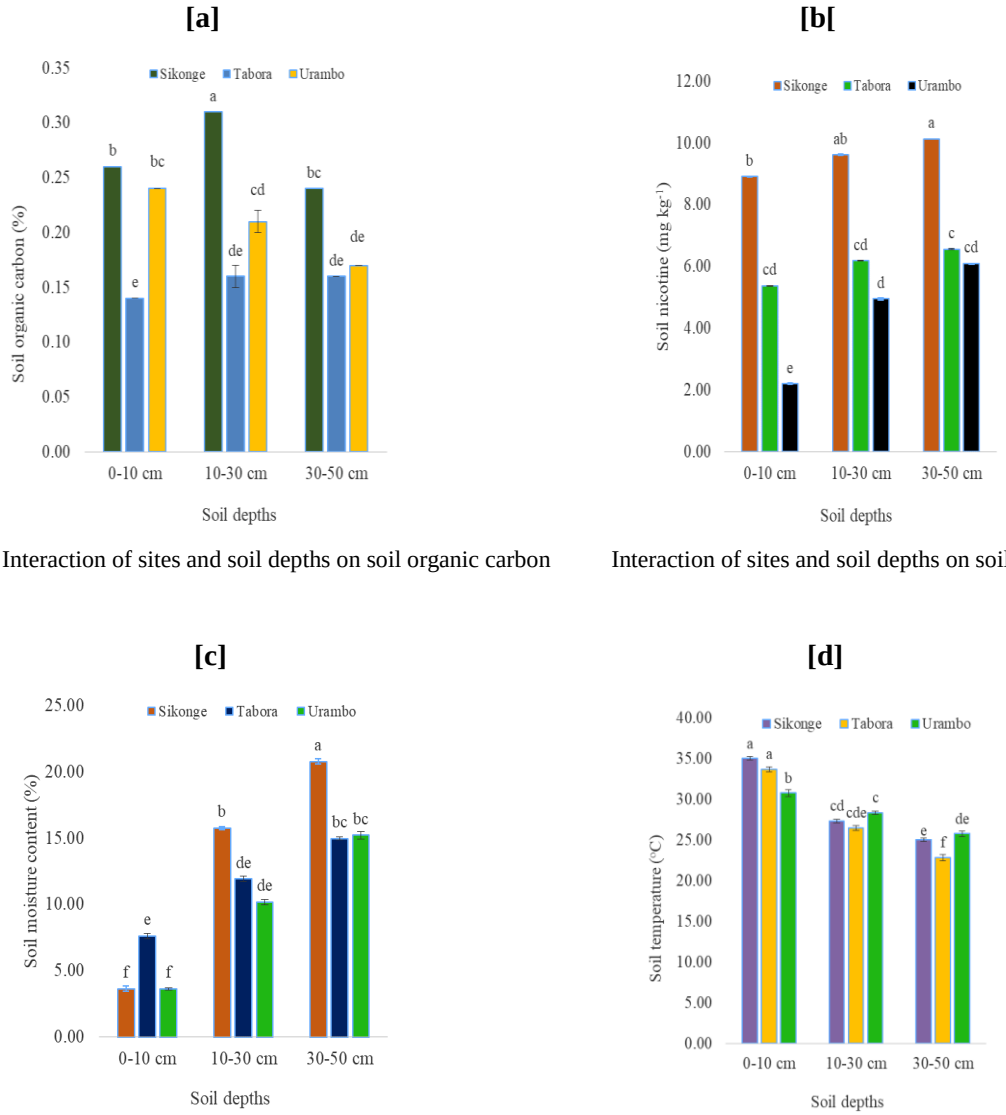
[d]



Interaction of sites and fertilizer on soil temperature

Figure 11: Interaction of sites and fertilizer on soil nicotine, OC, moisture and temperature

Sikonge had the highest SM (13.37%) and Urambo the lowest (9.66%). Soil temperature decreased in fertilized soils (Fig. 11d); the highest T was in Urambo (28.94°C) and the lowest was in Tabora (27.11°C). Results of the site and soil depth interaction indicated that OC was higher (0.31%) at 10–30 cm followed by that at 0–10 cm (0.26%) and the lowest was 0.24% at 30–50 cm in Sikonge soil (Fig. 12a). The nicotine content also increased with an increase in soil depth (Fig. 12b). The highest nicotine content was recorded at 30–50 cm (10.12 mg kg^{-1}) in Sikonge, while the lowest was at the same depth (6.09 mg kg^{-1}) in Tabora. Similarly, SM exhibited increasing trends at all sites as soil depth increased (Fig. 12c). The highest SM was at 30–50 cm in Sikonge (20.76%), and the lowest was at the same depth in Tabora (14.57%), which was not significantly different to that in Urambo (15.2%). Soil temperature at all sites exhibited a decreasing trend as soil depth increased (Fig. 12d). The highest T in Sikonge (35°C) was at 0–10 cm and the lowest (25°C) at 30–50 cm. The lowest T in Urambo (30.75°C) was recorded at 0–10 cm, while the lowest T in Tabora soil (22.83°C) was recorded at 30–50 cm.



Interaction of sites and soil depths on soil organic carbon

Interaction of sites and soil depths on soil nicotine

Interaction of sites and soil depths on soil moisture

Interaction of sites and soil depths on soil temperature

Figure 12: Interaction of sites and soil depths on soil OC, nicotine, moisture and temperature

4.3.3 Interaction effects of sites, fertilizer and soil depth on soil nicotine

The interactions among sites, fertilizer treatments, and soil depths significantly affected the nicotine in the soil (Fig. 13). In fertilized soils, the nicotine content increased significantly as soil depth increased relative to that of unfertilized soils. The highest soil nicotine content was 15.22 and 5.05 mg kg^{-1} in Sikonge at 30–50 cm in fertilized and unfertilized plots, respectively. The lowest soil nicotine content was recorded in Urambo at 10.04 and 2.15 mg kg^{-1} for fertilized and unfertilized soils, respectively. Tobacco nicotine increased as the soil depths increased with the highest nicotine of 10.12 mg kg^{-1} recorded at the depth of 30-50 cm

in fertilized loamy sand Sikonge soil and the lowest was 6.09 mg kg^{-1} at the same depth and soil when unfertilized (Fig. 12b). This signifies the importance of mineral N in nicotine production and its distribution into various sinks (Xi, Li & Zhang, 2008). Soil moisture increased at all sites with increase in soil depths, while soil temperature decreased with increase in soil depths but dictated by the soil type as they differ in texture. Our study involves soils with different textures such as loamy sand Sikonge, sandy loam Urambo, and sandy Tabora. In a similar study elsewhere, Nwanko & Ogagarue (2012) found that the mean soil temperature for clayey soil was 12.3℃, 28.6℃ for sandy soil and 28.7℃ for loamy soil and concluded that these temperatures are ideal for crop productivity. Nwanko & Ogagarue (2012) concluded that due to the high thermal inertia of the soil, the temperature fluctuations at the soil surface decreases as the depth of the ground increases.

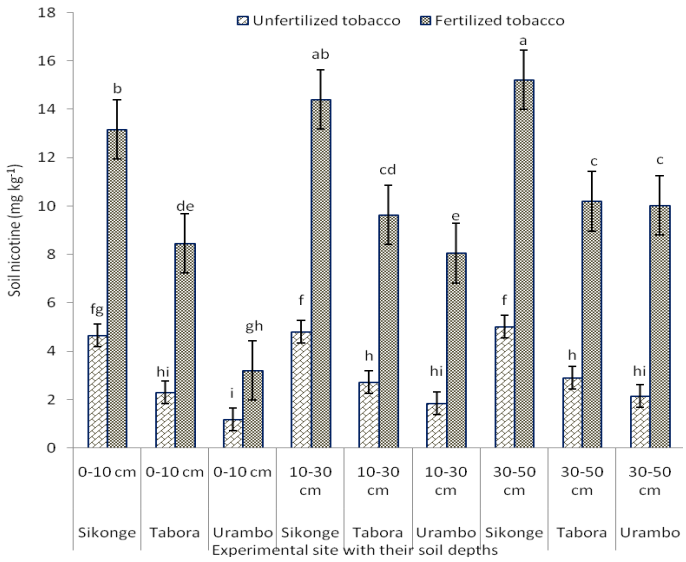


Figure 13: Interaction of sites, fertilizer treatments and soil depths on nicotine

4.3.4 Linkage of soil nicotine dynamics with soil moisture, temperature and pH

Dynamics of nicotine is also linked with moisture content as roots go further deep and diverted to access underground water (Hsiao & Xu, 2000). The soils from all sites with fertilizer application and variation in soil depths, the nicotine concentrations increased as the soil depth increased. Chen, Zeng, Singh and Chen (2005) found that the effects of different soil depths (0-60 cm), moistures and their interactions on net N-mineralization rates were significant at $P<0.05$. However, in this study, the net N-mineralization rates significantly decreased with increasing soil depths. Therefore, the increase in nicotine concentrations with

increased soil depths would be attributed with increase in N released for tobacco plant utilization and the dynamism is more strong as tobacco plant grows robust and vigorously (Image 4). Zou (2015) observed similar trend that the tobacco root systems have extensively branched root growth and exhibit highly plastic development to the soil depths penetration. The findings of the present study revealed that with the soil depths (0-50 cm), tobacco nicotine released into soils increased as the rooting depth increased but the increase was also determined by the levels of soil moisture. Thus, the effect of soil moisture on tobacco nicotine dynamics far exceeds the likely negative effect of soil temperature.

Image 4: Root architecture and penetrating depths of a tobacco plant

Soil pH did not really influenced amounts of nicotine retained in soils but more acid soils (low pH) are likely to favour the environment with which more nicotine in retained in soils (Rakić *et al*., 2010). The present study also showed that atmospheric temperatures in Sikonge (29° C), Tabora (27° C), and Urambo (25° C) agro-ecologies differed at relatively low ranges but had much influence on biosynthesis of nicotine in tobacco roots and that released into soils was similar to what was observed by Cheng *et al*. (2018) elsewhere. However, due to relatively higher rains in Sikonge (1050 mm), significantly higher nicotine contents were obtained in deeper (30–50 cm) tobacco root zones relative to shallow depths (0–30 cm) followed by Tabora (950 mm) and Urambo (890 mm) agro-ecologies. In soils where tobacco roots can penetrate beyond 50 cm given that moisture is promising, there are chances that more nicotine will be produced and retained in soils in larger quantities than those realized in

the present study. However, soil moisture should be considered in line with the adoption of appropriate crop production practices that will favour productivity of a tobacco plant. Therefore, based on the findings of this study, nicotine released in the soils observed to increase as soil depths increases, hence nicotine retained in soils may have significant residual impact to the subsequently cultivated crop in the same land. In all sites higher nicotine of 7.59 mg kg^{-1} was found at 30-50 cm, suggesting that shallow rooted crops would be useful as subsequent to tobacco. Nicotine content is less in shallow root zones and cannot disrupt availability of macronutrients such as P, K and proliferation of soil bacteria (Adediran *et al*., 2004; Moula *et al*., 2018)

4.3.5 Regression and correlation of nicotine with soil pH, OC, temperature and moisture

A multiple linear regression analysis results presented in Table 14 of the measured variables initially tabulated in Table 13 generated by regressing nicotine as a response variate (Y) with the fitted terms being constant (C), soil moisture (SM), organic carbon (OC), soil pH, and temperature (T) generated a regression model such that:

Nicotine (Y) = 76.1 + 0.024SM + 6.19OC + 0.042T – 13.13pH;

the coefficient of determination (R^2) accounted for is 84% and the standard error of observations is estimated to be ± 1.01.

Table 14: Multiple linear regression analysis of nicotine as a response variate and the measured variables in soil such as moisture, organic carbon, pH and temperature, as well as constant as the fitted terms

Fitted parameters	Estimate	s.e.	t(4)	t pr.	Variance (%)	Standard error of observations
Constant (C)	76.1	28.9	2.64	0.058		
Temperature (X_i)	0.042	0.216	0.2	0.855		
Soil pH (X_{ii})	-13.13	4.01	-3.27	0.031	84	1.01
Organic carbon (X_{iii})	6.19	7.02	0.88	0.427		
Moisture (X_{iv})	0.024	0.193	0.12	0.908		
Model	Nicotine (Y) = 76.1+ 0.042T+ 6.19OC+0.024SM– 13.13pH					

This model indicates that for every unit increase in soil moisture, organic carbon, and soil temperature the amount of nicotine produced is expected to increase by 0.024, 6.19, and 0.042%, respectively. However, at the same unit increase in soil reaction (i.e. decreases in acidity) the amount of nicotine would decrease by 13.13%. Further correlation analysis, however, clearly indicated that soil moisture (r = 0.57) and organic carbon (r = 0.45) had

positive but not significant relationship with nicotine retained in soils (Table 15). In addition, there was negative correlation between nicotine in soils and soil pH (r = -0.95; *P* = 0.0001) and soil temperature (r = -0.18).

Table 15: Correlation between nicotine and the measured variables in soils

SN	Parameters	Measured variables and their correlations				
		1	**2**	**3**	**4**	**5**
1	Moisture	-				
2	Nicotine	0.57	-			
3	Organic carbon	0.0002	0.45	-		
4	Soil pH	-0.65	-0.95 (0.0001)	-0.34	-	
5	Temperature	-0.83 (0.0054)	-0.17	0.16	0.25	-

Key: In brackets are the *P*-values of significant correlations

4.3.6 Summary results on the levels of nicotine released in soil and their dynamics

Nicotine level at Sikonge was as high as 9.55 mg kg^{-1} compared with Tabora and Urambo which had 6.04 mg kg^{-1} and 4.42 mg kg^{-1}, respectively, implying that nicotine level in soils varied among the different agro-ecologies. Twice the amount of nicotine was released into the soil from fertilized (21.60%) compared with unfertilized (10.07%) plants. However, nicotine concentration was not significantly different in the roots of fertilized (7.29 mg kg^{-1}) compared with unfertilized (7.12 mg kg^{-1}) plants. The dynamics of nicotine in soil was largely dependent on soil moisture and the depth at which tobacco roots can penetrate. Nicotine levels increased as soil moisture and root penetration depth increased in all sites. Therefore, since more nicotine accumulated in deeper soils, shallow (0–20 cm) rooted crops such as lettuce, potato and some maize variety are recommended as a subsequent crop to tobacco because at that depth, nicotine concentration is low, limiting macronutrient availability and the proliferation of soil bacteria.

4.4 To determine adsorption and desorption maximum levels of the released nicotine from tobacco plant by the soil using the best fitting Freundlich Model

4.4.1 Effect of tobacco cultivation on soil pH and nicotine degradation after 8 months

The nicotine released in soil and its residual effects on soil pH shown in Table 16 and Fig. 13. Results showed that soil pH (5.41) was lowered significantly (*P* <0.001) in loamy sand soil, and the nicotine released to this soil was 8.01 mg kg^{-1}. Sandy loam soil had the lowest nicotine (3.81 mg kg^{-1}) with a soil pH of 5.74. At 8 months after harvesting tobacco, soil pH in loamy sand, sandy and sandy loam soils increased from 5.41, 5.43 and 5.74 to 5.47, 5.45,

5.75, respectively. The increase in soil pH was attributed to the nicotine degradation by soil bacteria (Hu, Zhao, Li & Yu, 2019; Xia *et al*., 2019). At a period of 8 months after harvesting tobacco, the soil pH in unfertilized plots increased by 0.02 and nicotine decreased from 2.64 to 0.36 mg kg^{-1}. In the same period, soil pH in fertilized tobacco plots increased by 0.03, and the nicotine dropped from 10.03 to 1.12 mg kg^{-1}. Also, there was a decrease in soil pH with an increase in soil depth 8 months after harvesting tobacco leaves (Table 16).

Table 16: Soil pH and nicotine levels in soils after reaping tobacco leaves and at 8 months before planting maize

Description	At tobacco harvest		Desorbed at 8 months after tobacco harvest		Adsorbed/degraded at 8 months after tobacco harvest
	Soil pH	Nicotine total (mg kg^{-1})	Soil pH	Nicotine (mg kg^{-1})	Nicotine (mg kg^{-1})
Soils:					
Loamy soil	5.41 ± 0.03b	8.01 ± 1.02a	5.47 ± 0.03b	0.91 ± 0.11a	7.11 ± 0.91a
Sandy soil	5.43 ± 0.01b	5.40 ± 0.74b	5.45 ± 0.01b	0.68 ± 0.09b	4.71 ± 0.64b
Sandy loam soil	5.74 ± 0.02a	3.81 ± 0.63c	5.75 ± 0.02a	0.45 ± 0.06c	3.36 ± 0.57c
Fertilizers:					
Uncultivated soils	5.65 ± 0.04a	0.01 ± 0.00d	5.69 ± 0.04a	0.00 ± 0.00d	0.01 ± 0.00c
Unfertilized tobacco	5.56 ± 0.03b	2.64 ± 0.26c	5.58 ± 0.03b	0.36 ± 0.03c	2.28 ± 0.22b
Fertilized tobacco	5.47 ± 0.04c	10.03 ± 0.67b	5.50 ± 0.03c	1.12 ± 0.07b	8.90 ± 0.61a
Fertilized tobacco+SI	5.43 ± 0.04c	10.29 ± 0.62a	5.45 ± 0.04c	1.25 ± 0.06a	9.05 ± 0.56a
Depths (cm):					
0-10	5.61 ± 0.04a	4.79 ± 0.77c	5.62 ± 0.04a	0.54 ± 0.07c	4.25 ± 0.70c
10-30	5.52 ± 0.03b	5.87 ± 0.85b	5.55 ± 0.03b	0.71 ± 0.10b	5.16 ± 0.75b
30-50	5.45 ± 0.03c	6.56 ± 0.94a	5.50 ± 0.03b	0.78 ± 0.11a	5.78 ± 0.83a
3-Way ANOVA F-statistics					
Soils (S)	145.80***	850.56***	114.4***	546.90***	712.42***
Fertilizers (F)	30.70***	3863.19***	33.2***	2863.82***	3161.43***
Depths (D)	25.70***	150.57***	14.9***	155.24***	117.08***
S × F	4.10***	131.40***	6.7***	75.67***	112.60***
S × D	4.40***	26.51***	3.0*	6.89***	29.28***
F × D	1.80ns	46.24***	1.3ns	40.74***	37.36***
S × F × D	9.50***	9.85***	0.6ns	2.10*	10.51***

Values presented are means ± $SE_{\bar{x}}$ (Standard error of means); *, *** = significant at $P \leq 0.05$ and $P \leq 0.001$ respectively; ns = non-significant; SI = tobacco stalks incorporated after reaping the leaves. Means in the same category of evaluated interface sharing similar letter(s) do not differ significantly based on their respective Standard error (SE) at 5% error rate

The nicotine released in soils by tobacco roots and its residuals after 8 months differed significantly (P <0.001) across the soil textures (Table 16). The loamy sand soil had the highest nicotine initially released by the tobacco plants (8.01 mg kg^{-1}) through roots, residual nicotine (0.91 mg kg^{-1}), and the amount of nicotine adsorbed/degraded (7.11 mg kg^{-1}). The sandy soil had 5.40 mg kg^{-1} of nicotine initially released by the tobacco roots, residual nicotine (0.68 mg kg^{-1}), and the nicotine degraded (4.71 mg kg^{-1}). Sandy loam soil was the least with 3.81 kg^{-1} of nicotine released by the tobacco roots, residual nicotine (0.45 mg kg^{-1}), and the nicotine degraded (3.36 mg kg^{-1}). Effects of time exposure revealed that the acidic soil (Rakić *et al*., 2010) as in sandy soil (5.45) adsorbs more nicotine by 12.59% compared with the acidic, loamy sand soil (5.47) which adsorbed 11.36% of the nicotine. However, sandy loam soil with pH 5.75 adsorbed 11.81% of the nicotine. The observed differences in quantities of nicotine could be due to the variability in soil textures. A study conducted by Khairy *et al*. (1990) reported adsorption of nicotine on humic and clayed humic acid complex through the formation of H-bonds, indicating that acidic condition adsorbed nicotine. Furthermore, Mohammad, Amin, Nushad and El-Desoky (2013) observed that nicotine is adsorbed more to the cation exchange sites of the soil. The significantly (P <0.001) highest nicotine (10.29 mg kg^{-1}) was recorded in fertilized soils with the incorporation of tobacco stalks after harvesting the leaves. Fertilized tobacco soils with uprooted stalks after harvesting the leaves were the second for nicotine (10.03 mg kg^{-1}) while unfertilized soils recorded only 2.64 mg kg^{-1} of nicotine. Therefore, it indicates that nicotine released was less in unfertilized tobacco soils. Nicotine adsorbed/degraded was lower in unfertilized tobacco cultivated soils while in fertilized tobacco soils nicotine was high indicating that soil bacteria activities mostly influenced in the adequate soil nutrients (Camenzind, Hättenschwiler, Treseder, Lehmann & Rillig, 2018).

4.4.2 Nicotine adsorption in the soil: Fitting of nicotine sorption data into Freundlich Model

The Freundlich model was used to establish the maximum amount of nicotine adsorbed by soils or degraded by the soil bacteria and the amount nicotine desorbed or readily available such that:

$$\frac{x}{m} = KfP^{\frac{1}{n}} \qquad (1)$$

Where x/m was substituted by A, P by B and C such that;

$$A = KfB^{\frac{1}{n}} \quad (2)$$

And

$$A = KfC^{\frac{1}{n}} \quad (3)$$

The linear logarithmic forms of the above equations were:

LogA = 1/nLogB +LogKf (4)

And

LogA = 1/nLogC +LogKf (5)

Where:

- LogA is the logarithm of total nicotine released by the tobacco roots into the soil (measured immediately after harvesting of tobacco plants),
- LogB is the logarithm of nicotine in soils extracted (desorbed) from soils at 8 months after harvesting tobacco,
- LogC is the logarithm of nicotine assumed to be adsorbed/retained by soils or degraded by bacteria at 8 months after harvesting of tobacco.
- The intercept (*Kf*) is the adsorption capacity of the soil/adsorbent
- The slope (1/n) is the effect of concentration on the soil adsorption capacity and represents adsorption intensity.

4.4.3 Nicotine sorption isotherms

Nicotine adsorption and desorption isotherms based on the quantities of nicotine released by the tobacco roots into the soils upon harvest, is presented in Figs. 14 & 15. The plotting points from the origin (0,0) with depths followed an order of 0-10, 10-30, and 30-50 cm. Fertilizer conditions from the origin (0,0) of plotting followed a specific order. The order is the absolute control plots where no tobacco or any crop planted, unfertilized tobacco plots, fertilized tobacco plots with NPK+CAN, fertilized tobacco plots with NPK+CAN, and tobacco stalks incorporated after harvesting its leaves. Results indicated that the quantities of nicotine adsorbed by the soils and/or partly degraded by the soil bacteria increased with an increase in soil depths. Although all the studied soils indicated a significant increase for nicotine adsorbed with the increase in soil depth, the sandy loam soil showed the best description of the increase (Fig. 14). This finding suggests that the rate of the increase for nicotine adsorbed is dependent on the ratio of the soil particles as sandy and loamy sand soils were the poorer adsorbents than the sandy loam soil. Results also indicated that the soil

depths varying in texture did not significantly increase the quantities of nicotine desorbed from soils. Although these direct sorption isotherms provide a better representation of nicotine desorbed, still the rate of increase in desorption is generally independent of the initial amount of nicotine present in soils (Fig. 14).

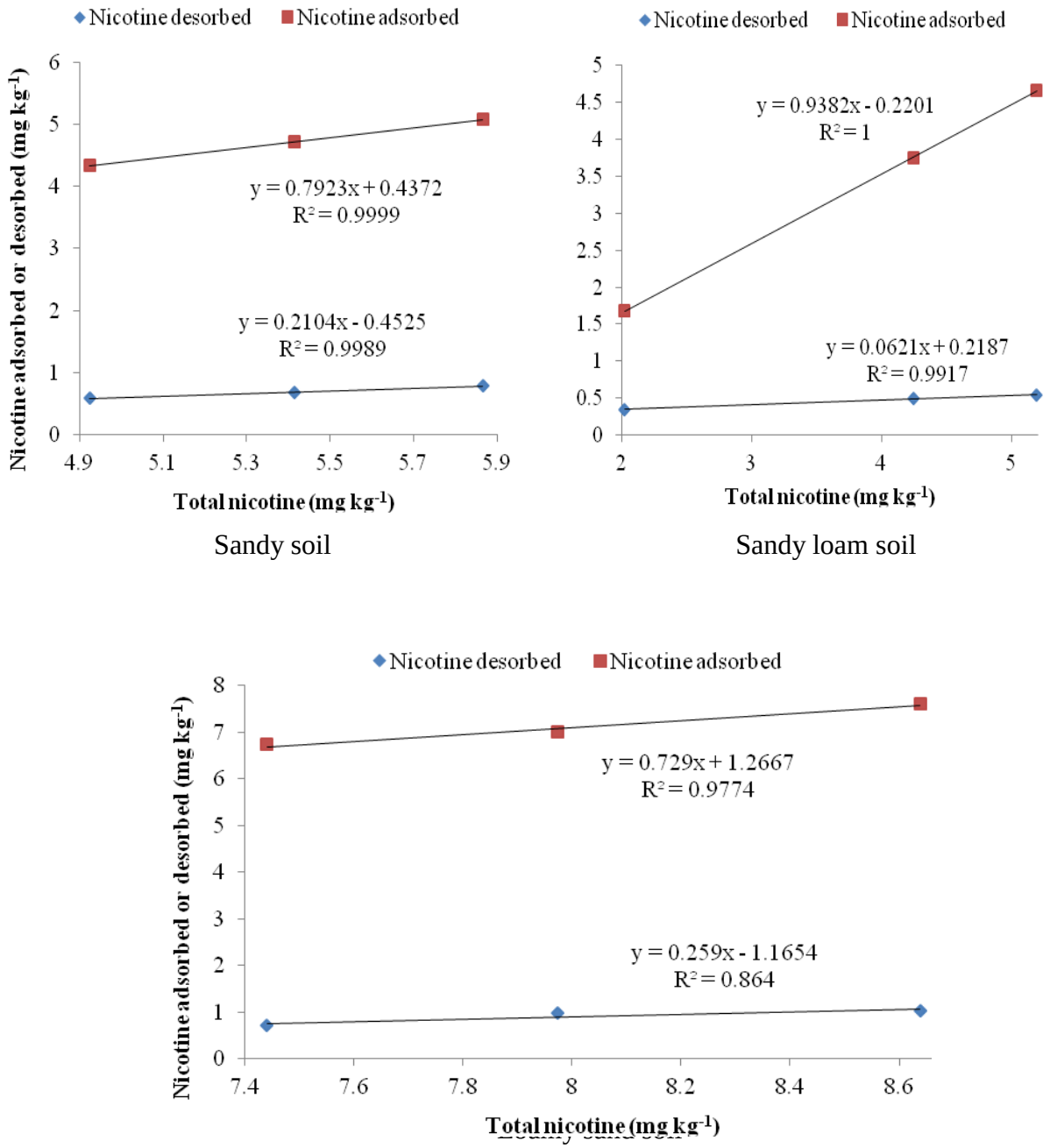
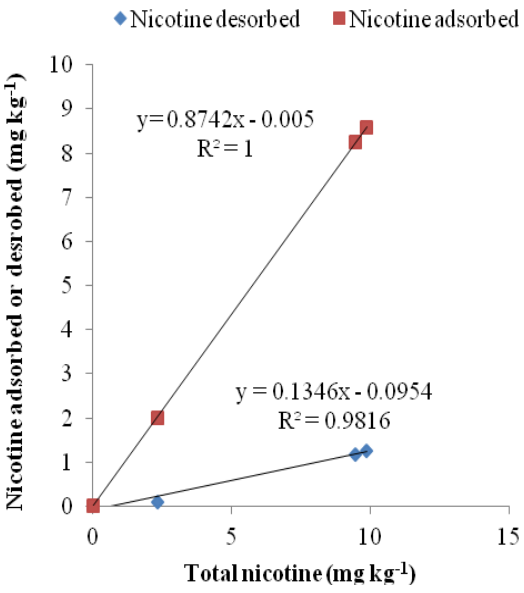


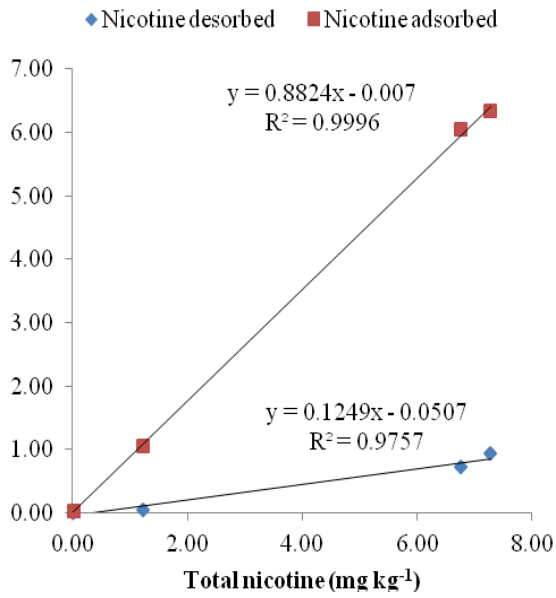
Figure 14: Nicotine adsorption and desorption isotherms as determined by the soil depths (0-10, 10-30, 30-50 cm) of sandy, sandy loam and loamy sand soils

The maximum amounts of nicotine adsorbed in sandy, sandy loam, and loamy sand soils were 8.74, 7.05, and 12.46 mg kg^{-1}, respectively suggesting that soils dominated by the finer

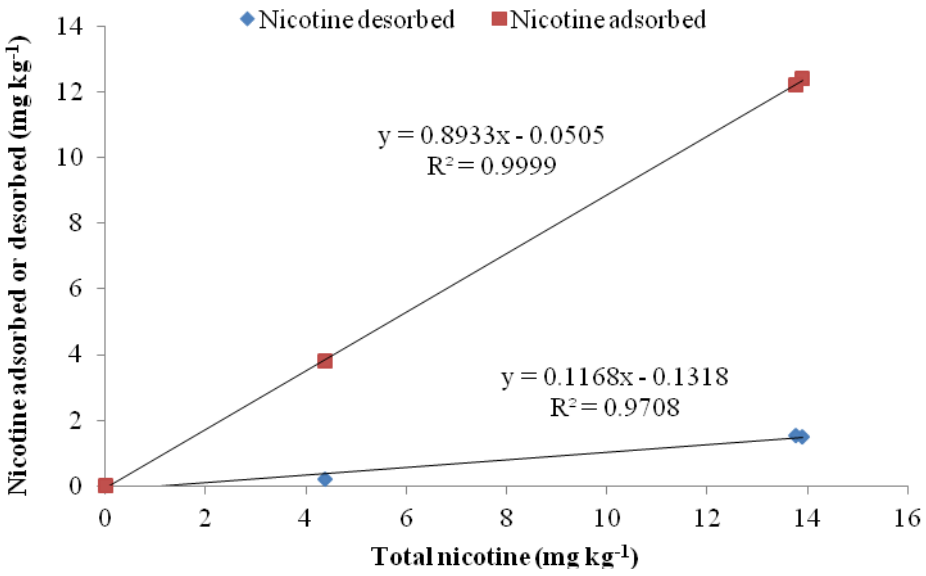
particles adsorb more nicotine than the soils with coarse particles (Fig. 15). On the other hand, the maximum quantities of nicotine desorbed were 1.25, 0.92 and 1.50 mg kg^{-1}, in sandy, sandy loam, and loamy soils, respectively. The quantities of nicotine desorbed followed a similar trend to that of nicotine adsorbed by the studied soils. However, it should be noted that the quantities of nicotine adsorbed could have taken different fates including retention by the soil particles, degradation by the soil bacteria (Hu *et al*., 2019; Xia *et al*., 2019), and transformation to forms which were not detected by the extraction method used in this study. Results also indicated that the quantities of nicotine adsorbed and desorbed in the studied soils following fertilizer application described by the coefficients of determination (R^2) which ranged from 98 to 100%. The adsorption of nicotine is better described in all soils (R^2 =99–100%) compared with the description of the nicotine desorption (R^2 =97–98%) phenomenon (Fig. 15).



Sandy soil



Sandy loam soil



Loamy sand soil

Figure 15: Portraits of nicotine adsorbed and desorbed (y-values in the equations) based on the total nicotine in soils (x-values in the equations) as determined by the fertilizers situations and tobacco cultivation (absolute control plots where no tobacco or any crop planted, unfertilized tobacco plots, fertilized tobacco plots with NPK+CAN, fertilized tobacco plots with NPK+CAN and tobacco stalks incorporated after harvesting its leaves) in sandy, sandy loam and loamy soils

4.4.4 Freundlich sorption isotherms for nicotine

The sorption (desorption and adsorption) isotherms of nicotine as determined by the depths of sandy, sandy loam, and loamy sand soils, is presented in Fig. 16. The effects of fertilizer application on the sorption behaviours of these soils to nicotine released by tobacco is presented in Fig. 17.

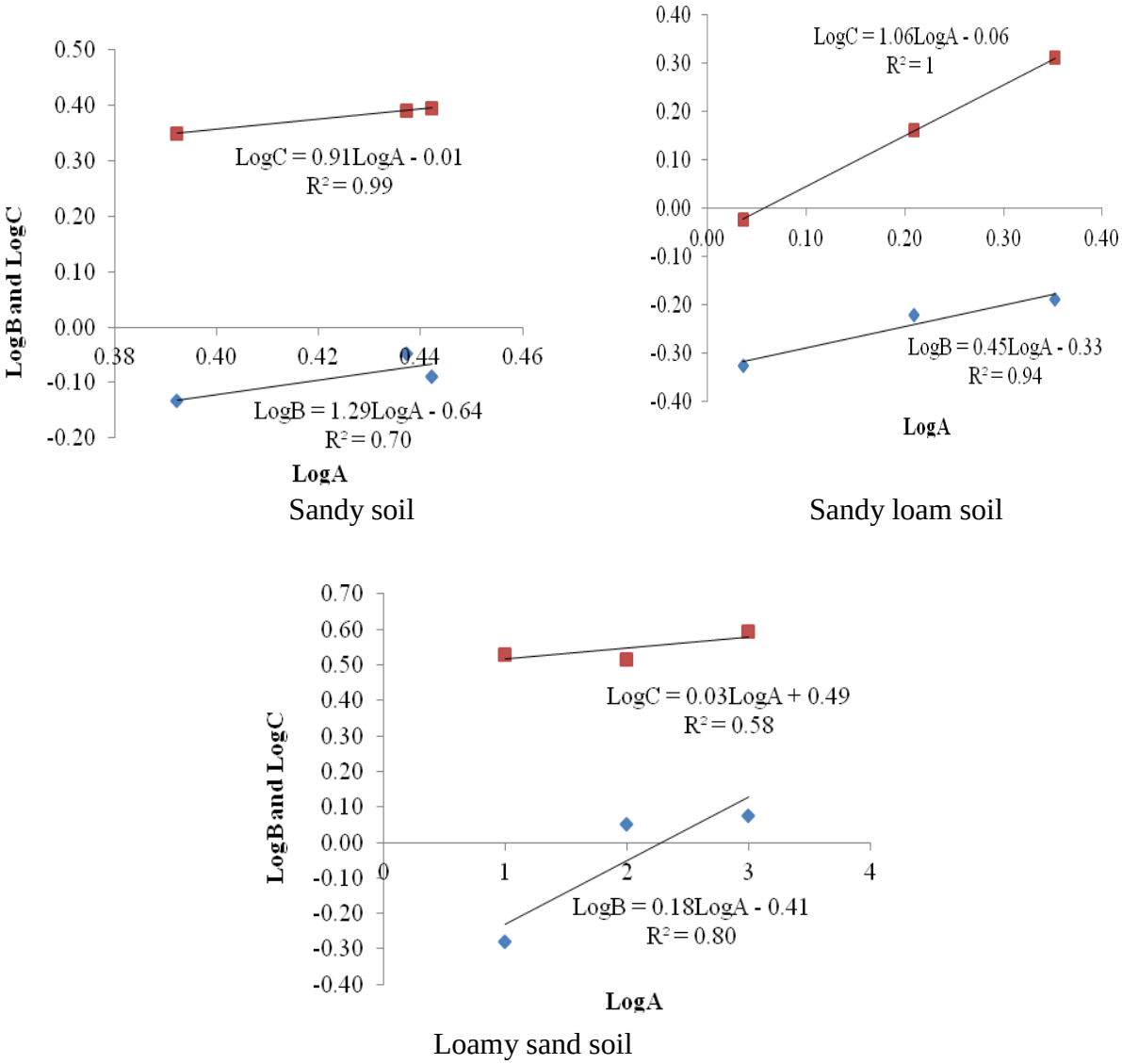
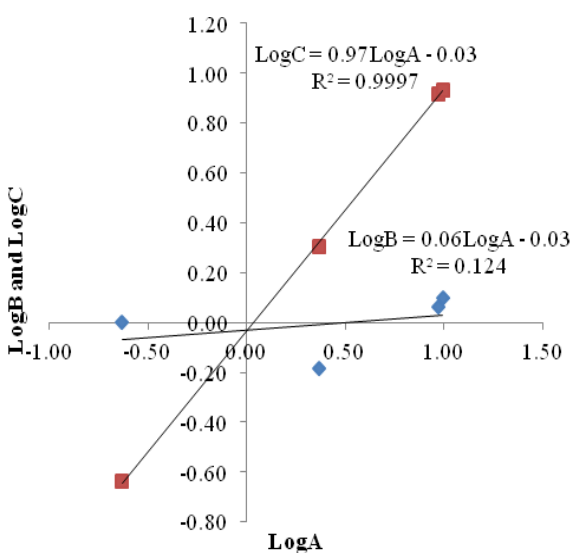
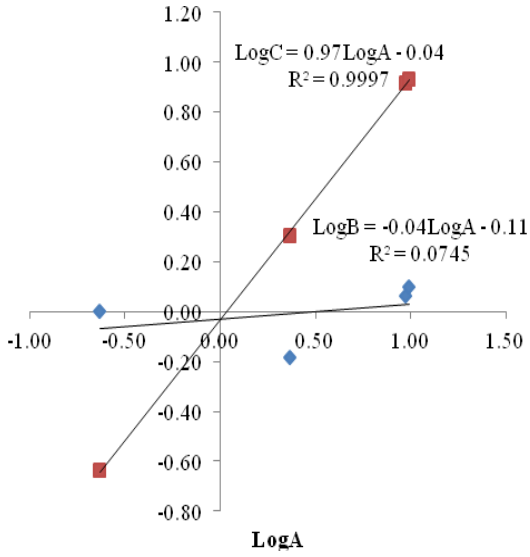


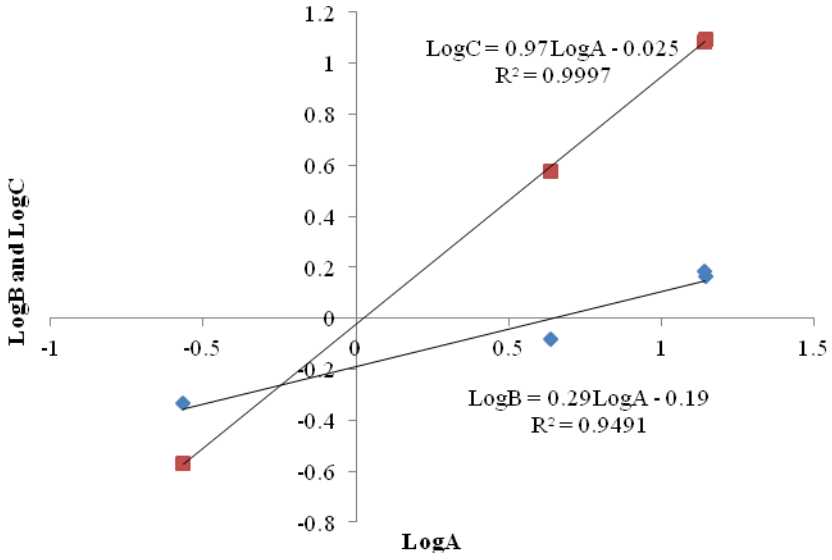
Figure 16: Freundlich sorption (desorption-LogB and adsorption-LogC) isotherms of nicotine as determined by the depths (0-10, 10-30, 30-50 cm) of sandy, sandy loam and loamy soils



Sandy soil



Sandy loam soil



Loamy soil

Figure 17: Freundlich sorption (desorption-LogB and adsorption-LogC) isotherms of nicotine as determined by the fertilizers situations and tobacco cultivation (absolute control plots where no tobacco or any crop planted, unfertilized tobacco plots, tobacco plots fertilized with NPK+CAN, tobacco plots fertilized with NPK+CAN and tobacco stalks incorporated after harvesting its leaves) in sandy, sandy loam and loamy soils

Further, the Freundlich sorption parameters of nicotine as determined by the soil depth and fertilizer application conditions on soils varying in texture, is presented in Table 17. The present study portraits higher magnitudes of Kf (adsorption capacity of the soil/adsorbent) and 1/n (concentration effect on the soil adsorption capacity and its adsorption intensity)

showing the natural separation of nicotine from the aqueous soil solution and indicates favourable adsorption in sandy and sandy loam soils compared with the loamy soil. Basher, Gupta and Chattre (2013) indicated that the intercept Kf value is an indication of the adsorption capacity of the adsorbent (e.g. soil) and the slope 1/n indicates the effect of concentration on the adsorption capacity and represents adsorption intensity. Further, 1/n also presents a measure of the deviation from the linearity of the adsorption and is used to verify the type of adsorption (Adnadjevic, Lazarevic & Jovanovic, 2009).

Results indicated that the highest maximum nicotine (2.81–4.61 mg kg^{-1} soil) was adsorbed in soils with sand texture characteristics as opposed to the lowest amount of nicotine adsorbed (0.72 mg kg^{-1} soil) in a loamy soil as affected by the soil depth parameter (Table 16). The highest maximum nicotine adsorbed in sandy soils is not well explained by the nicotine-bonding energies (1/n- values) although they display similar phenomena. The highest maximum nicotine adsorbed in soil, and the fertilizer conditions are ranging from 1.66–2.21 mg kg^{-1} soil. This is compared with the amount of nicotine desorbed in sandy soil as determined by the soil depth (0.45 mg kg^{-1} soil) and fertilizer conditions (0.18 mg kg^{-1} soil) (Table 21). Further, the maximum amount of nicotine desorbed was highest in soils with loamy texture characteristics as affected by both soil depth (0.89–1.12 mg kg^{-1}) and the maximum amount of nicotine desorbed in the loamy sand soil is higher than that adsorbed suggesting that much of nicotine are retained on the finer soil particles and less in the underlying soils.

Table 17: Freundlich sorption parameters of nicotine as determined by soil depth and fertilizer application on soils varying in textures

Treatments	Freundlich parameters	Soils					
		Sandy soil		Sandy loam soil		Loamy sand soil	
		Adsorption	Desorption	Adsorption	Desorption	Adsorption	Desorption
Soil depth	Y-(logKf)	-0.01	-0.64	-0.06	-0.33	0.49	-0.41
	Kf (mg kg^{-1})	4.61	0.45	2.81	1.12	0.72	0.89
	Slope (1/n)	0.19	1.29	1.06	0.45	0.03	0.18
	R^2	0.9997	0.6979	1.0000	0.9417	0.5812	0.7996
Fertilizers	Y-(logKf)	-0.03	-0.03	-0.04	-0.11	-0.025	-0.19
	Kf (mg kg^{-1})	0.18	0.18	3.22	2.21	3.69	1.66
	Slope (1/n)	0.97	0.06	0.97	-0.04	0.97	0.29
	R^2	0.9997	0.1240	0.9997	0.0745	0.9997	0.9491

Note: Kf (mg kg^{-1}) represents the maximum amount of nicotine adsorbed or desorbed/degraded in soils; Y-(logKf) is the intercept of the model; 1/n is the slope/gradient of the model; R^2 is the coefficient of determination

The relationships observed using the fitted Freundlich model between equilibrium nicotine concentrations and the nicotine-sorbed by the studied soils were linear (Figs. 16 & 17 and Table 17). Most values of the exponents (1/n) are less than one (1/n <1), which are also related to the type and nature of clay minerals found in soils differing in texture among other characteristics of the soils (Fytianos, Voudrias & Bozani, 2002). The values of the nicotine-binding energies suggest that the binding sites are more homogeneous indicating a high adsorptive capacity of the soil at high equilibrium concentrations of nicotine (Goncalves *et al*., 2013; Freitas, Netto, Correa, Xavier & Assis, 2018).

In the Freundlich equation model, the adsorption or desorption maximum (Kf) could be considered as a capacity factor associated with the coefficients of determination (R^2) implying that a soil having larger Kf-value has larger adsorbing capacity than a soil having smaller Kf-value (Hussain, Ghafoor, Anwar-Ul-Haq & Muhammad, 2003). Also, the R^2 explained well the suitability of the modified Freundlich equation model to the nicotine-sorption capacities of the studied soils by greater than 50%. Some exceptions observed on the desorption isotherms fitted for the data involved in sandy and sandy loam soils without or with the application of fertilizers. This finding suggests that soil texture, perhaps sandy characteristic, is an essential element to consider in fitting the Freundlich model for

contaminants desorbed by the soils as adsorbents. The R^2 values did not reverse from the Kf-values. The R^2 values demonstrated that linear fits for the adsorbed nicotine are very close for depths on one-side and fertilizer conditions on the other, except in the loamy sand soil where R^2 was equal to 0.5812. However, there is no closeness fit for the desorbed nicotine among depths and/or fertilizer conditions as determined by the R^2 values (Table 17). These findings are supported by other studies (Khairy *et al*., 1990; Sidhu, Narwal & Brar, 2004; Thakur, Tomar & Pandeya, 2004; Hannan, Ranjha, Rahmatullah & Niaz, 2007; Lazarevic, Jovanovic, Jevremovic, Nikolic & Adnadjevic, 2010; Rakić *et al*., 2010), which claim that the Freundlich isotherm describes better the adsorption of the data compared with other models like the Langmuir model. The Freundlich equation also fits best with low concentrations (Wu, Wu, Tseng & Juang, 2014).

Soil texture and the acidic condition reported having effects on various contaminants adsorbed and desorbed by the material (Rakić *et al*., 2010; Hanson, Cross, Bond & Jenkins, 2017). The soils with higher fractions in sand and silt particles are less attractive to contaminants compared with fine-textured soils as finer particles (e.g. clays) are electrically charged (Hanson *et al*., 2017). The sandy loam and loamy sand soils in the present study showed relatively higher nicotine desorption capacity compared with the sandy soil suggesting that finer soil particles have less bonding energies to nicotine hence easy of removal. According to Falciglia, Giustra and Vagliasindi (2011), soil texture influences contaminant sorption phenomena and remediation processes in desorption treatment. Falciglia *et al*. (2011) also indicated that the fine sandy soil exhibited the greatest extent of desorption. The findings of our study (Table 16) suggest that the adsorption of nicotine in soils is depended mainly on acidic soil levels (Rakić *et al*., 2010). The findings of the present study also suggest that the nicotine adsorbed by sandy, sandy loam, and loamy sand soils is likely to have residual effects on the subsequent crops and/or to the soil bacteria and thus require more studies to address options for remediation of these effects.

The present study revealed that nicotine adsorption in soils differing in texture favoured acidic soils. The direct and Freundlich fitted models were able to fit well the nicotine sorption isotherms, thereby generating the adsorption and desorption parameters in soils differing in texture. The Freundlich model showed more excellent proximity to the directly fitted experimental data. The quantities of nicotine adsorbed or degraded by the soil bacteria are dependent on the initial amount of nicotine produced by the tobacco plant, soil texture,

rooting depths of the tobacco plants, and use of NPK+CAN fertilizers and incorporation of tobacco stalks back to the soil after harvest. On the contrast, nicotine desorption is not directly linked to soil depths, soil texture and fertilization conditions but the Freundlich desorption isotherms present some useful fits of the data. Findings for this study, imply that the adsorbed nicotine is likely to have residual effects on the soil bacteria and/or to the subsequent crops hence a need for further study that addresses options for remediation of these effects.

4.4.5 Mitigation measures to reduce or remove residual nicotine from soils

Nicotine occurs naturally in smaller amounts varying from 0.002 to 0.007 mg kg^{-1} of soil or dry weight of a commodity (Domino, Hornbach & Demana, 1993). Previous studies indicated that there was no maximum residue level (MRL) set for nicotine in soils, but the official default set as 0.05 mg kg^{-1} soil by 2013 (Commission Regulation, 2013; Selmar *et al*., 2015). The findings indicated that the naturally occurring nicotine in uncultivated soils involved in the present study was 0.01 mg kg^{-1} soil. In soils where tobacco cultivated without application of any treatment, the nicotine increased from 0.01 to 2.64 mg kg^{-1} soil. Application of NPK + CAN fertilizers in tobacco-cultivated soils increased the nicotine from 2.64 to 10.03 mg kg^{-1} soil. Further, the average nicotine content recorded was 10.29 mg kg^{-1} soil following an application of NPK + CAN fertilizers and tobacco stalks incorporated back after harvesting the leaves.

Therefore, the alternative to intervene with this environmental effect caused by nicotine could be to cultivate non-food plants which are capable of reducing nicotine levels if the main food crop cultivated after tobacco. The fastest-growing inedible leguminous sunn hemp (*Crotalaria juncea* L.) can be planted soon after the tobacco crop has been harvested to intercede the tobacco crop and the intended main food crop. If a cereal crop like maize, for instance, is cultivated after the sun hemp next to tobacco, there are higher chances of both yield and health benefits derived from this technique (Lisuma *et al*., 2019). The practice will allow a cereal crop to escape coinciding with the extreme levels of nicotine in the same field. Furthermore, sun hemp will resist the effects of root-knot nematodes that would retard the performance of a cereal crop (Cook & White, 1996). Also, the subsequent cereal crop can benefit from improved soil nutrients and the fixed N in soil by the sun hemp (Márton, 2010).

4.4.6 Summary results on nicotine adsorption in soil after being released by the tobacco roots

Results showed that nicotine adsorption by the studied soils were increased by the soil acidity of the studied soils. The fitted Freundlich model of nicotine sorption isotherms indicated that the maximum nicotine adsorbed based on the soil depths (0–50 cm) ranged from 2.81 to 4.61 mg kg^{-1} in sandy loam and sandy soils. The maximum nicotine desorption at the same soil depths ranged from 0.89 to1.12 mg kg^{-1} in loamy sand and sandy loam soils. Application of $N_{10}P_{18}K_{24}$ and CAN 27% fertilizers recorded the maximum nicotine adsorption ranging from 3.22 to 3.69 mg kg^{-1} in sandy loam and loamy sand soils. Further, the desorption maximum of nicotine due to the effect of fertilizers ranged from 1.66 to 2.21 mg kg^{-1} in loamy sand and sandy loam soils. In conclusion, nicotine adsorbed by the soils is dependent on the soil reaction, textures, and fertilizer application and/or incorporation of tobacco stalks. This nicotine could have residual effects on the soil bacteria and/or to the subsequent crops hence, there is a need of including plants like sunn hemp (*Crotalaria juncea* L.) to intercede the tobacco crop and cereal food crops. This practice is expected to reduce or remove nicotine in soils to the lowest threshold of 0.05 mg kg^{-1} soil.

4.5 To investigate the effects of tobacco nicotine on availability of soil nutrients under fertilization

4.5.1 Effects of tobacco cultivation on soil reaction, organic carbon and nicotine

Results on the effects of tobacco cultivation and fertilizer application on soil pH, OC and nicotine before and after experiment are presented in Table 18. Soil pH for Sikonge, Tabora and Urambo were significantly different across the sites. The highest pH (5.79) was observed in Urambo. This was followed by Sikonge (5.58) and Tabora (5.47). Comparing the soil pH taken before the establishment of tobacco in the field (5.75), and the records taken after fertilization with NPK and CAN (5.52), the soil pH dropped by 0.23 units whereas in unfertilized plots (5.57) there was a drop of 0.18 units. Comparison between unfertilized tobacco (5.57) and fertilized tobacco (5.52) showed a pH drop of 0.05 units. Furthermore, results from this study showed significant interactions between sites and fertilizer application on soil pH. The pH of Sikonge soils was significantly reduced from 5.89 before experiment to 5.44 and 5.41 after unfertilized and fertilized tobacco harvesting respectively. Soil pH for Tabora was not affected significantly by tobacco cultivation and fertilization process when

compared with measurements taken before the field experimentation. At Urambo site, the soil pH was reduced significantly by the fertilization process (Fig. 18a).

[a] [b]

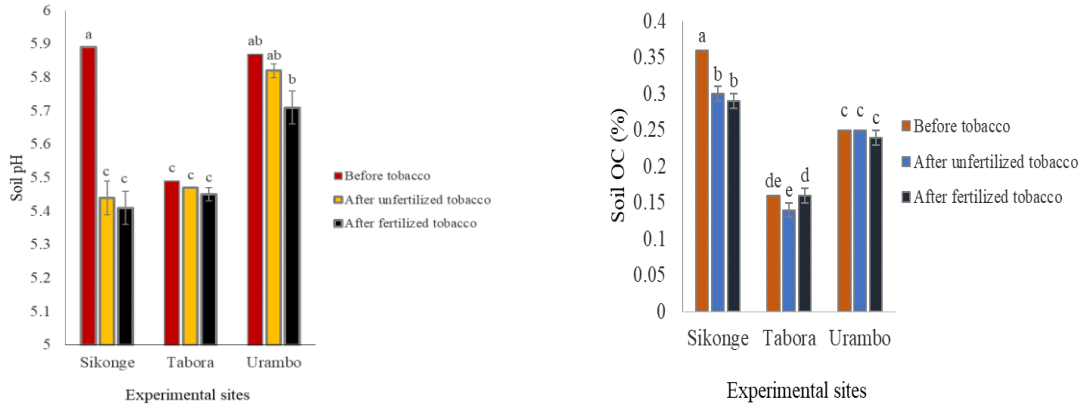


Figure 18: Effect of tobacco cultivation and fertilizer supply on soil pH and OC

Table 18: Selected soil macronutrients and properties of Sikonge, Tabora and Urambo experimental sites before and after experiments

Assessments	Soil measured variables								
	Soil pH	Organic carbon	Nitrogen	Nicotine	Phosphorus	Sulphur	Potassium	Calcium	Magnesium
		(%)			(mg kg^{-1})			(cmol(+) kg^{-1})	
Site:									
Sikonge	5.58 ± 0.09 b	0.32 ± 0.01 a	0.06 ± 0.00 a	5.93 ± 1.92 a	37.87 ± 1.42 a	4.80 ± 1.08 a	0.52 ± 0.00 a	1.56 ± 0.09 a	0.27 ± 0.01 a
Tabora	5.47 ± 0.00 c	0.15 ± 0.01 c	0.04 ± 0.00 b	3.97 ± 1.45 b	28.14 ± 6.32 c	3.45 ± 1.16 c	0.24 ± 0.01 b	0.95 ± 0.00 c	0.21 ± 0.01 b
Urambo	5.79 ± 0.03 a	0.25 ± 0.00 b	0.04 ± 0.00 b	1.51 ± 0.48 c	29.36 ± 3.77 b	3.52 ± 1.17 b	0.23 ± 0.00 b	1.25 ± 0.21 b	0.26 ± 0.01 a
Treatment:									
Soil before tobacco[+]	5.75 ± 0.06 a	0.25 ± 0.03 a	0.04 ± 0.00 c	0.01 ± 0.00 c	47.09 ± 1.58 a	8.47 ± 0.16 a	0.36 ± 0.04 a	0.60 ± 0.18 c	0.26 ± 0.01 a
Soil after tobacco – unfertilized[+]	5.57 ± 0.07 b	0.23 ± 0.02 b	0.05 ± 0.00 b	2.71 ± 0.52 b	23.81 ± 3.03 b	1.59 ± 0.24 c	0.32 ± 0.05 b	1.41 ± 0.09 b	0.23 ± 0.01 b
Soil after tobacco – fertilized	5.52 ± 0.05 b	0.23 ± 0.02 b	0.06 ± 0.00 a	8.69 ± 1.44 a	24.56 ± 2.77 b	1.72 ± 0.26 b	0.31 ± 0.05 b	1.76 ± 0.03 a	0.24 ± 0.01 b
2-Way ANOVA F Statistic									
Site (S)	25.09***	297.91***	48.20***	543.63***	226.13***	2442.6***	1561.49***	112.34***	25.09***
Treatment (T)	12.74***	9.46***	35.00***	2180.80***	1426*42***	65267.4***	31.20***	426.44***	6.73**
S × T	5.48**	7.68***	2.3ns	242.32***	197.45***	42.5***	9.32***	37.19***	2.81ns

Values presented are means ± SE (Standard Error); * ** *** significant at *$P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$ respectively*; ns non significant; Means in the same category of evaluated interface sharing similar letter(s) do not differ significantly based on their respective Least Significance Difference (LSD) value at 5% error rate.

[+] Details of correlation between soil parameters and bacterial diversity indices, multiple regression between bacteria diversity and soil parameters are shown in Appendix I Table 32-33.

Results indicate that tobacco had influence in dropping soil acidity to all sites. An increase in acidity (P <0.001) from 5.75 to 5.57 pH value was observed when tobacco was cultivated without fertilization. This indicates that there was an increase of H^+ concentration by 1.8. In principle for every 1-unit decrease in pH there is 10 times more much active H^+. An increase in acidity to 5.52 in soils where tobacco was cultivated with fertilization relative to 5.75 in non-tobacco cultivated soils, indicated that there was an increase of H^+ concentration by 2.3. Soil pH decreased from 5.57 to 5.52 with increase in acidity by 0.5 between tobacco cultivated without fertilization and with application of $N_{10}P_{18}K_{24}$ and CAN 27% fertilizers. This suggests that, the increase in soil acidity could be caused by the effect from tobacco plant. Other unclearly documented fates of other acid forming cations such H^+, and Al^{3+} in soils could have also increased acidity (Landon, 1991). The increase in acidy of the tobacco fertilized is higher than unfertilized tobacco cultivated soils. This substantiates the importance of nutrients N, P, K, and Ca on the growth of tobacco plant and its ability to increase acid forming cation (H^+) in soils through exudates of nicotine. Nicotine increases H^+ and reduces soil pH which is a dynamic and master parameter of all other soil parameters and biological population as well as their activities. Soil pH changes may result into significant spatial (Behera & Shukla, 2015) or/and temporal differences (Kairuki *et al.*, 2010).

The OC for Sikonge, Tabora and Urambo were significantly different across sites. The higher OC values were recorded in Sikonge (0.32%) followed by Urambo (0.25%) and Tabora (0.15%). The organic carbon in the soil decreased significantly by planting tobacco and supplying fertilizers (Table 18). For instance, there was a significant reduction in OC content from 0.25% to 0.23% by just cultivating tobacco and fertilizing tobacco with $N_{10}P_{18}K_{24}$ and CAN 27%. Furthermore, significant interactions were observed between sites and cultivating tobacco and fertilizer application. OC for Sikonge was significantly higher than the other two sites. The lowest organic matter content was reported in Tabora and followed by Urambo (Fig. 18b).

Across the sites OC differed significantly, despite of all experimental sites having low levels of OC, Sikonge at least had higher level of OC, followed by Urambo and Tabora which had the lowest level of OC. The OC decreased significantly (P <0.001) by 8% from 0.25% to 0.23% before installation of experimentation in unfertilized tobacco cultivated soils (Table 18). The difference in OC observed before experimentation and unfertilized or fertilized tobacco was similar. This is probably attributed to the inherent low OC of these soils and the

less time which was not enough for the tobacco resides and some weeds to decompose before sampling was done (Farooq *et al*., 2014).

Soil nicotine for Sikonge, Tabora and Urambo were significantly different across the sites. The highest soil nicotine of 5.93 mg kg^{-1} was observed in Sikonge, followed by Tabora (3.97 mg kg^{-1}) and Urambo (1.51 mg kg^{-1}). Before establishment of tobacco, soil nicotine was negligible (0.01 mg kg^{-1}). However, after harvesting unfertilized tobacco, the soil nicotine increased to 2.71 mg kg^{-1}. Upon tobacco fertilization with NPK and CAN, nicotine in soil increased significantly (P<0.001) to 8.69 mg kg^{-1}. Results showed significant interactions between sites and fertilizer application on soil nicotine. The highest increase of nicotine was observed in Sikonge soil with dramatic increase of nicotine from 0.01 mg kg^{-1} to 4.66 and 13.13 mg kg^{-1} for unfertilized and fertilized tobacco soils, respectively. Soil nicotine for Tabora soil increased from 0.01 mg kg^{-1} to 2.29 and 9.63 mg kg^{-1} for unfertilized and fertilized tobacco soils, respectively. The lowest increase of nicotine in soils observed in Urambo with an increase from 0.02 to 1.19 mg kg^{-1} and 3.31 mg kg^{-1} for unfertilized and fertilized tobacco soils, respectively (Fig. 19a).

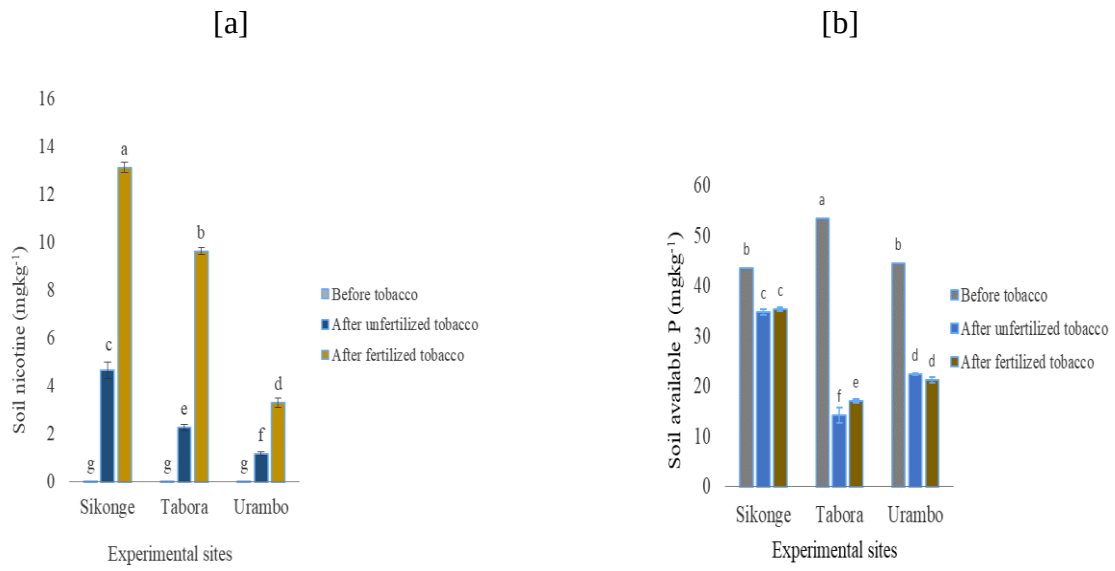


Figure 19: Effect of tobacco and fertilization on soil nicotine and soil P

Nicotine level in soils differed significantly across the sites (Table 18). Sikonge site had higher levels of nicotine (5.93 mg kg^{-1}) released in the rhizosphere followed by Tabora (3.97 mg kg^{-1}) and Urambo (1.51 mg kg^{-1}). These variations in nicotine was linked to the atmospheric temperature, the higher the atmospheric temperature, the higher the nicotine released in soils. Sikonge had higher atmospheric temperature of 29°C, followed by Tabora

and Urambo which had 27°C and 25°C respectively, and hence correlated with nicotine levels in the soils. Similar findings were also reported by Cheng *et al*. (2018) on high atmospheric temperature to induce nicotine biosynthesis and release to the soil environment. In fertilized tobacco cultivated soils, amount of nicotine increased from 0.01 to 8.69 mg kg^{-1} relative to the nicotine in soils before experimentation. Compared with the soils before the experiment (0.01 mg kg^{-1}), unfertilized tobacco increased nicotine content significant (P <0.001) to 2.71 mg kg^{-1} equivalent by 270% in soil. This suggested that the additional of nicotine into these soils was from tobacco plants. In addition, cultivation of tobacco in fertilized soils increased nicotine by 868% relative to that in soils before experimentation, and by 598% compared with unfertilized tobacco cultivated soils. These findings depict that application of NPK and CAN fertilizers resulted into increase in nicotine by 598% compared with cultivation of tobacco without fertilization in these soils.

4.5.2 Effects of tobacco cultivation on selected macronutrients before and after experiment

Total soil N (0.06%) for Sikonge was significantly higher (P<0.001) when compared with total soil N for Tabora and Urambo which had 0.04% each (Table 18). Total soil N increased significantly (P<0.001) from 0.04% for measurements taken before tobacco cultivation to 0.05% for treatments taken before tobacco cultivation. Furthermore, total soil N increased significantly to 0.06% in fertilized plots measured after tobacco cultivation. There were no interactive effects between sites and treatments for total soil N. The increase in soil total N could have been attributed to the nicotine released in the soils, that inhibited growth of soil bacteria by converting nitrate into inorganic form and hence N mineralization rate reduced to cause an increase of soil total N (Farooq *et al*., 2014). Furthermore, an increase of total N in the soils could be as a result of released nicotine accumulation in the rhizosphere of which one of its forming component is N. Thus, released nicotine in soils could also be mineralized and increase N in soils. It is obvious that tobacco plant creates the environment for increasing N in soils for its own advantage as this mineral is one of its nicotine component synthesized at the roots after being absorbed.

Available soil P was significantly (P<0.001) different across the three sites (Table 18). The highest available soil P of 37.87 mg kg^{-1} was recorded in Sikonge, followed by 29.36 mg kg^{-1} in Urambo and 28.14 mg kg^{-1} in Tabora. Soil samples collected after planting of tobacco without fertilization and fertilization reduced the available P significantly (P<0.001) from

47.09 mg kg^{-1} to 23.81 mg kg^{-1} and 24.56 mg kg^{-1} respectively. Significant interactions between sites and fertilizer application were observed in this study. Available soil P in Sikonge was reduced significantly (P<0.001) from 43.48 mg kg^{-1} before planting tobacco to 34.8 mg kg^{-1} in unfertilized tobacco with a little increase of 35.3 mg kg^{-1} after harvesting fertilized tobacco. Tabora site before tobacco cultivation had 53.31 mg P kg^{-1}. Data collected after harvesting tobacco in unfertilized plots showed that soil P was reduced significantly (P<0.001) to 14.22 mg kg^{-1} and increased slightly to 17.11 mg kg^{-1} in soil for fertilized tobacco plots. Urambo site showed a significant (P<0.001) decrease in P levels in the soil from 44.41 mg kg^{-1} before planting tobacco to 22.43 and 21.24 mg kg^{-1} in unfertilized and fertilized tobacco plots respectively (Fig. 19b).

Extractable soil S for Sikonge, Tabora and Urambo were significantly (P<0.001) different across the sites (Table 18). Sikonge site had the highest significantly extractable soil S (4.80 mg kg^{-1}) followed by Urambo (3.52 mg kg^{-1}) and Tabora (3.45 mg kg^{-1}). Before tobacco cultivation, the extractable S in the soil was 8.47 mg kg^{-1} and after cultivation, the extractable S in soil unfertilized tobacco plots was reduced significantly (P<0.001) to 1.59 mg kg^{-1}. Following fertilization of tobacco, extractable S in the soil increased significantly to 1.72 mg kg^{-1} when compared with unfertilized tobacco soil. Interaction between sites and treatments indicated that, extractable S levels were significantly decreased (P<0.001) in all experimental sites after tobacco cultivation (Fig. 20a). Before tobacco cultivation, extractable S in the soil were high; 9.12, 8.09, 8.19 mg kg^{-1} for Sikonge, Tabora and Urambo, respectively. However, after tobacco cultivation and harvesting, in unfertilized tobacco, extractable S were reduced to 2.54, 1.06 and 1.16 mg kg^{-1} for Sikonge, Tabora and Urambo, respectively. After tobacco cultivation and harvesting of tabacco, the application of fertilizer in tobacco increased soil extractable S in Sikonge (2.74 mg kg^{-1}) and Tabora (1.21 mg kg^{-1}), while in Urambo the extractable soil S was not significantly different in fertilized and unfertilized plots.

[a] [b]

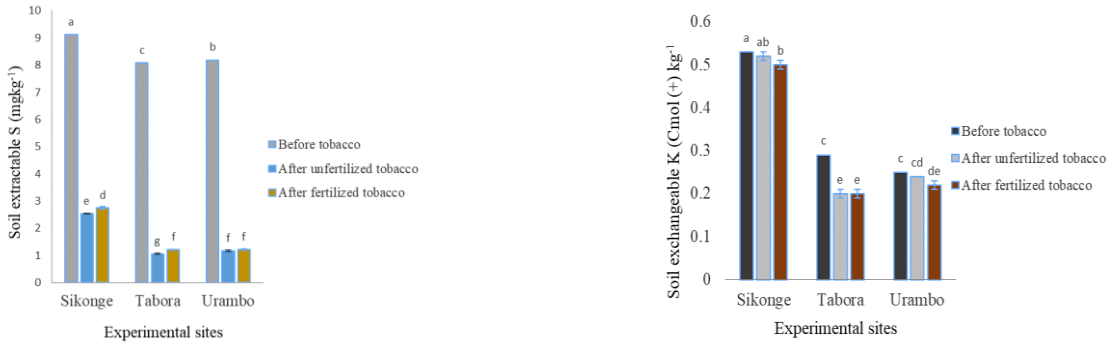


Figure 20: Effect of tobacco and fertilization on soil S and K

Exchangeable K for Sikonge soil was significantly (P<0.001) different when compared with Tabora and Urambo sites (Table 18). Exchangeble K in soils were 0.52 cmol (+) kg^{-1}, 0.24 cmol (+) kg^{-1} and 0.23 cmol (+) kg^{-1} for Sikonge, Tabora and Urambo respectively. Results from this study showed that soil samples collected after planting of tobacco without fertilization and with fertilization reduced soil K significantly (P<0.001) from 0.36 to 0.32 and 0.31 cmol (+) kg^{-1} respectively. There were interactive effects between sites and treatments for exchangeable soil K. The exchangeable soil K were higher in Sikonge and significantly lower in Tabora and Urambo respectively (Fig. 20b).

Cultivating tobacco with no fertilizer application resulted into reduced S, P, Mg, and K by 81, 49, 12, and 11% respectively. Released nicotine in the soils affect the soil chemistry and the levels of macronutrients, since nicotine is acidic, when mineralized influence solubilization of S, P, K and Mg to be readily available to the tobacco plant. Genetically, tobacco plant absorbing more of these nutrients for tobacco growth, seed formation, development and metabolism (Zhu & Lynch, 2004; Xu *et al*., 2008; Höller *et al*., 2010; Farooq *et al*., 2014) and hence leave very little nutrients to the soils. Application of NPK and CAN fertilizers elevated soil levels of N, P, S, Ca, and Mg by 20, 3, 8, 25, and 4% respectively relative to unfertilized tobacco cultivated soils (Table 18). These findings suggest that in situations where tobacco effect is masked by the application of N, P, K, and Ca nutrients, their expression as a magnitude of increase is also realized. It is also likely that apart from their deficiencies in soils, the availability of these nutrients is enhanced by the phyto-effect from tobacco roots (Smith, 2009; Reed *et al*., 2011). Interestingly, fertilization application in tobacco cultivated soils resulted into a decrease in soil available P, extractable S, exchangeable K, and Mg by 48, 80, 14, and 8%, respectively. The decrease of these nutrients

in soils when compared with unfertilized and fertilized scenarios, gave a signal that tobacco is a heavy nutrient feeder crop.

Soil exchangeable Ca differed significantly (P<0.001) across the sites. Sikonge had the highest soil exchangeable Ca of 1.56 cmol (+) kg^{-1} followed by Urambo (1.25 cmol (+) kg^{-1} in soil) and Tabora 0.95 cmol (+) kg^{-1}. The exchangeable Ca in soils was increased significantly (P<0.001) by cultivating tobacco with and without fertilization. Calcium levels in the soil before tobacco cultivation increased from 0.60 cmol (+) kg^{-1} to 1.41 and 1.76 cmol (+) kg^{-1} in unfertilized and fertilized plots respectively. Interactions between sites and treatments on soil exchangeable Ca was significant at P<0.001 (Fig. 21). In all sites, soil exchangeable Ca were significantly higher in soils collected in fertilized tobacco plots followed by unfertilized plots.

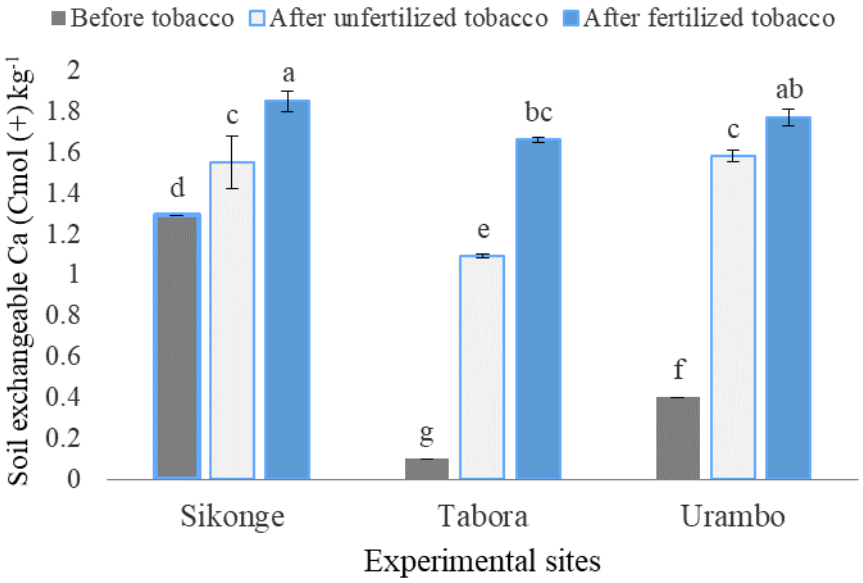


Figure 21: Effect of tobacco and fertilization on soil Ca

Calcium (Ca^{2+}) increased in the soil media due to the increase of soil acidity which decomposed initial levels of OC to these soil and release more Ca^{2+}. Through the decomposition resulted into lowering OC in soils (Fig. 18b). Hermiyanto, Winarso & Kusumandaru (2016) reported OC in soil to have great impact in improving biological, physical and chemical properties in the soil. Gulser, Demir and Ic. (2010) when incorporated tobacco wastes at different incubation periods, observed changes in soil properties including OC, indicating that nicotine has ability in modifying soil properties. The increase in total N by 50% and exchangeable Ca by 193% as a result of fertilization, suggesting that these

nutrients increased in soil from NPK and CAN fertilizers. However, the tobacco effect on the increase in exchangeable Ca^{2+} in the studied soil was by 1157% and for NPK and CAN fertilization was only 193%.

4.5.3 Relationships between nicotine contents in soils and macronutrients

Table 19 shows a multiple linear regression analysis results. Regressing soil nicotine as a response parameter (Y) while other macronutrients being constant N, K, Ca, Mg, S and P expressed a model as follows;

$$\text{Nicotine (Y)} = 21.57 + 327.29N + 47.71K - 15.96Ca - 8.15Mg - 1.46S - 0.61P$$

Table 19: A multiple linear regression analysis of nicotine as a response parameter and the measured macronutrients in soils

Fitted parameters	Coefficients	Standard Error	t-Stat	P-value	Lower 95%	Upper 95%
Intercept	21.570464	6.63464987	3.251183	0.007719	6.96769807	36.17322987
P (mg kg^{-1})	-0.60876671	0.174988087	-3.4789	0.005158	-0.99391289	-0.22362052
S (mg kg^{-1})	-1.46317501	0.501902323	-2.91526	0.014056	-2.567854574	-0.35849545
N (%)	327.291899	97.86795681	3.344219	0.006544	111.8859781	542.697819
Ca (cmol(+) kg^{-1})	-15.9599962	3.972355713	-4.01777	0.002024	-24.70309214	-7.2169002
Mg (cmol(+) kg^{-1})	-8.15136543	23.84940711	-0.34178	0.738953	-60.64355652	44.34082565
K (cmol(+) kg^{-1})	47.7142636	11.67659588	4.086316	0.001801	22.01424932	73.41427779

Table 20: Correlations between nicotine and the measured macronutrients in soils

Parameters	Nicotine	P	S	N	Ca	Mg	K
1. Nicotine (mg kg^{-1})	1						
2. P (mg kg^{-1})	-0.58	1					
3. S (mg kg^{-1})	-0.76	0.90	1				
4. N (%)	0.84	-0.42	-0.59	1			
5. Ca (cmol(+) kg^{-1})	0.72	-0.80	-0.78	0.79	1		
6. Mg (cmol(+) kg^{-1})	-0.33	0.34	0.46	0.05	0.02	1	
7. K (cmol(+) kg^{-1})	0.18	0.37	0.33	0.49	0.23	0.57	1

The coefficient of determination (R^2) being 95%. This model depicts that N and K both are positively statistically significant at *P=0.01 and P<0.001 respectively*; whereby a unit increase of N and K leads to an increase of 327.19 and 47.71 nicotine level in the soil respectively. Negatively significant relationship at *P=0.01* was observed in nicotine levels against P, S and Ca macronutrients. The results show that a unit increase of P, S and Ca leads to a decrease level of nicotine in soils by 0.61, 1.46 and 15.96 respectively. The association between nicotine and soil P, S, N, Ca, Mg and K as shown in Table 20, indicated significant

positive correlation of nicotine released in rhizosphere with N, Ca and K macronutrients. An increase of a unit of N, Ca and K will lead to an increase of 0.84, 0.72 and 0.18 of nicotine level in soil respectively. The results further show that, the effects of N and Ca is strong while for K is weak. Furthermore, P, S and Mg have negative relationship with nicotine. This indicates that an increase of 1 unit of P, S and Mg will lead to a decrease of 0.58, 0.76 and 0.33 of nicotine level respectively. Such linear relationship is strong with P and S contrary with Mg which revealed a weak relationship.

Therefore, correlation between nicotine and soil macronutrients confirmed that released levels of nicotine in soils resulted into significantly increase of soil total N and exchangeable Ca while nicotine effects on exchangeable K was weak and for this study showed a decreasing trend. Furthermore, released nicotine levels in soil reduced significantly extractable P, available S and exchangeable Mg. This study revealed that nicotine reduced the presence of P, S and Mg in the soil for creating favourable conditions on N mobilization and its uptake as a precursor for nicotine synthesis, while also uptaking Ca to influence biomass production. However, mechanisms for nicotine in reducing the presence of certain nutrients prompt a need for further investigation.

4.5.4 Effects of tobacco cultivation on selected micronutrients before and after experiment

Across the sites B, Cu and Mn differed significantly, Fe for Urambo differed significantly with Sikonge and Tabora respectively, while for Zn Sikonge differed significantly with Tabora and Urambo respectively (Table 21). Micronutrients evaluation indicated that Cu^{2+}, Fe^{2+}, Mn^{2+}, and Zn^{2+} increased significantly (P <0.001) while B decreased significantly (P <0.001) under tobacco cultivation conditions. Extractable soil B (0.33 mg kg^{-1}) in Sikonge was significantly (P <0.001) higher in comparison to Urambo and Tabora with recorded values of 0.28 and 0.22 mg kg^{-1}, respectively (Table 21). Extractable B decreased significantly (P <0.001) from 0.32 mg kg^{-1} to 0.28 mg kg^{-1} before tobacco cultivation in unfertilized tobacco soils. Extractable B decreased further to 0.24 mg kg^{-1} in fertilized tobacco soils. There were interaction effects between sites and B treatments (Fig. 22a). Soil extractable B in Tabora and Urambo reduced both in unfertilized and fertilized tobacco plots. Surprisingly in Sikonge site B increased significantly from 0.33 to 0.37 mg kg^{-1} for unfertilized tobacco soils, however B decreased in soil to 0.30 mg kg^{-1} in fertilized tobacco soils and fertilized tobacco soils, respectively.

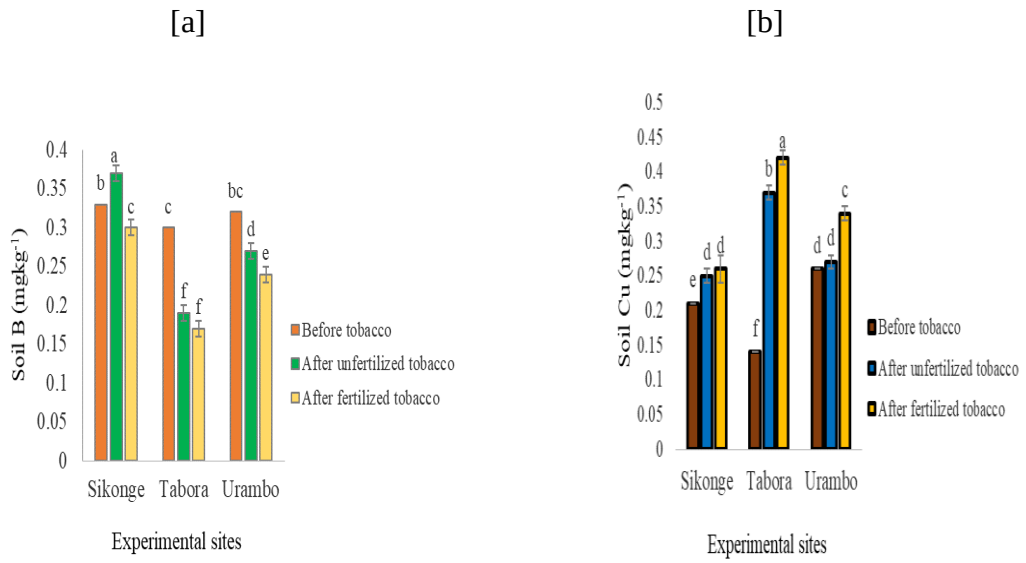


Figure 22: Effect of tobacco and fertilization on soil B and Cu

Boron decreased from 0.32 to 0.28 mg kg^{-1} soil in soils before experimentation relative to that in unfertilized tobacco soils. The solubility of B decreased from 0.32 to 0.24 mg kg^{-1} soil between soils before experimentation and in tobacco fertilized soils. On the other hand, the amount of B decreased from 0.28 to 0.24 mg kg^{-1} soil between unfertilized and fertilized tobacco cultivated soils. Therefore, solubility of B decreased by 4% in soils before experimentation relative to unfertilized tobacco cultivated soils, while between fertilized and before experiment, solubility of B decreased by 8%. These findings indicate that application of NPK and CAN fertilizers in the studied soils resulted in a decrease in B by 4% as it is the case for the cultivation of tobacco without fertilization. The decrease of B in soils as nicotine levels increases in soils could be related to the role of B in tobacco plants. More B absorbed from soils reported in improving sugars, nicotine, organic acids and amino acids contents (Lopez-Lefebre *et al*., 2002). These findings are in line with other similar observations (Steiner & do Carmo Lana, 2013).

Table 21: Selected soil properties of the Sikonge, Tabora and Urambo experimental sites as affected by the course of tobacco cultivation

Assessment	Measured variables in soils							
	Soil pH	Organic carbon (%)	Nicotine	Boron	Copper	Iron	Manganese	Zinc
			(mg kg^{-1}) soil					
Site:								
Sikonge	5.58 ± 0.09 b	0.32 ± 0.01 a	5.93 ± 1.92 a	0.33 ± 0.01 a	0.24 ± 0.01 c	22.43 ± 2.10 a	29.28 ± 1.26 b	0.58 ± 0.06 a
Tabora	5.47 ± 0.00 c	0.15 ± 0.01 c	3.97 ± 1.45 b	0.22 ± 0.02 c	0.31 ± 0.04 a	22.31 ± 2.41 a	18.79 ± 1.73 c	0.32 ± 0.06 b
Urambo	5.79 ± 0.03 a	0.25 ± 0.00 b	1.51 ± 0.48 c	0.28 ± 0.01 b	0.29 ± 0.01 b	21.38 ± 2.03 b	31.21 ± 1.79 a	0.38 ± 0.01 b
Treatment:								
Soil before tobacco[+]	5.75 ± 0.06 a	0.25 ± 0.03 a	0.01 ± 0.00 c	0.32 ± 0.00 a	0.20 ± 0.02 c	13.60 ± 0.24 c	20.10 ± 2.05 c	0.32 ± 0.05 c
Soil after tobacco – unfertilized[+]	5.57 ± 0.07 b	0.23 ± 0.02 b	2.71 ± 0.52 b	0.28 ± 0.02 b	0.30 ± 0.02 b	24.63 ± 0.24 b	29.24 ± 1.96 b	0.40 ± 0.00 b
Soil after tobacco – fertilized	5.52 ± 0.05 b	0.23 ± 0.02 b	8.69 ± 1.44 a	0.24 ± 0.02 c	0.34 ± 0.02 a	27.89 ± 0.57 a	29.93 ± 1.84 a	0.56 ± 0.07 a
2-Way ANOVA F Statistic:								
Site (S)	25.09***	297.91***	543.63***	133.93***	35.49***	4.10*	1461.21***	24.77***
Treatment (T)	12.74***	9.46***	2180.80***	69.43***	118.53***	696.15***	985.93***	21.37***
S x T	5.48**	7.68***	242.32***	19.90***	43.50***	4.51**	12.44***	5.13**

Similar letter(s) do not differ significantly based on their respective Least Significance Difference (LSD) value at 5% error rate.Values presented are the means ± SE (Standard Error); *, **, *** significant at $P \leq 0.05$, $P \leq 0.001$, $P < 0.001$ respectively; ns = non-significant.

[+] Details of correlation between soil parameters and bacterial diversity indices, multiple regression between bacteria diversity and soil parameters are shown in Appendix I Table 32-33.

Extractable soil Cu was significantly (P <0.001) high in Tabora (0.31 mg kg^{-1}) followed by Urambo and Sikonge with values of 0.29 and 0.24 mg kg^{-1} respectively. Extractable Cu increased significantly (P <0.001) from 0.20 mg kg^{-1} before tobacco cultivation to 0.30 mg kg^{-1} after unfertilized tobacco cultivation. Extractable Cu increased further in soil to 0.34 mg kg^{-1} after fertilized tobacco cultivation.

There were interaction effects between sites and Cu (Fig. 22b). With exception to Sikonge site, soil extractable Cu in Tabora and Urambo increased significantly both in unfertilized and fertilized tobacco plots. Copper in soils increased from 0.2 to 0.3 mg kg^{-1} soil between before experiments and unfertilized tobacco soils (Table 21). On the other hand, Cu^{2+} in soils increased from 0.2 to 0.34 mg kg^{-1} soil between the soils before experiments and fertilized tobacco soils. Further, Cu^{2+} in soils increased from 0.3 to 0.34 mg kg^{-1} soil between the unfertilized tobacco and fertilized tobacco soil. Tobacco influence Cu^{2+} increase in unfertilized soils by 50%, indicating that there is probably a positive association between Cu^{2+} and tobacco rhizosphere and other soil modification (Giller, 2001; Farooq *et al*., 2014). Further to that, application of NPK and CAN fertilizers in tobacco plants resulted to increase in Cu^{2+} by 70%. This indicates that, the influence of N, P, K, and Ca nutrients and tobacco crop increased Cu^{2+} solubility by 20% in these soils. However, the soil increase in Cu^{2+} as a result of these nutrient elements is 13%. The influence of these nutrients on the increase in Cu was also reported by Giller (2001), Bryson and Mills (2014) and Rengel (2015).

Extractable Fe for Sikonge and Tabora were significantly (P <0.001) higher than Urambo. Recorded Fe values in soils were 22.43, 22.31 and 21.38 mg kg^{-1} for Sikonge, Tabora and Urambo respectively. The Fe in the soils increased significantly from 13.60 to 24.63 mg kg^{-1} after unfertilized tobacco cultivation. After fertilized tobacco cultivation, Fe levels in soil increased to 27.89 mg kg^{-1} (Table 21). Significant interactions were observed between sites and Fe. Across the sites Fe levels increased significantly after unfertilized and fertilized tobacco cultivation (Fig. 23a).

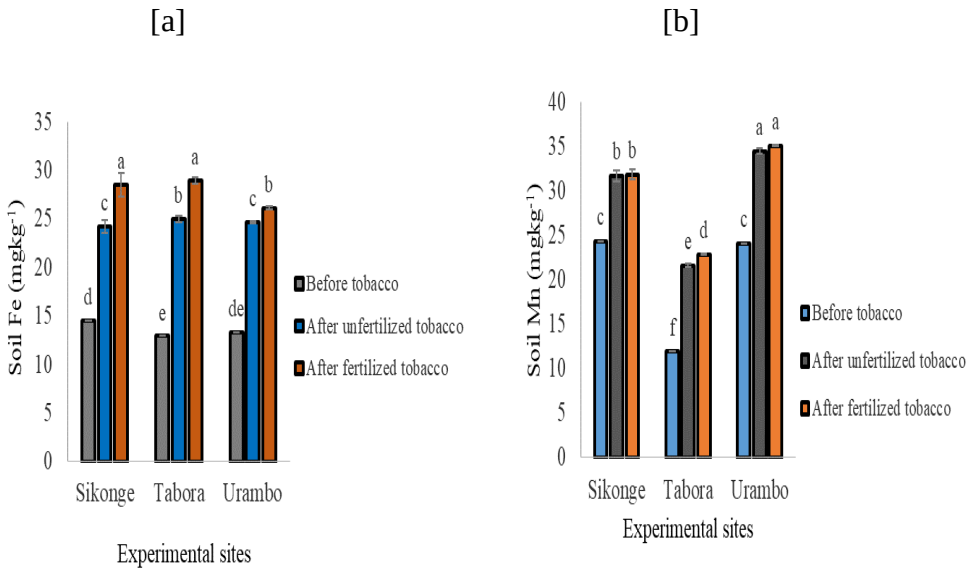


Figure 23: Effect of tobacco and fertilization on soil Fe and Mn

The amount of Fe^{2+} increased from 13.60 to 24.63 mg kg^{-1} soil between the soils before experimentation and unfertilized tobacco soils (Table 21). Furthermore, Fe^{2+} in soils increased from 13.60 to 27.89 mg kg^{-1} soil between the soils before experiments and fertilized tobacco soils. Therefore, Fe^{2+} increased from 24.63 to 27.89 mg kg^{-1} soil between unfertilized tobacco and fertilized tobacco soils. Influence of tobacco increased Fe^{2+} by 81%, while that of both tobacco and fertilizers is by 105% and for the nutrients N, P, K and Ca is 13%. This finding suggests that tobacco in soils has the highest influence in increases of Fe^{2+} solubility in the studied soils. This observation is concurrent with other studies conducted by Farooq *et al*. (2014).

All experimental sites, Mn levels in soil was significantly (P<0.001) different with values of 29.28, 31.21 and 18.79 mg kg^{-1} in Sikonge, Urambo and Tabora respectively (Table 21). Before tobacco cultivation and after unfertilized tobacco cultivation, the increase of Mn in soils was significantly (P<0.001) from 20.10 to 29.24 mg kg^{-1}. Concentration of Mn increased significantly (P<0.001) by application of fertilizer from 29.24 to 29.93 mg kg^{-1}. There were significant interactions between sites and fertilizer application on soil Mn (Fig. 23b). Soil Mn levels to all sites increased significantly following cultivation of unfertilized tobacco from 24.32, 11.90, 24.07 mg kg^{-1} to 31.65, 21.60 and 34.47 mg kg^{-1} in Sikonge, Tabora and Urambo respectively. Tabora site had significant increase in soil Mn following fertilizer application. However, for Sikonge and Urambo there was no significant increase in Mn levels in the soil. Manganese increased from 20.10 to 29.24 mg kg^{-1} soil between before

experiments and unfertilized tobacco soils (Table 21). Further, Mn^{2+} in soils increased from 20.10 to 29.93 mg kg^{-1} soil between the soils before experiments and fertilized tobacco soils. The quantities of soluble Mn^{2+} in soils increased from 29.24 to 29.93 mg kg^{-1} soil between the unfertilized tobacco and fertilized tobacco soils. Influence of tobacco in the increase of Mn^{2+} in soils was by 46%, while that of both tobacco and fertilizers was by 49% and the nutrients N, P, K, and Ca was only is 2%. This finding suggests that tobacco crop displays the highest influence in increasing Mn^{2+} in the studied soils. This observation is concurrent with other studies conducted by Rengel (2000), Porter, Bajita-Locke, Hue and Strand (2004) and Sparrow and Uren (2014).

Extractable Zn for Urambo and Tabora were significantly (P <0.001) lower than Sikonge. Urambo, Tabora and Sikonge had Zn values of 0.38, 0.32 and 0.58 mg kg^{-1} respectively. Zinc levels in the soils increased significantly from 0.32 to 0.40 mg kg^{-1} after unfertilized tobacco cultivation. After fertilized tobacco cultivation, Zn levels in soil increased to 0.56 mg kg^{-1} (Table 21). Significant interactions were observed between sites and Zn treatments (Fig. 24). In Sikonge and Urambo, Zn levels in soils did not increased significantly, following fertilization only significant increase in Zn levels recorded in Sikonge. Tabora site, Zn level in soil increased significantly both in unfertilized and fertilized tobacco plots.

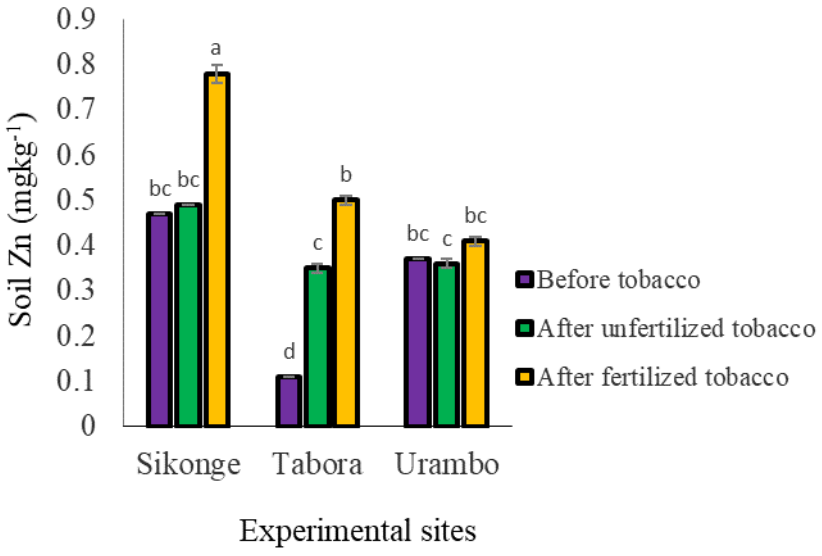


Figure 24: Effect of tobacco and fertilization on soil Zn

Zinc increased from 0.32 to 0.40 mg kg^{-1} soil between the soils before experiments and unfertilized tobacco soils (Table 21). Zinc also increased from 0.32 to 0.56 mg kg^{-1} soil before experimentation and fertilized tobacco soils. Furthermore, Zn^{2+} in soils increased from

0.40 to 0.56 mg kg^{-1} soil between the unfertilized tobacco and fertilized tobacco. Influence of tobacco in the increase of Zn^{2+} in soils was by 25%, while that of both tobacco and fertilizers was by 75% and the nutrients N, P, K, and Ca only was 40%. This finding suggests that these nutrients display the highest influence in increases of Zn^{2+} in the studied soils, however tobacco contribution in increasing Zn^{2+} levels in soils is not neglected. Similar observation was also reported in other related studies (Fässler, Robinson, Gupta & Schulin, 2010; Farooq *et al*., 2014).

4.5.5 Relationship between nicotine concentration in soils and micronutrients

Regressing soil nicotine as a response variable while other micronutrients kept constant (Table 22), gave the following model:

Nicotine (Y) = 95.42+95.58B+64.92Cu+41.12OC+0.47Fe-25.89SoilpH-9.34Zn-0.20Mn
The coefficient of determination (R^2) was 96%.

The model narrates that B, Cu^{2+}, Fe^{2+} and OC are positively influenced by nicotine contents in soils. Meaning that a unit increase of B, Cu^{2+}, Fe^{2+} and OC led to an increase of 95.58, 64.92, 0.42 and 41.12 nicotine level in the soil respectively. On the other side, Mn^{2+}, Zn^{2+} and soil pH are negatively influenced by soil nicotine. The results show that a unit increase of Mn^{2+}, Zn^{2+} and soil pH led to a decrease level of nicotine in the soils by 0.20, 9.34 and 25.89 respectively. However, correlations between nicotine and soil B, Cu^{2+}, Fe^{2+}, Mn^{2+}, Zn^{2+}, OC and soil pH (Table 23), showed significant positive correlations between nicotine in soils with Cu^{2+}, Fe^{2+}, Mn^{2+}, and Zn^{2+}. Negative correlations observed between nicotine in soils with OC, Soil pH and B. Correlations results are consistent with the observed trends of these micronutrients in studied soils.

Table 22: A multiple linear regression analysis of nicotine as a response parameter and the measured micronutrients in soil

Parameters	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Intercept	95.42434313	34.90534505	2.733803175	0.021055512	17.65038767	173.1982986
B (mg/kg)	95.58563717	41.95593708	2.278238643	0.045923021	2.101983691	189.0692906
Cu (mg/kg)	64.92308337	27.71679316	2.342373556	0.041172614	3.166219676	126.6799471
Fe (mg/kg)	0.475368305	0.252771336	1.880625838	0.089431575	-0.087841329	1.038577938
Mn (mg/kg)	-0.203960288	0.126413294	-1.613440181	0.13772393	-0.485626661	0.077706084
Zn (mg/kg)	-9.339325774	7.857166985	-1.188637812	0.262043109	-26.8461848	8.167533251
OC (%)	41.11614161	15.0755355	2.727342032	0.02129032	7.525755258	74.70652796
Soil pH	-25.88681638	8.139113946	-3.180544781	0.00981037	-44.02189238	-7.751740375

Table 23: Correlations between nicotine and the measured micronutrients in soils

Parameters	Nicotine	B	Cu	Fe	Mn	Zn	OC	Soil pH
1. Nicotine (mg/kg)	1							
2. B (mg/kg)	-0.47	1						
3. Cu (mg/kg)	0.52	-0.82	1					
4. Fe (mg/kg)	0.88	-0.70	0.76	1				
5. Mn (mg/kg)	0.44	-0.14	0.50	0.63	1			
6. Zn (mg/kg)	0.74	-0.04	0.42	0.65	0.67	1		
7. OC (%)	-0.06	0.49	-0.16	-0.09	0.52	0.41	1	
8. Soil pH	-0.70	0.43	-0.11	-0.54	0.15	-0.20	0.58	1

4.5.6 Summary results on effects of tobacco cultivation to the soil nutrients levels

Unfertilized tobacco plant influences the increase of nicotine to the rhizosphere, the macronutrients Ca (135%) > N (25%) and decrease in the order of S (81%) > P (49%) > Mg (12%) > K (11%). The sole effect of NPK and CAN 27% fertilizers increased further nicotine, Ca (25%) > N (20%) > S (8%) > Mg (4%) > P (3%) and decrease in K (3%) on the rhizosphere. Both tobacco plant and NPK + CAN fertilizers on the rhizosphere increased Ca (193%) > N (50%) and decreased S (80%) > P (48%) > K (14%) > Mg (8%). Leaf concentrations in fertilized tobacco increased in the following order Ca (197%) > K (28%) > P (27%) > S (26%) > N (18%) > Mg (12%).

Unfertilized tobacco soils had increased micronutrients concentration in the following order: Fe^{2+} (81%) > Cu^{2+} (50%) > Mn^{2+} (46%) > Zn^{2+} (25%) and decreasing B by 4%. Fertilizing the tobacco with $N_{10}P_{18}K_{24}$ and CAN 27% resulted to increased concentrations of Zn^{2+} (40%) > Cu^{2+} = Fe^{2+} (13%) > Mn^{2+} (2%) and decreasing B by 14%.

4.6 To determine the effects of nicotine on subsequent maize crop yield in different soil textures under fertilization

4.6.1 Maize flowering time, yields and harvesting index in the 1st cropping season before tobacco cultivation

Results of maize flowering time, biological yield, grain yield and harvest index (HI) are shown in Table 24. Maize planted at Sikonge flowered after 52.50 days and did not differ significantly with maize planted at Tabora which flowered after 53.17 days. Urambo maize took 53.33 days to flower, and this duration was significantly ($P \leq 0.001$) longer than maize planted in Sikonge. These results suggest that time for maize flowering did no vary

significantly because they were planted on the same day starting with Tabora, Urambo and Sikonge.

Table 24: Maize biological and grain yield in the 1st cropping season before tobacco cultivation

Assessment	Time to flowering (Day)	Biological yield (t ha^{-1})	Grain yield (t ha^{-1})	Harvest Index (%)
Site				
Sikonge	52.50 ± 0.85 b	18.61 ± 2.18 a	2.30 ± 0.66 b	11.03 ± 2.73 b
Tabora	53.17 ± 0.70 ab	18.45 ± 2.19 a	2.17 ± 0.63 c	10.48 ± 2.15 c
Urambo	53.33 ± 0.92 a	17.22 ± 1.64 a	2.36 ± 0.67 a	12.46 ± 2.69 a
Treatments				
Unfertilized maize crop	54.78 ± 0.22 a	13.62 ± 0.04 b	0.82 ± 0.02 b	6.02 ± 0.14 b
Fertilized maize crop	51.22 ± 0.22 b	22.57 ± 0.44 a	3.73 ± 0.04 a	16.62 ± 0.48 a
2-Way ANOVA F-statistics				
Sites (S)	3.5ns	44.33***	49.34***	75.56***
Treatment (T)	170.7***	4566.81***	32025***	6100.29***
S x T	1.2ns	37.87***	11.39***	28.67***

Values presented are means ± $SE_{\bar{x}}$ (Standard error of means); *** = significant at $P \leq 0.001$; ns = non-significant. Means in the same category of evaluated interface sharing similar letter(s) do not differ significantly based on their respective Standard error (SE) at 5% error rate

Biological yield did not differ significantly ($P \leq 0.001$) across the sites. The biological yield did not differ significantly as the amount of rainfall and sunshine (Table 25) also did not vary widely at all the sites. Grain yield differed significantly ($P \leq 0.001$) across the sites. The highest and significant grain yield was recorded in Urambo at 2.36 t ha^{-1}, and this was followed by Sikonge and Tabora with 2.30 and 2.17 t ha^{-1}, respectively. The initial B, Cu^{2+}, Zn^{2+} (Table 11) could have influenced the maize grain and biological yields in Urambo and Sikonge than in Tabora (Lisuma, Semoka & Semu, 2006; Ghaffari *et al*., 2011; Eteng, Asawalam & Ano, 2014). The grain yield to all sites corresponded with harvest indexes (HI) which had 12.46, 11.03 and 10.48% of HI for Urambo, Sikonge and Tabora, respectively.

Table 25: Weather data on rainfall and temperature during the 2017/18 cropping season

Month	Sikonge			Tabora			Urambo		
	Rain (mm)	Min Temp (°C)	Max Temp (°C)	Rain (mm)	Min Temp (°C)	Max Temp (°C)	Rain (mm)	Min Temp (°C)	Max Temp (°C)
October	32.21	21	33	32.52	20	31	28.65	18	28
November	39.6	20	32	36.25	18	29	37.98	17	25
December	195.1	18	29	182.36	17	27	160.01	17	26
January	142.52	17	26	140.15	16	25	138.12	16	24
February	147.13	18	28	145.5	17	26	134.71	17	25
March	182.6	17	27	150.65	16	26	155.53	16	24
April	196.3	17	26	147.1	17	25	124.33	16	24
May	114.55	16	28	115.02	16	27	100.65	16	25
June	0	16	30	0.5	16	30	10.1	16	27
Average	**1050.01**	**17.78**	**28.78**	**950.05**	**17.00**	**27.33**	**890.08**	**16.56**	**25.33**

Unfertilized maize plots took 54.78 days equivalent to 55 days to flower, while fertilized maize plants achieved an early flowering time of 51.22 days. Biological yield (22.57 t ha^{-1}) was highest in fertilized maize plots than unfertilized maize plots (13.62 t ha^{-1}) which were also correlated with HI to both unfertilized (6.02%) and fertilized (16.62%) maize plots. Fertilized maize had significantly ($P \leq 0.001$) higher grain yield of 3.73 t ha^{-1} than unfertilized maize which gave 0.82 t ha^{-1}. The highest significant for early flowering time and increase of biological and grain yields to the fertilized maize plots (Table 26) was as a result of fertilizer application (NPK) which had an impact on these parameters (Njoroge, Otinga, Okalebo, Pepela & Merckx, 2018).

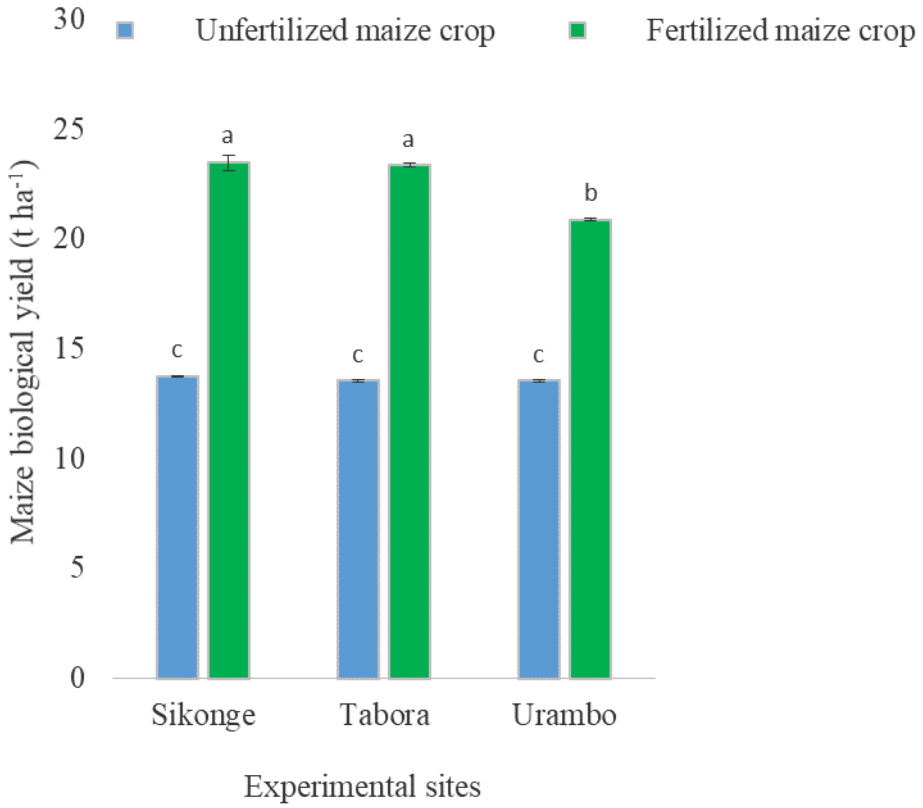


Figure 25: Interaction effects between sites and fertilizer on biological yield in 1st crop

There were no interactions between the sites and fertilization application on the time of flowering. However, there was significant interactions between sites and fertilizer application on biological yield (Fig. 25), grain yield (Fig. 26a) and harvest index observed (Fig. 26b). Sikonge and Tabora had the highest biological yield of 23.47 and 23.35 t ha^{-1}, respectively, compared to Urambo (20.87 t ha^{-1}). Interaction of sites and fertilizer applications on grain yield was significantly higher ($P \leq 0.001$) at Urambo (3.85 t ha^{-1}) followed by Sikonge (3.77 t ha^{-1}) and the lowest being Tabora with 3.57 t ha^{-1} (Fig. 26a). Harvest index (HI) was

significantly ($P \leq 0.001$) higher in Urambo (18.47%), followed by 16.10% in Sikonge and the lowest HI was 15.29% in Tabora (Fig. 26b). These interactions effects (Table 24) were resulted based on the NPK application fertilizer (Njoroge *et al*., 2018).

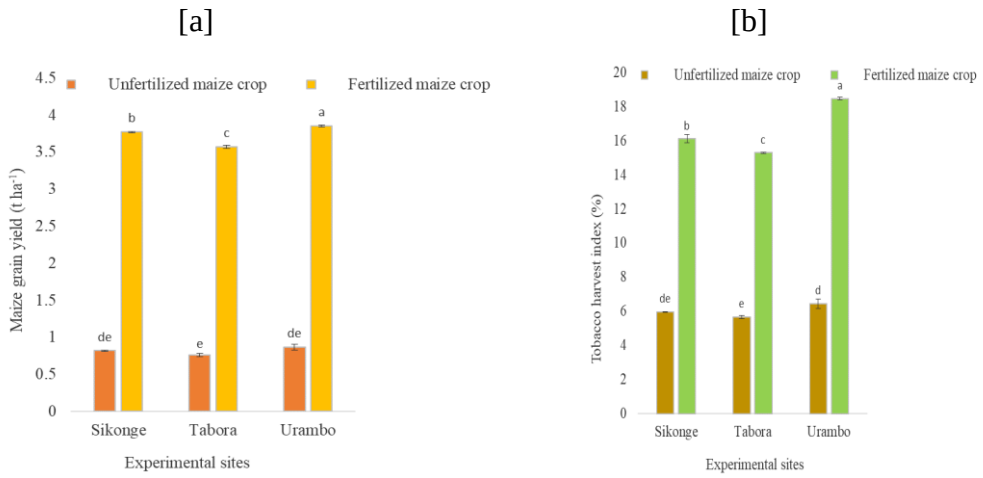


Figure 26: Interaction between sites and fertilizer on grain yield and harvest index (HI) in 1st crop

4.6.2 Maize leaf nutrients assessment in the 1st cropping season

Maize leaf nutrients for N, P, K, Ca and Cu were assessed under different fertilizer treatments (Table 26). Maize leaf N and P did not differ significantly in all sites. Urambo had the highest maize leaf K (1.86%), Ca (0.21%) and Cu (11.11 mg kg^{-1}), followed by Sikonge which had 1.76% of K, 0.19% of Ca and 9.13 mg kg^{-1} of Cu. Maize leaf nutrient concentrations were low in unfertilized maize plant with 1.56 %N, 0.20% P, 1.48% K, 0.17% Ca and 8.51 mg kg^{-1} Cu. Upon fertilization, nutrient leaf concentrations increased significantly ($P \leq 0.001$) for N (3.31%), P (0.25%), K (2.07%), Ca (0.21%) and Cu (10.47 mg kg^{-1}). There were no interaction effects between sites and fertilizer treatments across the sites.

Table 26: Maize nutrient leaf concentrations in the 1st cropping season

Assessment	N (%)	P (%)	K (%)	Ca (%)	Cu (mg kg^{-1})
Site					
Sikonge	2.56 ± 0.37 a	0.23 ± 0.01 a	1.79 ± 0.15 ab	0.19 ± 0.01 ab	9.13 ± 0.49 b
Tabora	2.37 ± 0.51 a	0.23 ± 0.01 a	1.68 ± 0.11 b	0.17 ± 0.01 b	8.24 ± 0.42 c
Urambo	2.38 ± 0.34 a	0.22 ± 0.01 a	1.86 ± 0.15 a	0.21 ± 0.01 a	11.11 ± 0.42 a
Treatments					
Unfertilized maize crop	1.56 ± 0.12 b	0.20 ± 0.00 b	1.48 ± 0.02 b	0.17 ± 0.01 b	8.51 ± 0.43 b
Fertilized maize crop	3.31 ± 0.11 a	0.25 ± 0.01 a	2.07 ± 0.06 a	0.21 ± 0.01 a	10.47 ± 0.43 a
2-Way ANOVA F-statistics					
Sites (S)	0.77ns	0.58ns	3.18ns	4.25*	186.15***
Treatment (T)	154.23***	24.50***	94.55***	29.82***	247.88***
S x T	3.76ns	0.68ns	1.22ns	1.58ns	0.25ns

Values presented are means ± SE (Standard Error); *, *** significant at $P \leq 0.05$ and $P < 0.001$ *respectively*; ns= non-significant; Means in the same category of evaluated interface sharing similar letter(s) do not differ significantly based on their respective Least Significance Difference (LSD) value at 5% error rate

4.6.3 Persistence of nicotine released by tobacco plant to the 2nd cropping season

The effects of growing tobacco crop on soil pH and residual nicotine in soils at planting time for maize crop are shown in Table 27. Soil pH and nicotine residues in soil were both significantly ($P \leq 0.001$) different across the sites. Urambo had the highest soil pH (5.80) followed by Sikonge and Tabora with 5.61 and 5.46, respectively. The results of this study indicated that tobacco cultivation in loamy sand, sand and sandy loam soils reduced significantly soil pH when compared with the soils before tobacco cultivation. Soil pH differed significantly across the sites. Soil pH in Urambo was found to be 5.80, while for Sikonge and Tabora the pH reached 5.61 and 5.46 respectively. The soils before tobacco cultivation, loamy sand soil in Sikonge site had a drop of soil pH by 0.28 units which was significantly large in comparison with the sandy loam soil in Urambo and sand soil in Tabora which had a drop of soil pH by 0.07 and 0.03 units, respectively. Therefore, soil pH drop was higher in loamy sand soil of Sikonge than sand soil of Tabora.

Table 27: Residual effects of tobacco cultivation[+] on soil pH and nicotine after 8 months

Assessment	Soil pH	Soil nicotine (mg kg^{-1})
Site		
Sikonge	5.61 ± 0.06 b	0.60 ± 0.14 a
Tabora	5.46 ± 0.02 c	0.46 ± 0.12 b
Urambo	5.80 ± 0.02 a	0.27 ± 0.08 c
Treatments		
T1: Unfert ZM>Unfert ZM	5.75 ± 0.06 a	0.00 ± 0.00 d
T2: Unfert ZM>Unfert NT	5.58 ± 0.05 b	0.32 ± 0.06 c
T3: Fert ZM>Fert ZM	5.76 ± 0.06 a	0.00 ± 0.00 d
T4: Fert ZM>Fert NT	5.57 ± 0.03 b	0.86 ± 0.10 b
T5: Fert ZM>Fert NT + SI	5.47 ± 0.02 c	1.05 ± 0.08 a
2- Way ANOVA F-statistics		
Site (S)	92.30***	132.48***
Treatment (T)	28.50***	678.85***
S x T	10.70***	26.52***

Values presented are means ± $SE_{\bar{x}}$ (Standard error of means); *** = significant at $P \leq 0.001$; ns = non-significant. Means in the same category of evaluated interface sharing similar letter(s) do not differ significantly based on their respective Standard error (SE) at 5% error rate.

[+]The residual nicotine in Table 27 is a result of tobacco planted in the 1st cropping season. Detailed for the tobacco yields and leaf nicotine are shown in Appendix II Table 34-37

With regard to the applied treatments, the soil under previously unfertilized maize (T1) had significantly ($P \leq 0.001$) higher soil pH of 5.75 which was similar with T3 that was previously planted with fertilized maize (5.76). Previously unfertilized tobacco plot (T2) had soil pH of 5.58 of which did not differ significantly ($P \leq 0.001$) with previously fertilized tobacco plot (T4) which had soil pH of 5.57. The lowest soil pH of 5.47 was recorded in T5, previously grown tobacco followed by incorporation of tobacco stalks in the ridges. There were significant interactions between sites and treatments on soil pH (Fig. 25). The highest significant soil pH of 5.89 recorded in previously unfertilized maize plot T1 and previously fertilized maize plot T3 (5.90) for Sikonge site. The lowest soil pH of 5.28 was recorded in previously fertilized tobacco followed by incorporation of tobacco stalks T5 of Sikonge (Fig. 27). These results indicate that the soil pH was lowered significantly in unfertilized tobacco plots than unfertilized maize plots. Upon fertilization, soil pH was reduced significantly in tobacco plots than in maize plots. The lowest soil pH of 5.47 resulted following the incorporation of tobacco stalks in soils, and this is confirming that tobacco contributed to the lowering soil pH (Farooq *et al*., 2014). Of all the sites, Sikonge site had a significant reduction in soil pH followed by Urambo and Tabora, which soil pH reduced at the lowest rate.

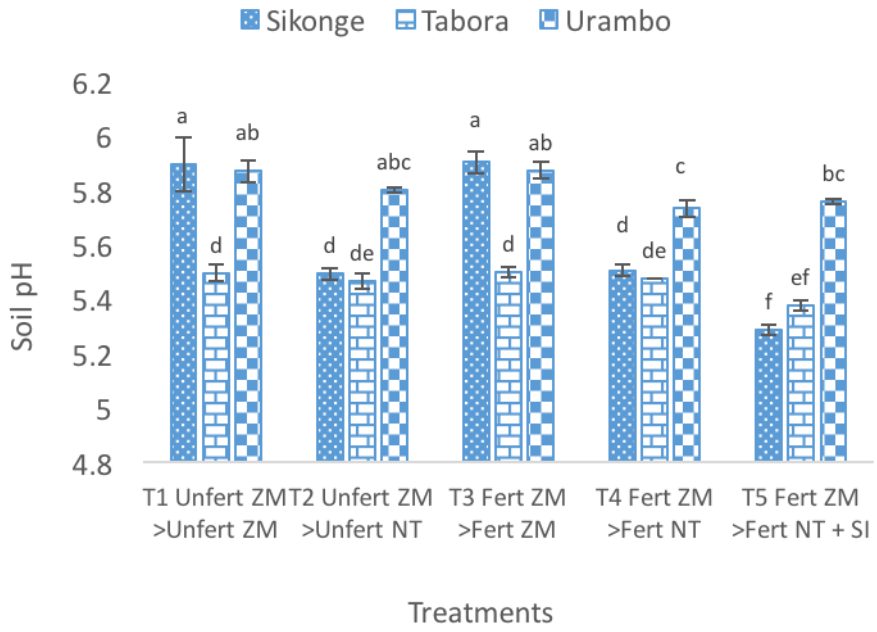


Figure 27: Effects of tobacco cultivation on soil pH after 8 months

Nicotine residues in the soil were significantly ($P \leq 0.001$) higher in Sikonge reaching 0.60 mg kg^{-1}, followed by Tabora and Urambo with nicotine levels of 0.46 and 0.27 mg kg^{-1}, respectively. In the previously unfertilized (T1) maize and fertilized maize (T3), there was no nicotine residues in their soils, while previously unfertilized tobacco plot T2 had the lowest nicotine residual of 0.32 mg kg^{-1}. Previously fertilized tobacco plot (T4) had nicotine level of 0.86 mg kg^{-1}, and the highest significantly ($P \leq 0.001$) nicotine residual of 1.05 mg kg^{-1} was recorded in T5 which was previously fertilized tobacco followed by incorporation of tobacco stalks.

There were significant interactions between the sites and the treatments on soil nicotine (Fig. 28). The highest significant ($P \leq 0.001$) soil residual nicotine of 1.30 mg kg^{-1} was recorded in Sikonge to the T5 (previously fertilized tobacco plot followed by tobacco stalks incorporation after harvesting tobacco leaves). The lowest significant ($P \leq 0.001$) nicotine residual of 0.14 mg kg^{-1} was recorded in T2 of Urambo site, previously unfertilized tobacco. These results suggest that nicotine persistence in soils for a period longer than 8 months. The loamy sand soils of Sikonge retained higher nicotine in soils (0.60 mg kg^{-1}) followed by Sand loam soils of Urambo which had nicotine persistence reaching 0.46 mg kg^{-1} and sandy loam soil of Urambo with 0.27 mg kg^{-1}. Retention of nicotine in sand soils was higher compared with sandy loam soils and this could be related to the acidic soils (Rakić *et al.*, 2010). The highest nicotine persistence of 1.05 mg kg^{-1} was recorded in T5 which was incorporated with tobacco

stalks, indicating that if farmers do not uproot tobacco stalks in their fields, nicotine persistence in soils will be high in the next cropping season. However, if uprooting tobacco stalks immediately after harvesting the leaves, the residual nicotine in soils will be reduced (0.86 mg kg^{-1}).

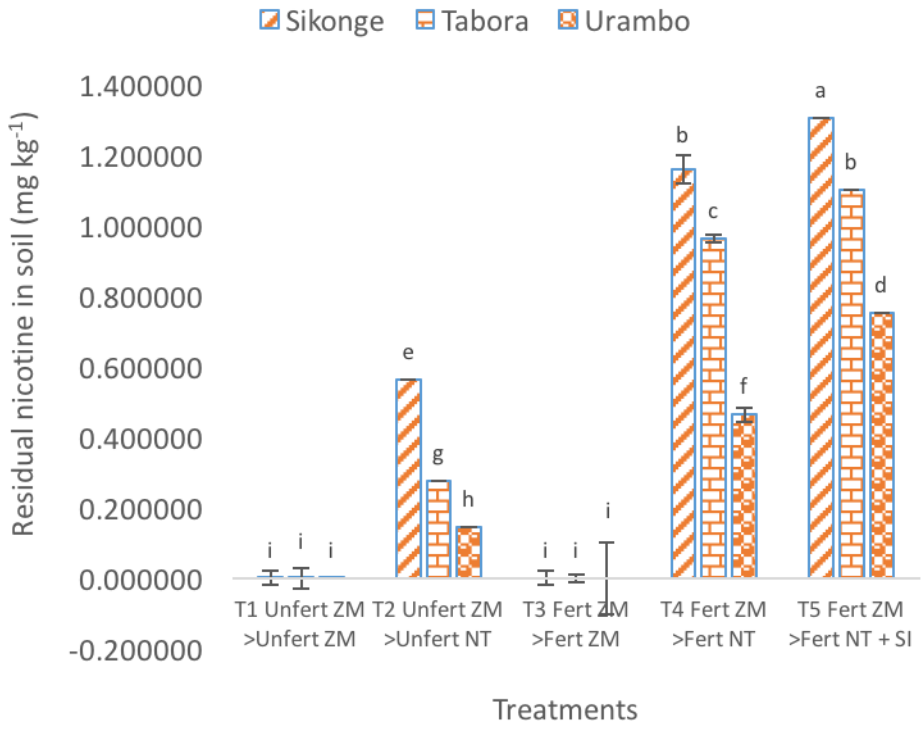


Figure 28: Effects of tobacco cultivation on nicotine persistence after 8 months

4.6.4 Effects of tobacco cultivation on subsequent maize yield in the 2nd cropping season

Effects of tobacco cultivation to the subsequent maize yield in the 2nd cropping season is shown in Table 28. Biological yield, grain yield and harvest index differed significantly ($P \leq 0.001$) across the sites. Time for maize flowering did not differ significantly ($P \leq 0.001$) in all experimental sites (Table 28). However, time of flowering for subsequent maize crop was significantly reduced in the 2nd cropping season as a result of nicotine residues than in the first cropping season where there were no nicotine residues (Table 24). Before tobacco cultivation, maize flowering took 52.50, 53.17 and 53.33 days in Sikonge, Tabora and Urambo, respectively (Table 24). Maize planted as subsequent crop after tobacco in the second year flowered after 50.73, 49.20 and 51.80 days for Sikonge, Tabora and Urambo, respectively (Table 28).

Table 28: Effects of tobacco cultivation to the subsequent maize yield in the 2nd season

Assessment	Flowering time (Days)	Biological yield (t ha^{-1})	Grain yield (t ha^{-1})	Harvest Index (%)
Site				
Sikonge	50.73 ± 0.99 a	20.25 ± 1.18 a	2.36 ± 0.32 c	11.05 ± 1.23 c
Tabora	49.20 ± 3.12 a	18.46 ± 1.01 b	2.52 ± 0.33 b	12.87 ± 1.28 b
Urambo	51.80 ± 0.96 a	17.10 ± 0.76 c	2.79 ± 0.33 a	15.55 ± 1.41 a
Treatments				
T1: Unfert ZM>previous Unfert ZM	55.00 ± 0.83 a	15.02 ± 0.55 b	1.13 ± 0.07 d	7.61 ± 0.61 c
T2: Unfert ZM>previous Unfert NT	55.55 ± 0.82 a	13.96 ± 0.11 b	1.05 ± 0.09 d	7.57 ± 0.67 c
T3: Fert ZM>previous Fert ZM	51.55 ± 0.24 ab	20.93 ± 0.74 a	3.86 ± 0.05 a	18.70 ± 0.86 a
T4: Fert ZM>previous Fert NT	47.89 ± 0.42 b	21.97 ± 0.95 a	3.53 ± 0.12 b	16.42 ± 1.13 b
T5: Fert ZM>previous Fert NT+SI	42.89 ± 4.37 c	21.12 ± 0.92 a	3.21 ± 0.09 c	15.48 ± 0.86 b
2-Way ANOVA F-statistics				
Site (S)	0.69ns	16.54***	20.29***	34.54***
Treatment (T)	6.80***	57.09***	465.81***	110.13***
S x T	1.12ns	2.41*	0.83ns	2.12ns

Values presented are means ± SE (Standard Error); *, *** significant at $P \leq 0.05$ and $P < 0.001$ *respectively*; ns= non-significant; Means in the same category of evaluated interface sharing similar letter(s) do not differ significantly based on their respective Least Significance Difference (LSD) value at 5% error rate

Time to flowering was significant (P<0.001) early (42.89 days) in T5 planted with fertilized maize which in previous season was planted with fertilized tobacco followed by incorporation of tobacco stalks immediately after harvesting. This treatment was followed by T4 (47.89 days) planted with fertilized maize of which in the previous season was planted with fertilized tobacco with uprooted stalks after harvesting. The 3rd treatment in flowering was T3 (51.55 days) of which was planted with fertilized maize and previous season planted with the same fertilized crop. There was no significant difference between T2 (unfertilized maize previous planted with unfertilized tobacco), and T1 planted with unfertilized maize and previously planted with unfertilized maize as both took 55 days to flower.

No interaction effects were observed between sites and treatments on the time for flowering. These results indicated that residual nicotine in soils has a strong effect in hastening the flowering time of maize. The early flowering time in tobacco plots resulted in the early growth stimulation of maize (Rizvi *et al*., 1989; Farooq *et al*., 2014). The residual nicotine in the soils to the subsequent unfertilized maize crop after unfertilized tobacco did not have an impact to hasten the flowering of maize as the nicotine residual levels were significantly low to cause the effect. Zhou *et al*. (2014) reported early maize growth was influenced by released tobacco nicotine in soils, and therefore, the growth improvement on maize could be associated to have an impact on the early maize flowering.

Unlike in the 1st cropping season whereby maize biological yield did not differ significantly across the sites (Table 24), the biological yield differed significantly ($P \leq 0.001$) across the sites in the 2nd cropping season following planting maize as subsequent crop after tobacco. In the 1st cropping season, the biological yield was 18.61, 18.45 and 17.22 t ha^{-1} for Sikonge, Tabora and Urambo (Table 24) whereby biological yield in the 2nd cropping season was 18.25, 18.46 and 17.10 t ha^{-1}, respectively (Table 28). In treatments, biological yield did not differ significantly in all fertilized treatments T4, T3 and T5 which had 21.97, 20.93 and 21.12 t ha^{-1}, respectively. Fertilized treatments T2 and T1 had a biological yield of 13.96 and 15.02 t ha^{-1} which did not differ significantly. There were significant interactions between sites and treatments of which Sikonge had higher significant biological yields to almost all the treatments, however the highest significantly ($P \leq 0.001$) biological yield recorded in Sikonge was in T4, previous fertilized tobacco and T5, previously fertilized tobacco with tobacco stalks incorporated which had 25.21 and 24.05 t ha^{-1}, respectively. The lowest

biological yield (13.67 t ha^{-1}) was recorded in Urambo on T2, previously unfertilized tobacco (Fig. 29).

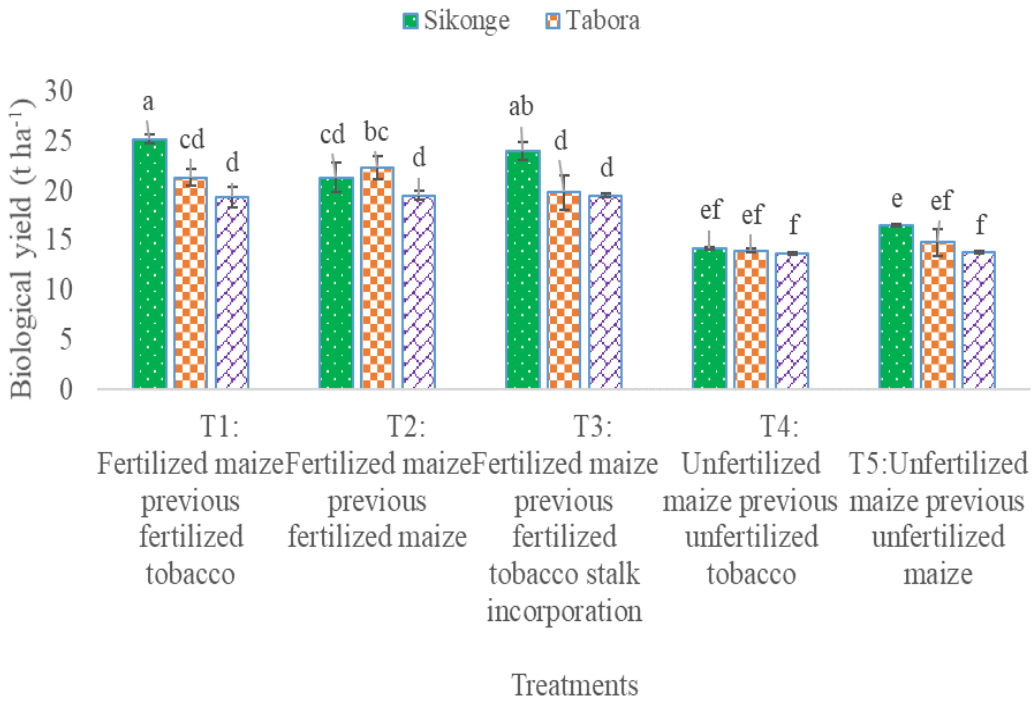


Figure 29: Influence of residual nicotine on maize biological yield in the 2nd year

These results indicate that residual nicotine in soils increased more the maize vegetative growth and hence the maize biological yield was significantly high in treatments which had highest residual nicotine than those with lowest or without residual nicotine (Image 5). In comparisons with the sites, Sikonge site had higher nicotine residual levels with significantly highest biological yields, while Urambo site had the lowest residual nicotine with significantly lowest biological yields. Therefore, nicotine persistence in soils which was more in fertilized tobacco plots influenced the increase of subsequent maize biological yield. Nicotine persistence in soils has been associated on N mineralization in tobacco soils (Hu *et al*., 2018), and therefore as a result of this influenced more biological yields to the subsequent maize crop.

(a)

(b)

Image 5: Maize stem planted in nicotine residual plot (a) and at no nicotine residual (b)

In the 1st cropping season, maize grain yields were 2.36, 2.30 and 2.17 t ha^{-1} in Urambo, Sikonge and Tabora, respectively (Table 24). The residual levels of NPK in 1st cropping season, NPK applied fertilizer in the 2nd cropping season (Table 28), and adequate rainfalls (Table 29) influenced significantly maize grain yield reaching 2.79, 2.52 and 2.36 t ha^{-1} in Urambo, Tabora and Sikonge, respectively in the 2nd cropping season (Table 28).

Table 29: Weather data on rainfall and temperature during the 2018/19 cropping season

Month	Sikonge			Tabora			Urambo		
	Rain (mm)	Min Temp (°C)	Max Temp (°C)	Rain (mm)	Min Temp (°C)	Max Temp (°C)	Rain (mm)	Min Temp (°C)	Max Temp (°C)
October	39.95	21	32	37.5	20	31	28.4	20	29
November	51.45	20	31	39.33	19	29	37.27	18	27
December	194.4	18	28	181.29	17	28	161.55	17	26
January	125.1	18	27	123.45	16	27	140.2	15	25
February	147.5	18	28	148.11	16	25	145.42	15	26
March	155.25	18	29	153.34	17	27	154.35	17	24
April	219.9	18	28	165.26	17	26	123.37	17	25
May	106.35	17	27	99.29	16	26	95.77	17	24
June	0.5	17	30	0.9	16	28	0.1	16	27
Average	**1040.40**	**18.33**	**28.89**	**948.47**	**17.11**	**27.44**	**886.43**	**16.89**	**25.89**

The results revealed that the maize grain yield was significantly ($P \leq 0.001$) higher at 3.86 t ha^{-1} in fertilized maize plots (T3) which did not be after tobacco crop. T3 had leaf concentration of 0.33% P and 2.44% K (Table 30) within the leaf critical ranges given by Landon (1984). It was this treatment which also had significant maize harvest index (HI) of 18.70% than the rest of the treatments. The fertilized maize crop after fertilized tobacco crop (T4) was the second to have a significant yield of 3.53 t ha^{-1} with HI of 16.42%. However, to the plots where stalks of fertilized tobacco were incorporated in soils (T5), maize yield were

reduced significantly further to 3.21 t ha^{-1} with HI of 15.48% than the T4 plots of which tobacco stalks uprooted. T5 attained 2.87% of leaf N concentration which was at the marginal level of critical range (Landon, 1984). The findings of this research indicates that maize yields are higher 3.86 t ha^{-1} in plots not subsequent to tobacco. Thus, if farmers will plant maize as a subsequent crop after tobacco, they should expect yield reduction by 0.33 t ha^{-1}. The lowest grain yield obtained from unfertilized maize treatments T2 (1.05 t ha^{-1}) and T1 (1.13 t ha^{-1}) which did not differ significantly. There was no significant interaction between sites and treatments on grain yield.

During the harvesting time of maize cobs from maize planted after tobacco crop (T2, T4, T5) observed too many cobs outgrowths and deficient grain filling than maize cobs from T1, T3 which were not after tobacco crop (Image 6). The outgrowth of cobs could result from maize plants absorbing more nicotine from the soil. Nicotine reported having effects in distorting DNA and RNA transcription and cause incomplete growth (Yazdani, 2014). The most affected plots to all sites with deficient grains was in T5 (previously incorporated with stalks). The deficient grain filing was due to the residual nicotine levels in the soils which resulted into limiting P and K nutrients in maize (Moula *et al*., 2018) and hence maize flag leaf (Table 30) indicated levels of these nutrients to be below the critical levels of 0.25-0.40% for P and 1.8-2.5% of K as given by Landon (1984).

(a) (b)

Image 6: (a) Maize cobs with outgrowth planted after tobacco (b) Maize with cobs from control plot

The HI in the 1st cropping season for maize was 12.48, 11.03 and 10.48% for Urambo, Sikonge and Tabora, respectively (Table 24). In the 2nd cropping season, HI for maize improved substantially, and the highest significantly ($P \leq 0.001$) HI 15.55% was recorded in Urambo, followed by 12.87% in Tabora, and the lowest 11.05% in Sikonge (Table 28). In treatments, HI in T3 was 18.70% significantly higher than T4 and T5 which had 16.42 and

15.48%, respectively, when compared with unfertilized T2 (7.57% HI) and T1 (7.61% HI). There was no significant interaction between sites and treatments effect on HI.

4.6.5 Effects of residual soil nicotine on maize leaf nutrient concentrations in the 2nd year

Maize leaf nutrients for N, P, K, Ca^{2+} and Cu^{2+} assessed under different fertilizer treatment (Table 30). With exception to maize plant leaf Cu^{2+} which was high for Sikonge site indicating that more nicotine residuals influenced solubility of Cu^{2+}, all the measured nutrients were significantly ($P \leq 0.001$) high in Urambo site followed by Tabora despite having no significantly different with Sikonge site.

Table 30: Influence of nicotine on subsequent maize leaf nutrient concentrations in 2nd cropping season

Assessment	N (%)	P (%)	K (%)	Ca (%)	Cu (mg kg^{-1})
Site					
Sikonge	2.14 ± 0.14 b	0.19 ± 0.01 b	1.60 ± 0.10 b	0.30 ± 0.01 b	27.02 ± 3.20 a
Tabora	2.18 ± 0.14 b	0.21 ± 0.01 b	1.64 ± 0.10 b	0.32 ± 0.01 b	24.66 ± 3.18 b
Urambo	2.24 ± 0.13 a	0.26 ± 0.02 a	1.78 ± 0.13 a	0.43 ± 0.01 a	24.05 ± 2.40 b
Treatments					
T1: Unfert ZM>previous Unfert ZM	1.33 ± 0.02 e	0.19 ± 0.01 c	1.65 ± 0.01 b	0.28 ± 0.02 d	9.83 ± 0.54 e
T2: Unfert ZM>previous Unfert NT	1.90 ± 0.04 d	0.19 ± 0.01 c	1.11 ± 0.02 d	0.33 ± 0.03 c	29.58 ± 1.07 c
T3: Fert ZM>previous Fert ZM	2.27 ± 0.03 c	0.33 ± 0.02 a	2.44 ± 0.07 a	0.35 ± 0.01 b	15.30 ± 1.28 d
T4: Fert ZM>previous Fert NT	2.57 ± 0.02 b	0.23 ± 0.00 b	1.61 ± 0.02 bc	0.39 ± 0.01 a	34.59 ± 1.23 b
T5: Fert ZM>previous Fert NT+SI	2.87 ± 0.02 a	0.16 ± 0.01 d	1.54 ± 0.04 c	0.39 ± 0.01 a	36.90 ± 0.66 a
2-Way ANOVA F-statistics					
Site (S)	7.72**	26.82**	14.74***	136.06***	6.69**
Treatment (T)	708.46***	60.44***	238.20***	32.62***	235.88***
S x T	1.48ns	7.72***	1.53ns	4.15**	2.86*

Values presented are means ± SE (Standard Error); *, **, *** significant at $P \leq 0.05$, $P \leq 0.01$ and $P < 0.001$ *respectively*; ns= non-significant; Means in the same category of evaluated interface sharing similar letter(s) do not differ significantly based on their respective Least Significance Difference (LSD) value at 5% error rate

Fertilized maize plants which were planted after fertilized tobacco plants (T4, T5) had significantly higher leaf N, Ca and Cu. However, T5 had very significant leaf N (2.87%), Ca (0.39%) and Cu (36.90 mg kg^{-1}) concentration than T4 which had leaf concentration reaching 2.57% N, 0.35% Ca and 34.59 mg kg^{-1} Cu. Fertilized maize plants which were planted in a previously maize plots (T3) had significantly ($P \leq 0.001$) higher leaf concentration of P (0.33%) and K (2.44%). It was in this T3 which had the highest significantly ($P \leq 0.001$) grain yield of 3.86 t ha^{-1} than the rest treatments. The highest grain yield in this treatment (T3) signify the role of P and K for grain filling (Liu, Yu, Liu & Konijn, 2006; Setiyono, Walters, Cassman, Witt & Dobermann, 2010; Annes *et al*., 2016; Laekemariam, Kibret, Mamo & Gebrekidan, 2016; Pavuluri *et al*., 2016).

The T4 was the next to have higher significant ($P \leq 0.001$) grain yield of 3.35 t ha^{-1} followed by T5 (3.21 t ha^{-1}). The yield impacts for T4 than T5 (Table 26) contributed as a result of higher nutrient P and K (Liu *et al*., 2006; Setiyono *et al*., 2010; Annes *et al*., 2016; Laekemariam *et al*., 2016; Pavuluri *et al*., 2016). In unfertilized treatments, maize plants planted in previous tobacco plot (T2) had significantly ($P \leq 0.001$) high leaf N (1.90%), Ca (0.33%) and Cu (29.58 mg kg^{-1}) than the maize plants planted in a previous maize plot (T1). The later treatment had only significantly ($P \leq 0.001$) higher K (1.65%) of maize leaf than T2 which had 1.11% of leaf K. Based on the high leaf K content for the T1 enabled this treatment to have more yield of 1.13 t ha^{-1} compared with T2 which had 1.05 t ha^{-1}, however, the yield did not differ significantly to both treatments. Furthermore, both T1 and T2 had 0.19% of P, hence did not differ significantly.

Interaction effects of sites with fertilizer treatments in maize cultivation were highly significant ($P \leq 0.001$) on leaf P, Ca and Cu (Fig.30a, 30b, 30c). In fertilized treatments, T3 and T4 in Urambo site had the highest leaf P (0.43%) and Ca (0.48%), respectively. These nutrients leaf concentrations in particular for P influenced high grain yields in Urambo site (Njoroge *et al*., 2018), as these treatments generally had the highest grain yields of 3.86 and 3.53 t ha^{-1}, respectively. The lowest grain yield recorded in T3 of Sikonge, which had 0.27 and 0.32% of leaf P and Ca, respectively. Low interaction effects for P and Ca in resulted into the lowest yield (Annes *et al*., 2016; Laekemariam *et al*., 2016) in this site due to the impact of higher nicotine residuals. Sikonge had the highest leaf Cu (38.69 mg kg^{-1}) in T5 and the lowest in Sikonge 11.97 mg kg^{-1} in T3. In unfertilized treatments, T1 of Urambo had the highest leaf P (0.23%) and Cu (11.49 mg kg^{-1}) and the lowest for both nutrients recorded in

T1 for Sikonge with 0.17% P and 8.79 mg kg^{-1} Cu. For Ca in unfertilized treatments, the highest recorded in Urambo in T2 (0.45%) and the lowest in the same treatment in Sikonge, reaching 0.25%.

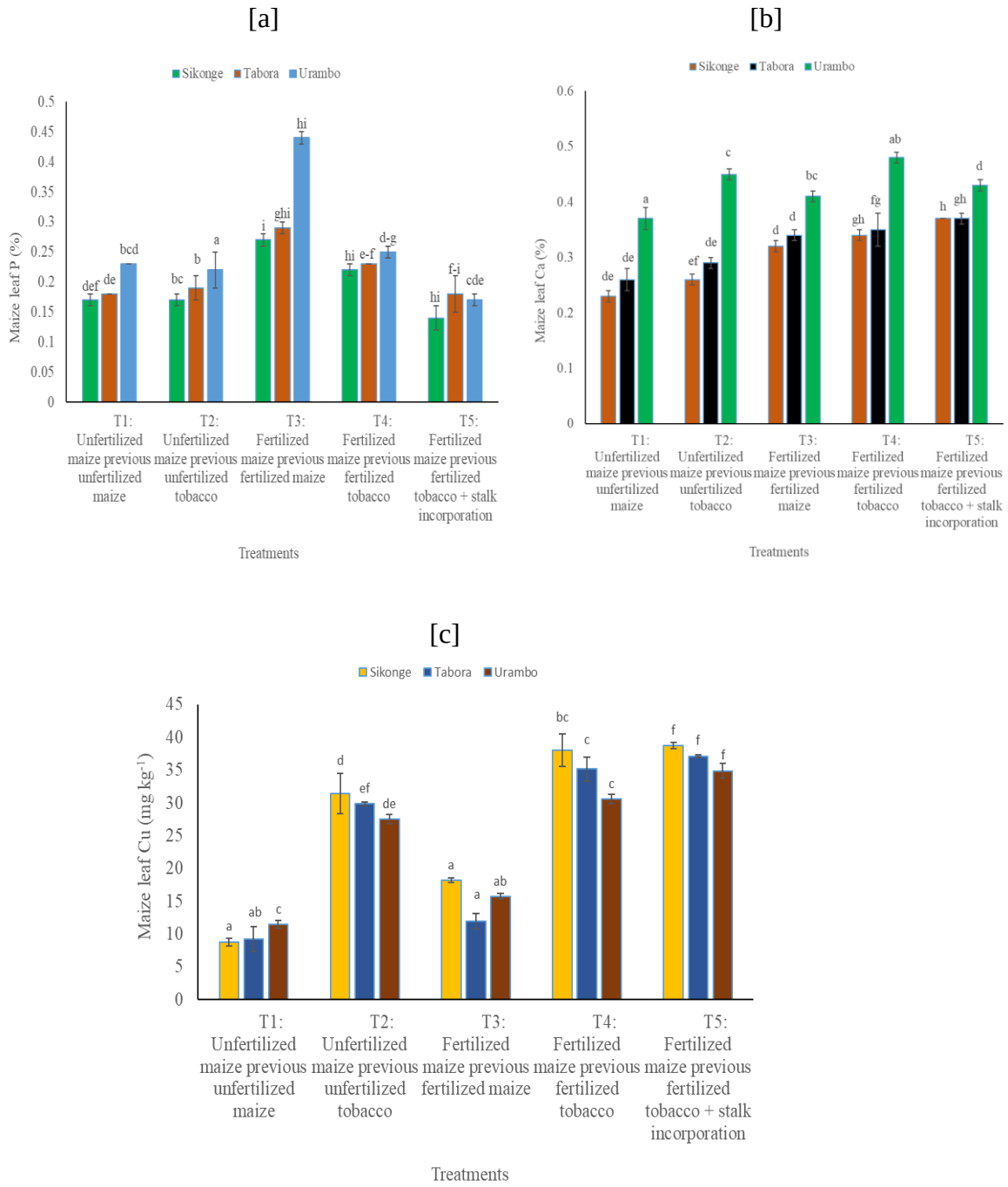


Figure 30: Interaction effects of residual nicotine on maize leaf P, Ca and Cu

4.6.6 Levels of absorption of residual soil nicotine by maize roots to the grain

The levels of nicotine absorption by the maize roots, and it apportions to the maize plant parts are shown in Table 31. Nicotine absorbed by the maize roots did not differ significantly (*P*

≤0.001) across the sites. Sikonge site had significantly ($P \leq 0.001$) nicotine levels in maize stem and grain, while the Tabora site had significantly ($P \leq 0.001$) nicotine levels in leaf. Even though the levels of nicotine absorbed by the maize roots (Image 7) is generally low, maize planted in Sikonge site absorbed 0.49% of nicotine in roots, 0.29% of nicotine in stem, 0.03% to the flag leaf, 0.001% to the maize grain and making the overall total of 0.811%. In Tabora site 0.46% of nicotine absorbed by roots, 0.28% of nicotine reaching the maize stem, 0.05% of nicotine to the flag leaf, 0.0004% reaching to the maize grain making the overall nicotine total of 0.790% in maize plant. Urambo had the lowest nicotine levels absorbed by the maize plant reaching 0.43%, 0.26%, 0.03%, 0.0001% and 0.720% in roots, stem, leaf, maize grain and overall maize plant totals, respectively.

Table 31: Levels of nicotine absorbed by the maize roots and its distribution to plant parts

Assessment	Maize root Nic conc (%)	Maize stem Nic conc (%)	Maize leaf Nic conc (%)	Maize grain Nic conc %	Whole plant Nic conc (%)
Site					
Sikonge	0.49 ± 0.08 a	0.29 ± 0.05 a	0.03 ± 0.00 b	0.0010 ± 0.0003 a	0.811 ± 0.140 a
Tabora	0.46 ± 0.08 a	0.28 ± 0.05 ab	0.05 ± 0.01 a	0.0004 ± 0.0001 b	0.790 ± 0.134 ab
Urambo	0.43 ± 0.08 a	0.26 ± 0.04 b	0.03 ± 0.01 b	0.0001 ± 0.0001 b	0.720 ± 0.133 b
Treatments					
T1: Unfert ZM> Unfert ZM	0.14 ± 0.03 d	0.07 ± 0.01 d	0.03 ± 0.00 bc	0.0000 ± 0.0000 b	0.240 ± 0.056 c
T2: Unfert ZM> Unfert NT	0.64 ± 0.02 b	0.42 ± 0.01 b	0.04 ± 0.01 b	0.0000 ± 0.0000 b	1.100 ± 0.032 b
T3: Fert ZM> Fert ZM	0.06 ± 0.01 c	0.04 ± 0.01 d	0.02 ± 0.01 c	0.0000 ± 0.0000 b	0.120 ± 0.013 d
T4: Fert ZM> Fert NT	0.68 ± 0.01 b	0.38 ± 0.01 c	0.07 ± 0.01 a	0.0011 ± 0.0003 a	1.131 ± 0.026 b
T5: Fert ZM> Fert NT+SI	0.79 ± 0.02 a	0.47 ± 0.01 a	0.06 ± 0.01 a	0.0014 ± 0.0006 a	1.321 ± 0.038 a
2-Way ANOVA F-statistics					
Site (S)	1.86ns	4.48***	2.98ns	12.83***	3.05ns
Treatment (T)	153.92***	387.48***	16.66***	21.82***	215.67***
S x T	0.073ns	0.09ns	0.24ns	3.67**	0.05ns

Values presented are means ± SE (Standard Error); **, *** significant at $P \leq 0.01$ and $P < 0.001$ *respectively*; ns= non-significant; Means in the same category of evaluated interface sharing similar letter(s) do not differ significantly based on their respective Least Significance Difference (LSD) value at 5% error rate

(a) (b)

Image 7: Underground root structure of tobacco (a) and maize (b)

This study indicates that there was a correlation of residual nicotine in soils (Table 27) and levels of nicotine absorbed by maize plants (Table 31). Sikonge site had higher nicotine residuals (0.60 mg kg^{-1}) followed by Tabora (0.46 mg kg^{-1}), and Urambo had the lowest nicotine residuals in soil reaching 0.27 mg kg^{-1}. Even though the overall total of nicotine absorbed by maize plants in Sikonge (0.811%) did not differ significantly ($P \leq 0.001$) with nicotine absorbed by plants in Tabora (0.790%), levels of nicotine absorbed in Sikonge was slightly higher than Tabora. Similarly, overall nicotine absorbed by maize plants in Urambo site (0.720%) was lower than overall nicotine absorbed by maize plants in Tabora; however, both overall nicotine levels did not differ significantly at $P \leq 0.001$. Despite nicotine residual in soils differing across the sites (Table 27), levels of nicotine absorbed by the maize plants did not differ across the sites (Table 31). On this, indicates that maize root absorption of the variety DKC8053 has similar absorption capacity, however as nicotine flow through the stem, leaf and grain differs but not significantly based on the pressure gradient within the plant itself.

In fertilized maize treatment plots, T5 which previously incorporated with tobacco stalks had the significantly ($P \leq 0.001$) levels of nicotine absorbed in roots (0.79%), stem (0.47%), leaf (0.06%), maize grain (0.001%) and whole maize plant reaching 1.321%. Next to the T5 was the T4 which previously planted tobacco, but tobacco stalks uprooted after harvesting tobacco leaves. Maize plants planted in these treatments, absorbed 0.68% of nicotine in roots,

reaching nicotine levels of 0.38% in the stem, 0.07% in flag leaf, 0.0011% in maize grain with an overall total of only 1.131%. Generally, nicotine absorbed by maize plants observed to range from 0.06 – 0.79% in roots, 0.42 – 0.47% in stems, 0.004 – 0.006% in leaf and 0.0000 – 0.001 in grain. These results indicate that if tobacco stalks not uprooted after harvesting leaves, residual nicotine levels in the soil will increase significantly.

Furthermore, even though tobacco stalks uprooted, maize planted as a subsequent crop after tobacco, still absorb nicotine levels from the soil. The fact of nicotine absorption despite uprooting tobacco stalks, indicates that fine roots remains in the soil and increasing nicotine residuals as they decompose. Studies conducted by Farooq *et al*. (2014) also reported residual nicotine levels in soils after uprooting tobacco stalks before planting a subsequent crop.

The lowest treatment to absorb nicotine was on T3, which was planted with maize not after tobacco. This treatment absorbed 0.06% of nicotine in roots, 0.04% in the stem, 0.02% in leaf, 0.00% in maize grain and with an overall total of 0.12%. To the surprise, unfertilized maize treatments not after tobacco crop (T1), absorbed more nicotine in roots (0.14%), this could have been due to the contamination caused through water runoff from residual nicotine plots to the soil nicotine-free plots such as T1 or T3. Furthermore, this study observed that, analysed control samples (with no nicotine) in the laboratory found to have some residual nicotine levels, indicating that levels of N captured as nicotine since it is one of nicotine component. The absorbed nicotine levels by the roots reduced on its concentration towards upward of plants until reaching the grain where the levels were extremely low. Residual nicotine become available to the maize plant roots proximity through the increased mass flow of nutrients (Ca>Mg>N>S>K>P) as nicotine is N containing compound (Oliveira *et al*., 2010). Immediately after nicotine absorbed by maize roots transported through the xylem to the different plant parts. Results for this study indicates that, even though low nicotine levels observed to the flag leaf, still at the maize cobs composed of cob alpha-cellulose (CAC) have great ability of binding or absorbance of foreign substances from entering the seed (Audu-Peter, Ojile & Bhatia, 2004). The overall levels of nicotine detected in maize grain ranged from 0.0000 – 0.001% and considered negligible equivalent to the levels of nicotine detected in eggplant, potato, tomato, pepper, tea, cauliflower and wild mushrooms (Siegmund, Leitner & Pfannhauser, 1999; Moldoveanu *et al*., 2016; Ikka *et al*., 2018).

There was an interaction effect between sites and grain nicotine residual in fertilized maize plots (Fig. 31). Fertilized T5 previously incorporated with tobacco stalks had significantly (*P*

≤0.001) higher nicotine residuals followed by T4 previously uprooted tobacco stalks in Sikonge, Tabora and Urambo than the rest treatments. However, these levels are still low but should be not neglected. This study observed that maize roots have functional absorption capacity of nicotine and therefore risk of absorbing nicotine by the maize plant could be minimize by planting first unedible leguminous crop immediately after tobacco.

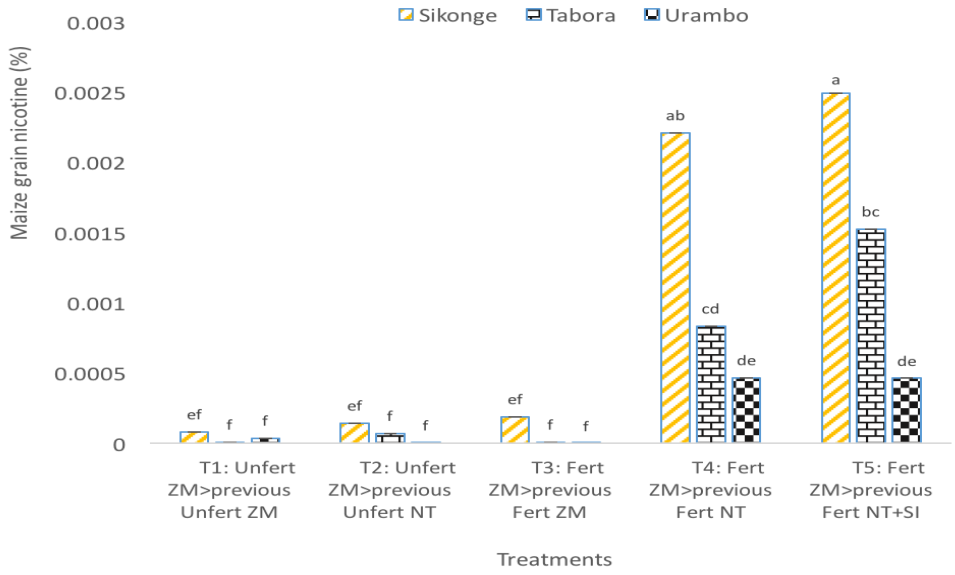


Figure 31: Interaction effect of tretaments and maize grain nicotine

4.6.7 Summary results on effects of nicotine to the subsequent maize yield

Results indicated that biological yield of unfertilized maize grown as subsequent crop after unfertilized tobacco attained 13.96 t ha^{-1} with grain yield of 1.05 t ha^{-1}, while the biological yield of fertilized maize grown as a subsequent crop to fertilized tobacco plot attained 21.97 t ha^{-1} with grain yield of 3.53 t ha^{-1}. Maize grown in previously fertilized tobacco incorporated with tobacco stalks had a bit lower biological yield of 21.12 t ha^{-1} with reduced grain yield (3.12 t ha^{-1}). Fertilized maize grown in previously fertilized maize plot had a slightly lower biological yield of 20.93 t ha^{-1} but had the highest significant grain yield of 3.86 t ha^{-1}. Therefore, results revealed that residual nicotine in soils influenced more maize biological yields than grain yields.

4.7 To determine the effect of nicotine on the diversity of bacteria in the soil and linking with their influence on soil fertility

Two kingdoms archaea and bacteria were identified in all the three sites (Fig. 32). In tobacco plots, archaea counts were 19.48, 16.34 and 3.83% in loamy sand, sandy loam and sand soil,

respectively. Archaea kingdom existed in fewer population in purely sand soil, as the soil particles decreased in size the archaea kingdom kept on increasing. The increasing in richness for archaea from sand soil towards sandy loam and loamy sand soils could be associated with increasing moisture content. Recent studies have indicated archaea richness to be higher to some different soils and the archaea population was correlated positively with the presence of abundant soil moisture, finest soil forest soils and in the fertility soils (Richter *et al*., 2014; Tupinambá *et al*., 2016). In the same tobacco plot, bacteria kingdom counts in loamy sand, sandy loam and sand soil were 80.52, 83.66 and 96.18%, respectively. Bacteria kingdom dominated in all soil types with the almost equal population; however, in sand soil the bacteria population increased substantially. Bacteria reported to be more abundantly at the rhizosphere than in bulk soils, the soil types, on the other hand, found to be significant parameter affecting bacterial diversity in soils (Grządziel & Galazka, 2018; Khan *et al*., 2018).

In maize plots, archaea counts were 17.95, 16.97 and 18.73% in loamy sand, sandy loam and sand soil, respectively. The diversity of archaea was almost equal as the soil moisture favours their abundance in those soils (Richter *et al*., 2014). Bacteria count were 82.05, 83.05 and 81.27% in loamy sand, sandy loam and sand soil respectively. Both kingdoms archaea and bacteria in each soil types were abundant in almost the same richness, indicating that maize crop favours archaea and bacteria at the rhizosphere than tobacco crop do. In fallow plots no archaea kingdom count in loamy sand soil, but in sandy loam and sand soil, the count was 18.2 and 15.33% respectively. Bacteria kingdom dominated by 100, 81.80 and 84.67% in loamy sand, sandy loan and sand soil, respectively. This indicates that bacteria exist more abundantly in different soil types (Grządziel & Galazka, 2018).

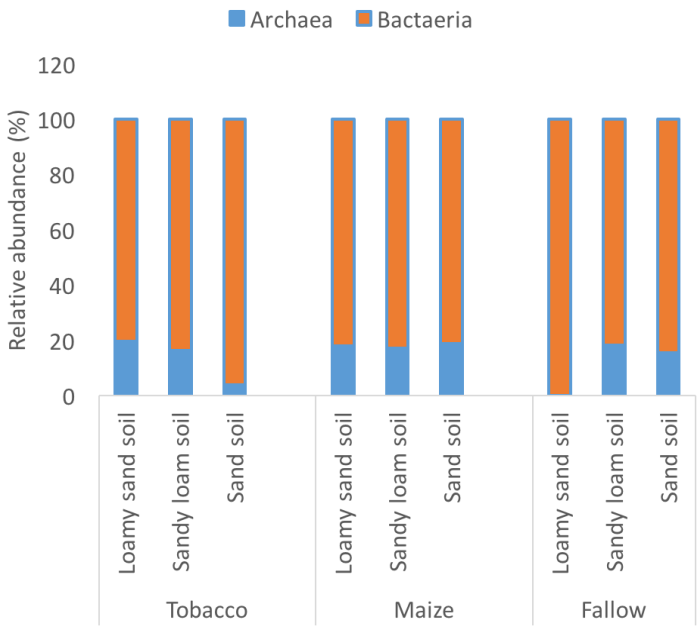


Figure 32: Relative abundance of archaea and bacteria kingdom

4.7.1 General distribution of soil bacteria phylum in tobacco, maize and fallow plots

The 375 429 classifiable sequences in this study, were correlated with 12 relative abundance bacterial phyla from each experimental site (Sikonge, Tabora and Urambo) covering all crops (Fig. 33). To all cropping systems the dominant phyla spotted as *Actinobacteria* (36.21%), *Proteobacteria* (26.27%), *Chloroflexi* (9.03%), *Acidobacteria* (8.74%), *Planctomycetes* (5.78%), *Gemmatimonadetes* (5.41%), *Firmicutes* (4.95%) and *Bacteroidetes* (2.04%). Bacterial phyla with < 1% abundance excluded, and not considered as dominant. The bacterial distribution at the phylum level differed in the different cropping system and different relative abundance.

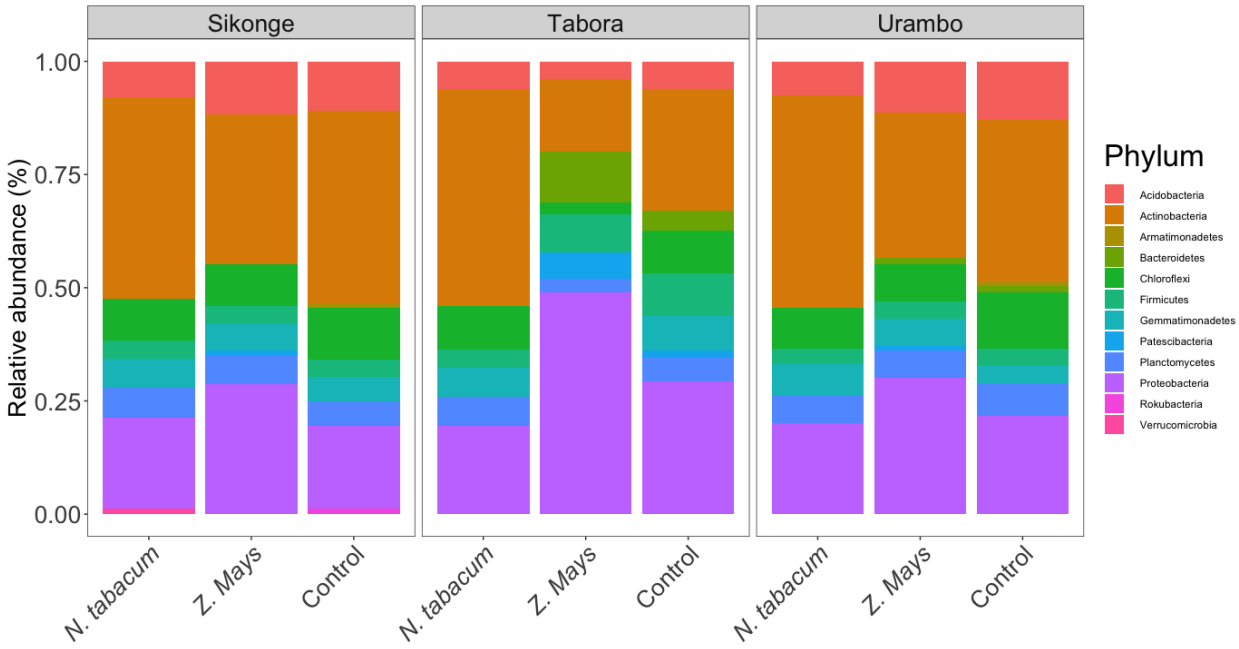


Figure 33: Relative abundances (%) of phylum for each cropping system in Sikonge, Tabora and Urambo

4.7.2 Comparison of soil bacteria phylum in tobacco, maize and fallow plots in different soil types

Relative abundances of major phyla in different soil types, cropping systems and total phyla to each crop are indicated in Figures 34 & 35, respectively. In tobacco rhizosphere the following phyla ranked in their abundance levels as identified through 16S rRNA gene, *Proteobacteria* (11.89%), *Actinobacteria* (8.97%), *Acidobacteria* (2.99%), *Chloroflexi* (2.24%), *Firmicutes* (1.77%), *Planctomycetes* (1.69%), *Bacteroidetes* (1.40%) and *Gemmatimonadetes* (1.31%).

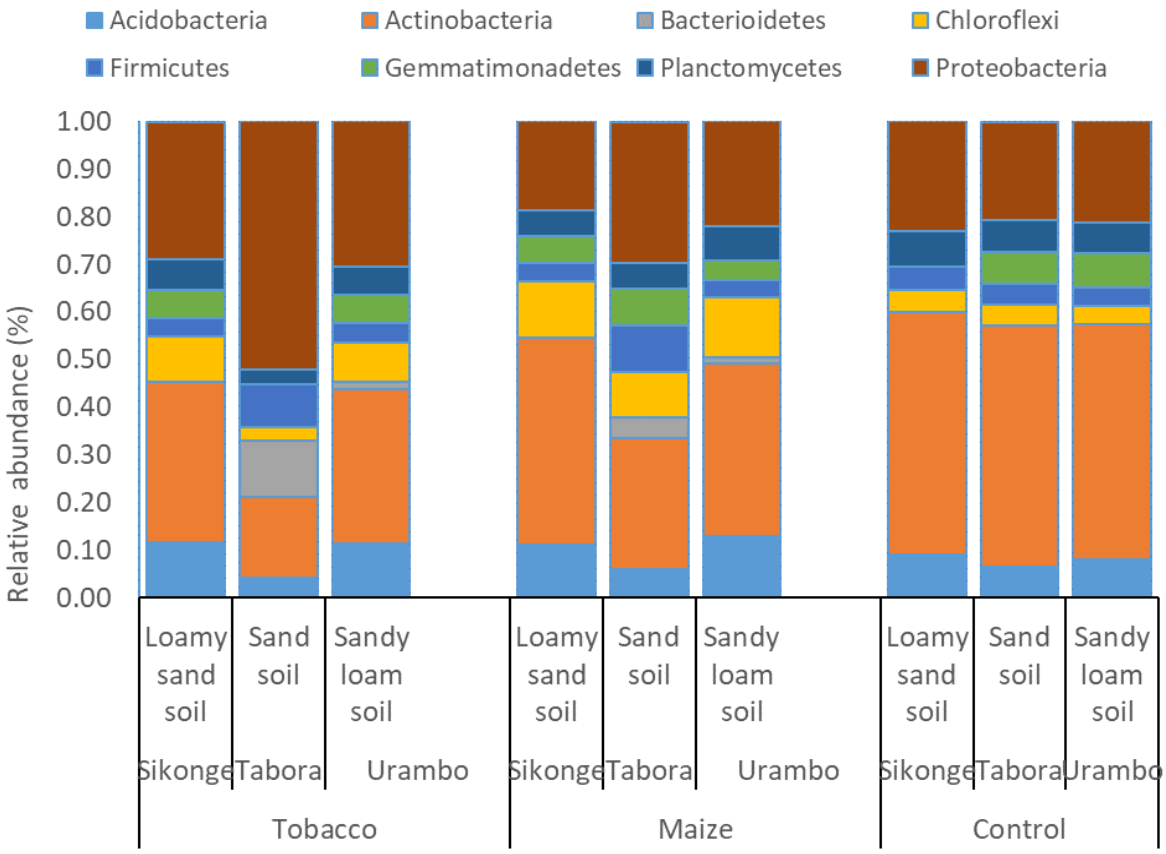


Figure 34: Relative abundancies of bacteria phyla in different soil types and cropping systems

Proteobacteria for tobacco was abundant in Tabora sand soil (5.36%), followed by Urambo sandy loam soil (3.38%) and 3.15% in Sikonge loamy sand soil. *Actinobacteria* in tobacco plots were most dominant by 3.64% in loamy sand soil of Sikonge, followed by sandy loam soil in Urambo (3.59%) and Tabora sandy soil by 1.75%. *Acidobacteria* abundance were 1.28% in loamy sand soil (Sikonge), 1.27% in sandy loam soil (Urambo) and 0.43% in Tabora sandy soil. *Chloroflexi* in tobacco plots abundance was 1.03% in Sikonge (loamy sand soil), 0.92% in Urambo (sandy loam soil) and 0.29% in Tabora sand soil. *Firmicutes* abundance were 0.92% in the sandy soil of Tabora, 0.43% in Urambo sandy loam soil and 0.42% in loamy sand soil (Sikonge). *Planctomycetes* in tobacco plots abundance were 0.70% in Sikonge loamy sand soil, 0.66% in Urambo sandy loam soil and 0.33% in Tabora sandy soil. *Bacterioidetes* were 1.23% in abundance for Tabora sand soil and 0.17% in Urambo sandy loam soil. *Gemmatimonadetes* abundance in loamy sand soil of Sikonge and sandy loam soil of Urambo were 0.64 and 0.66%, respectively.

In maize rhizosphere, our results reveal that abundant phyla as identified through 16S rRNA gene colonized by *Actinobacteria* (11.74%), *Proteobacteria* (7.71%), *Chloroflexi* (3.71%), *Acidobacteria* (3.32%), *Planctomycetes* (1.99%), *Gemmatimonadetes* (1.90%), and *Firmicutes* (1.86%). In maize plots, the distribution of phyla abundances was as follows; *Actinobacteria* were 4.77% in loamy sand soil (Sikonge), 3.99% in sandy loam soil (Urambo), and 2.98% in the sandy soil of Tabora. *Proteobacteria* was 3.24% in Tabora sandy soil, 2.43% in sandy loam soil of Urambo and 2.04% in Sikonge loamy sand soil. *Chloroflexi* were 1.39% in Urambo sandy loam soil, 1.27% in Sikonge loamy sand and 1.04% in Tabora sandy soil. *Acidobacteria* were mostly abundant by 1.44% in Urambo sandy loam soil, 1.21% in Sikonge loamy sand soil and 0.66% in Tabora sandy soil. *Planctomycetes* were 0.80% in Urambo sandy loam soil, 0.60% in Sikonge loamy sand soil and 0.59% in Tabora sandy soil. *Gemmatimonadete*s were 0.84% in Tabora sandy soil, 0.61% in Sikonge loamy sand soil and 0.45% in Urambo sandy loam soil. *Firmicutes* were 1.04% in Tabora sandy soil, 0.43% in Sikonge loamy sand soil and 0.40% in Urambo sandy loam soil.

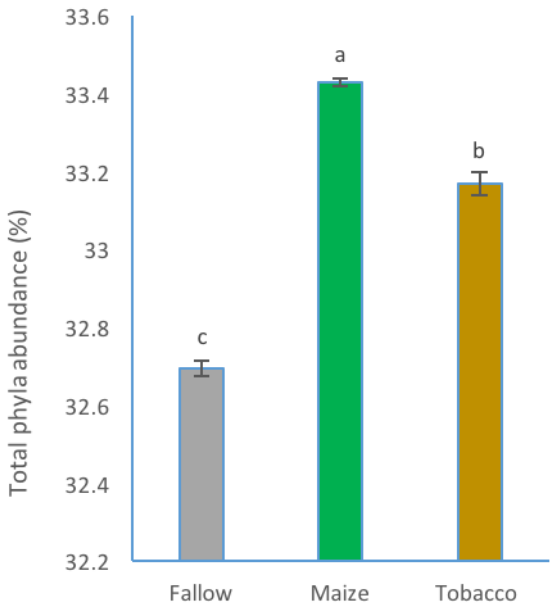


Figure 35: Total phyla abundance in fallow, maize and tobacco

Fallow plots considered as control plots of which no any planted crops except weeds in order to study bacteria phyla abundances in their natural environment. The abundances of phyla in control plots were; *Actinobacteria* (15.48%), *Proteobacteria* (6.65%), *Chloroflexi* (3.09%), *Acidobacteria* (2.43%), *Gemmatimonadetes* (2.19%), *Planctomycetes* (2.10%) and *Firmicutes* (1.31%). In control plots the distribution of phyla abundances were as follows;

Actinobacteria abundance were dominant by 5.31% in Tabora sandy soil, 5.21% in Urambo sandy loam soil and 4.97% in Sikonge loamy sand soil. *Proteobacteria* abundance were 2.25% in Sikonge loamy sand soil, 2.23% in Urambo sandy loam soil and 2.17% in Tabora sandy soil. *Chloroflexi* were 1.06% in Tabora sandy soil, 1.03% in Sikonge loamy sand soil and 1.00% in Urambo sandy loam soil. *Acidobacteria* abundances were 0.89% in Sikonge loamy sand soil, 0.84% in Urambo sandy loam soil and 0.70% in Tabora sandy soil. *Gemmatimonadetes* were 0.71% in Tabora sandy soil, 0.77% in Urambo sandy loam soil and 0.01% in Sikonge loamy sand soil. *Planctomycetes* were 0.73% in Sikonge loamy sand soil, 0.69% in Tabora sandy soil and 0.67% in Urambo sandy loam soil. *Firmicutes* were 0.45% in Sikonge loamy sand soil and Tabora sandy soil respectively, and 0.40% in Urambo sandy loam soil.

4.7.3 Principal component analysis (PCoA) of bacterial phyla based on crops and locations

Most abundant distribution of bacteria phyla shared in all crops, but in relative abundance (Fig. 34). Total phyla abundance was significantly ($p<0.05$) higher in maize (*Zea mays* L.) than in tobacco (*Nicotiana tabacum* L.) and fallow plots (Fig. 35). Bacterial phyla under maize crop distributed almost equally and their relative abundance were significantly higher than bacterial phyla in tobacco crop. Total bacteria phyla in fallow plots were the least than tobacco and maize, respectively. The PCoA score revealed that the maize treatment clustered together and separated away from tobacco treatment with the 70.6% power of separation in the first principal component (Fig. 36 & 37).

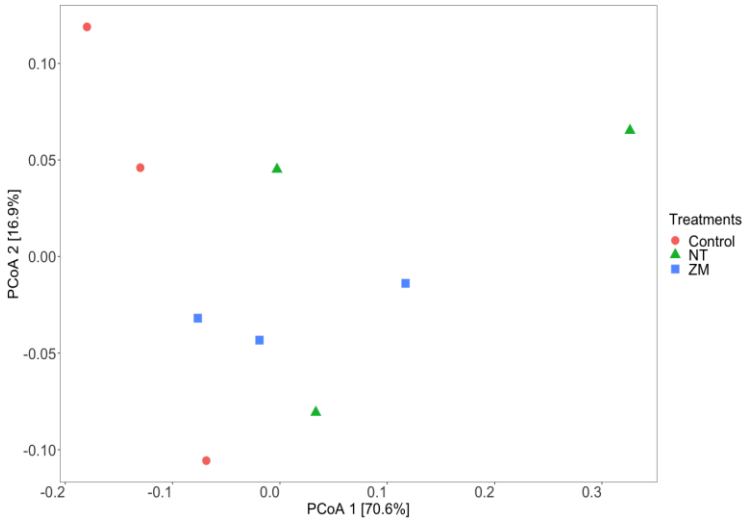


Figure 36: PCoA of the crop treatments based on bacteria phyla abundance

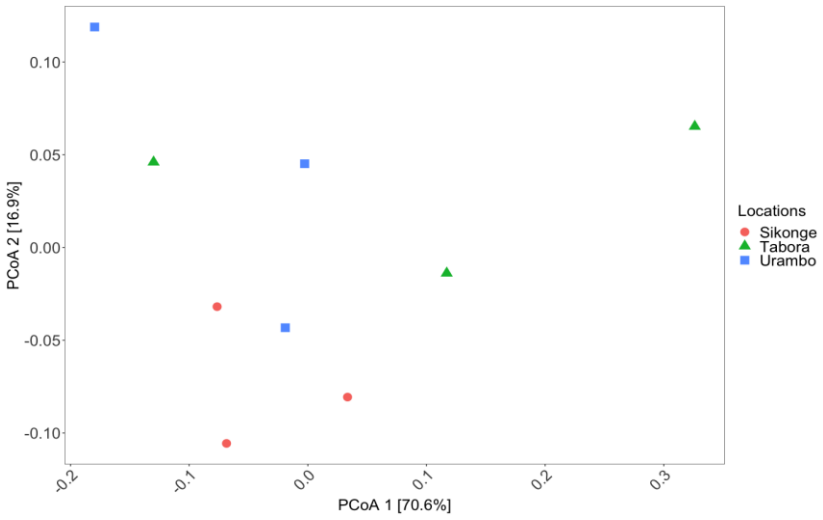


Figure 37: PCoA of the crop locations based on bacteria phyla abundance

4.7.4 Composition of phyla community variation with crops and locations

Comparison of bacteria phyla community varying with treatments (fallow, maize, tobacco) performed across the locations. The significant abundant phyla across the locations along with fallow, maize and tobacco crops were *Actinobacteria*, *Acidobacteria*, *Proteobacteria*, *Chloroflexi*, *Planctomycetes*, *Firmicutes*, *Gemmatimonadetes* and *Bacterioidetes* (Fig. 38).

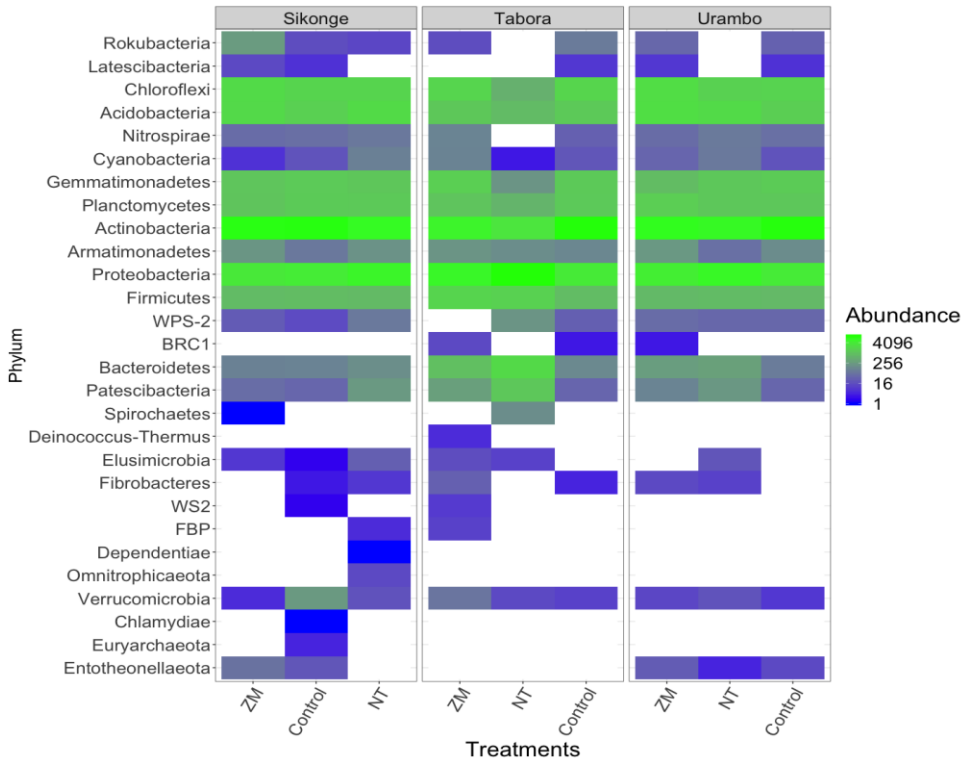


Figure 38: Heatmap indicating the phyla relative abundance in ZM (maize), NT (tobacco) and control

4.7.5 Identified bacteria species through 16S rRNA gene in tobacco, maize and fallow plots

Bacteria species identified in tobacco, maize and fallow plots are shown in Fig. 39, 40 & 41. In tobacco plots, a total of fifteen (15) species were identified. The loamy sand soil had the following species; *Nonomuraea monospora* (5.57%) of phylum *Actinobacteri*a, *Thermomicrobium roseum* (3.99%) of *Chloroflexi* phylum, *Staphylothermus marinus* (3.47%) under *Crenarchaeota* phylum and 86.97% species were unknown. In sandy loam soil, the species identified included the following; *Staphylothermus marinus* (7.18%), *Caminibacter hydrogeniphilus* (4.82%) under *Proteobacteria* phylum, *Nonomuraea monospora* (4.29%) under *Actinobacteria* phylum and 83.71% were unknown. In sand soil among the species identified through 16S rRNA gene are; *Chryseobacterium gleum* (15.30%), *Chryseobacterium arthrosphaerae* (9.50%) and *Chryseolinea serpens* (6.26%) under Bacteroidetes phylum, *Serratia nematodiphilia* (7.00%) under Proteobacteria phylum, *Herbaspirillium seropedicae* (3.92%), *Massilia aurea* (2.85%), *Serratia marcescens*_subsp (2.14%), *Acinetobacter calcoaceticus* (2.11%), *Enterobacter asburiae* (2.04%) under *Proteobacteria phylum* and unknown species were 48.87%.

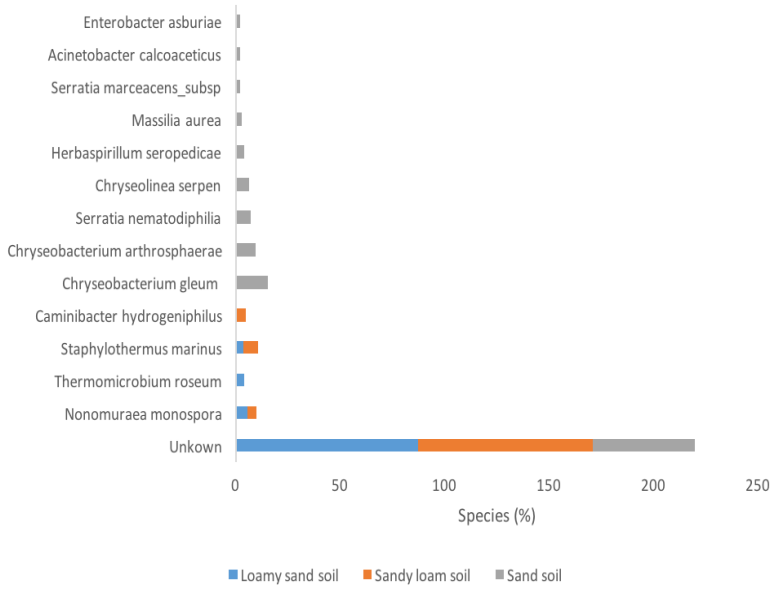


Figure 39: Dominant bacteria species in the tobacco rhizosphere

In maize plots, eleven (11) bacteria species were identified (Fig. 40). Among the identified species in loamy sand soil are the following; *Fictibacillus gelatine* (4.07%) under *Firmicutes* phylum, *Methanopyrus kandleri* (3.60%) under *Euryarchaeota* phylum, *Pyrobaculum aerophilum* (3.49%) under *Crenarchaeota* phylum, *Nonomuraea monospora* (3.49%) under *Actinobacteria* phylum, *Thermomicrobium roseum* (3.26%) under *Chloroflexi* phylum, *Methylocella silvestris* (3.14%) under *Proteobacteria* phylum and 78.95% were unknown. Sandy loam soil unknown species were reaching 96.35%, other identified specie was *Methanopyrus kandleri* (3.65%) under *Euryarchaeota* phylum. Sand soil species were *Nonomuraea monospora* (10.16%) under *Actinobacteria* phylum, *Methanopyrus kandleri* (7.78%), *Methylohalobius crimeensis* (4.77%) under *Proteobacteria* phylum, *Caldivirga maquilingensis* (3.21%) under *Crenarchaeota* phylum and 74.08% of unknown species.

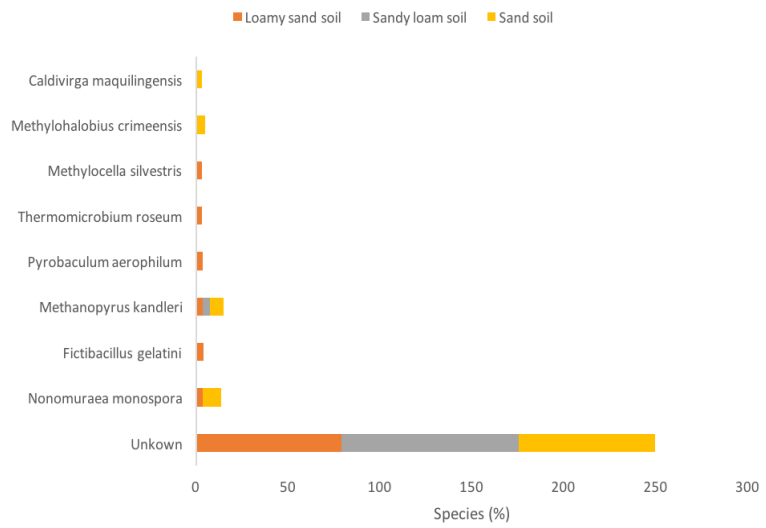


Figure 40: Dominant bacteria species in the maize rhizosphere

In fallow plots eight (8) bacteria species were identified (Fig. 41). In loamy sand soil, the bacteria species identified were *Gaiella occulta* (9.09%), *Conexibacter arvalis* (5.63%), under *Actinobacteria* phylum, *Gemmatimonas aurantiaca* (6.86%) under *Gemmatimonadetes* phylum, *Aquisphaera giovannonii* (3.3%) under *Planctomycetes* phylum and 75.11% unknown species. Species identified in sandy loam were *Methanopyrus kandleri* (4.16%) and *Gordonibacter pamelaeae* (3.71%) under *Actinobacteria* phylum and 92.13% were unknown species. In sand soil, the identified species were *Allochromatium phaeobacterium* (5.12%) under *Proteobacteria* phylum and *Methanopyrus kandleri* (3.38%) and 87.23% of unknowm species.

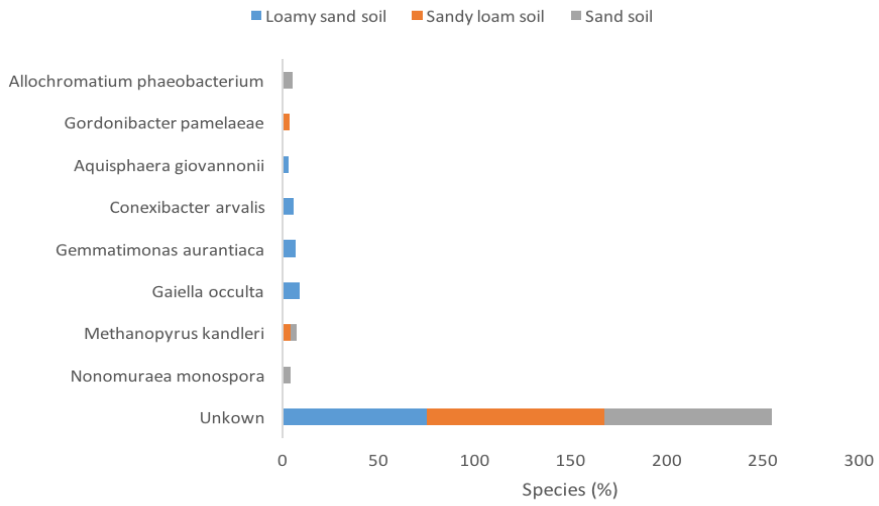


Figure 41: Dominant bacteria species in the fallow rhizosphere

4.7.6 Factors associated with bacteria phyla increasing in their abundances in soil

Proteobacteria and *Actinobacteria* phyla were most abundant in tobacco plot covering 11.89 and 8.97%, respectively. Other phyla were *Acidobacteria*, *Chloroflexi*, *Firmicutes*, *Planctomycetes, Bacteroidetes* and *Gemmatimonadetes* in 2.99, 2.24, 1.77, 1.69, 1.40 and 1.31% proportions, respectively. These bacteria phyla were identified in the studied areas for the first time, their proportions indicating their suitability and withstanding ability to the tobacco rhizosphere. The trends showed to increase in their abundances towards the fine-textured soils for the *Acidobacteria, Chloroflexi, Bacteroidetes* and *Gemmatimonadetes*. *Firmicutes* and *Proteobacteria* showed an increasing trend of their abundances towards coarse-textured soils. The phyla identified in our study in the tobacco plots, were almost similar with the phyla reported by Wu *et al*. (2016) in tobacco fields of which the dominant phyla included; *Proteobacteria*, *Acidobacteria*, *Bacteroidetes*, *Gemmatimonadetes* and *Actinobacteria*. Tobacco is composed of massive roots structure and phylum abundance falling to *Proteobacteria*, *Bacteroidetes*, *Actinobacteria*, *Planctomycetes*, observed to increase towards the delicate roots of tobacco crop (Lei *et al*., 2017; Saleem, Law, Sahib, Pervaiz & Zhang, 2018). Thus, soil type, tobacco plant with the delicate roots and root hairs subjected to the release of nicotine as exudates to the soil and influence diversity of phylum groups and bacteria species (Dey, Pal & Tilak, 2012; Saleem *et al*., 2018).

In the coarse-textured soil, bacteria species were dominant than in the fine-textured soils. Similarly, bacterial species under *Proteobacteria* phylum seems to be dominant in sand soils, while bacterial species under *Actinobacteria* phylum being dominant towards fine-textured soils. In overview, *Proteobacteria* and *Actinobacteria* phyla have great tolerance to all soil types where tobacco is grown. These phyla could be having great abilities in tolerating and degrading nicotine levels in the soil. However, other phyla such as *Bacterioidetes* and *Firmicutes* should be not neglected as they found to be beneficial for the plant growth promotion (Kyselková *et al*., 2009; Kim *et al*., 2013; Basharat *et al*., 2018). This study has identified other phyla that dominated in the tobacco plots to be *Acidobacteria*, *Eurychaeota, Crenarchaeota, Armatimonadetes* and *Chloroflexi*. *Actinobacteria* (11.74%), *Proteobactaria* (7.71%), *Chloroflexi* (3.71%), *Acidobacteria* (3.32%), *Planctomycetes* (1.99%), *Gemmatimonadetes* (1.90%), *Firmicutes* (1.86%) and *Bacteroidetes* (0.64%).

This study revealed a higher proportion of *Actinobacteria,* and *Proteobacteria* phyla with the abundance of 11.74 and 7.71% respectively, in maize plots. Next to these phyla were

Chloroflexi, Acidobacteria, Planctomycetes, Gemmatimonadetes and *Firmicutes* in the proportional abundance of 3.71, 3.32, 1.99, 1.90 and 1.86 %, respectively. With exception to *Proteobacteria* phylum, the rest of the phyla increased in their proportional abundance when compared to the tobacco phyla proportions. *Actinobacteria* and *Chloroflexi* phyla increased significantly in the maize plots than tobacco plots by 2.77 and 1.47% respectively. The small increase in *Acidobacteria, Planctomycetes, Gemmatimonadetes* and *Firmicutes* phyla were by 0.33, 0.30, 0.59 and 0.09% in comparison from the tobacco phyla proportions.

These results indicate maize crop to be a hotspot of bacterial infestation than tobacco crop (Li *et al*., 2014). Fine roots of maize exudate metabolites different from tobacco (nicotine), and influence an increase of bacteria proportions (Dey *et al*., 2012; Li *et al*., 2014) than tobacco which was considered to release nicotine in soils and hence suppress the number of bacteria (Lisuma, Mbega & Ndakidemi, 2019). In this perspective, bacteria phyla in maize plots were in large proportions than in the tobacco plots (Niu *et al*., 2017). Maize rhizosphere, as similar to tobacco rhizosphere, showed a trend of *Actinobacteria*, *Acidobacteria*, *Chloroflexi*, and *Planctomycetes* increasing towards fine-textured soils (from sandy soil, sandy loam to loamy sand soils). On the other side, *Bacteroidetes* (not reported in this study had 0.64% abundance), *Firmicutes* and *Proteobacteria* increased in abundance from loamy sand, sandy loam to sandy (coarse textured) soil. The most abundant phyla reported in this study were in similar with other studies that reported dominant phyla in maize to be *Proteobacteria, Bacteroidetes, Actinobacteria* and *Firmicutes* (Pereira, Ibáñez, Rosenblueth, Etcheverry & Martínez-Romero, 2011; Li *et al*., 2014; Verma, Yadav, Khannam, Saxena & Suman, 2017).

In the fallow plots which were control in this study, the bacteria phyla belonging to *Actinobacteria, Gemmatimonadetes* and *Planctomycetes* their proportions were highest than tobacco and maize crops by reaching 15.48, 2.19 and 2.10%, respectively. Other phyla proportions were at 6.65, 3.09, 2.43 and 1.31% for *Proteobacteria, Chloroflexi*, *Acidobacteria*, and *Firmicutes* respectively. Abundances of these phyla, in general, were in low proportions, indicating that crop rhizosphere influences large proportions and diversity of bacteria. *Acidobacteria*, *Actinobacteria*, *Proteobacteria* and *Bacteroidetes* also have been observed to be abundant in no-till land (Yin *et al*., 2010; Figuerota *et al*., 2012; Aslam, Yasir, Yoon, Jeon & Chung, 2013; Dong, Liu, Yan, Zhang & Zhang, 2017a; Dong *et al*., 2017b). In current study, similar phyla results were observed, in addition to that *Chloroflexi*, *Gemmatimonadetes*, *Planctomycetes,* and *Firmicutes* were found in the control plots. These

phyla also have been recently observed in no-till land (Dong *et al.*, 2017b; Yin *et al.*, 2017). Bacteria reported to be more abundant at the crop rhizosphere than in bulk soils, the soil types on the other hand also were found to be the significant parameter affecting bacterial diversity in soils (Grządziel & Galazka, 2018; Khan *et al.*, 2018). However, Helgason, Walley and Germida (2009) in their study, indicated that bacteria phylum was not consistent in no-till soils.

4.7.7 Soil bacteria species and their diversities in tobacco, maize and fallow plots

Different crops (maize, tobacco) revealed to have influences on the soil chemical properties and exudates of metabolites. Bacteria phyla proportions and diversity in maize crop were higher than in tobacco crop across the experimental locations (Fig. 42). Thus, different crops may change bacteria phyla or their proportions in different locations. The alpha diversity for Tabora versus Sikonge had *p=0.54*; *p=1* value for the observed and Shannon diversity index, respectively. Urambo versus Sikonge had *p=0.35*; *p=1* value for the observed and Shannon diversity index, respectively. These results depict that through observation, Sikonge had more proportion of bacteria species; however, some of them also were found in Tabora. Tabora had more diversified

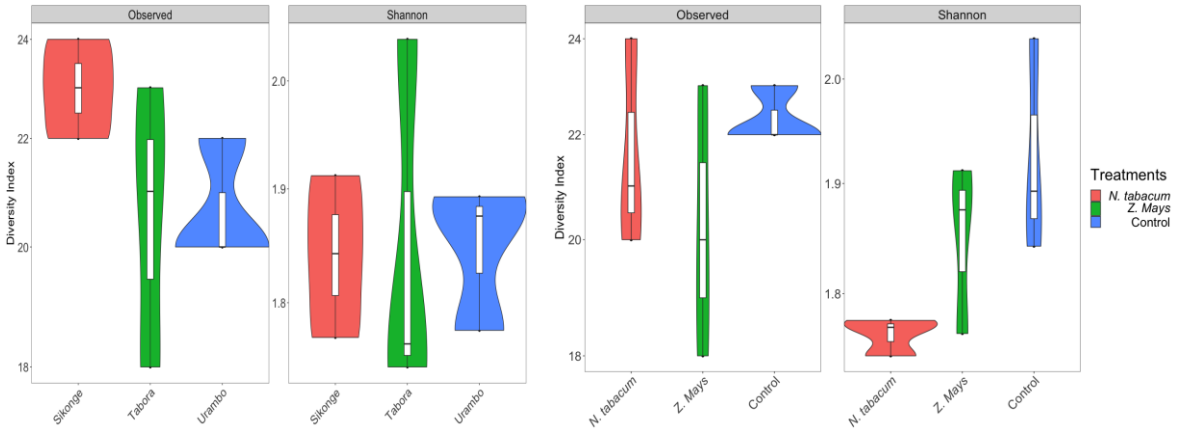


Figure 42: Observed and Shannon diversity index showing location and treatment Phylum level diversity

bacterial species than Sikonge and Urambo but with minimum average. Urambo versus Tabora had both *p=1* values for the observed and Shannon diversity index, respectively, indicating that the Shannon diversity index in these locations did not differ significantly. The none diversity performances in these locations could be as a result of similar soil type, whereby both sites dominated by sandy soil.

Concerning treatment crops, alpha diversity for tobacco versus control/fallow plots and maize versus tobacco had *p=1*; *p=0.8* value for the observed and Shannon diversity index, respectively. Maize versus control/fallow plots had *p=1*; *p=0.3* for observed and Shannon, respectively. These results revealed that through observation, tobacco had more bacterial species in proportions. However, through the Shannon diversity index, bacterial species in tobacco were less in proportions and with different species in comparison to bacterial species in maize and fallow plots. Bacteria species in Maize crop showed a general uniformity throughout soil textures. Bacteria species under fallow plots observed to be more in the coarse and fine-textured soils.

Through the Shannon diversity index, tobacco had bacterial species dominated more by *Proteobacteria* and *Bacteroidetes* than the rest of the phyla. However, majority of the bacteria species such as *Staphylothermus marinus*, *Caminibacter hydrogeniphilus*, *Chryseobacterium gleum*, *Chryseobacterium arthrosphaerae*, *Chryseolinea serpens*, *Serratia nematodiphilia*, *Herbaspillium seropedicae*, *Massilia aurea*, *Serratia marcescenes_subsp*, *Acinetobacter calcoaceticus* and *Enterobacter asburiae* were only found in tobacco plots.

Maize crop based on the Shannon diversity index observed to have a large proportion of *Actinobacteria* phylum and had more proportions of bacteria species in a wide range. Some of these bacteria such as *Nonomuraea monospora*, *Thermomicrobium roseum* and *Methanopyrus kandleri* were also observed in tobacco plots. Other species such as *Pyrobaculum aerophilum*, *Methylocella silvestris*, *Methylohalobius crimeensis* and *Caldivirga maquilingensis* only were identified only in maize plots. Fallow plots observed to have bacteria species not found neither in tobacco nor maize plots. These species include *Gaiella occulta*, *Conexibacter arvalis*, *Gemmatimonas aurantiaca* and *Aquisphaera giovannonii*. In addition to this, fallow and maize plots had bacteria phyla which did not separated widely based on PCoA results (Fig. 36 & 37).

4.7.8 How does soil bacteria species influence soil fertility in the tobacco-maize cropping system

Bacteria species showed an increasing trend towards coarse-textured soil (sand soil of Tabora) with acidic soil (pH = 5.49) than Urambo sandy loam soil and Sikonge loamy sand soil which had soil pH of 5.87 and 5.89, respectively (Tables 18 & 21). The Bacteria population depends on soil pH and types of soils (Marschner, Crowley & Yang, 2004;

Lehtovirta, Prosser & Nicol, 2009). Tobacco generally preferred sand soils for its growth and the crop seems to be favoured in terms of nutrition through the different number of bacteria species. Effects of NPK fertilizers added in soils also significantly induced positive effect on the soil bacterial abundances. Unavailability of N and particularly for P in soils have reported limiting bacteria diversity and abundance (Marschner *et al*., 2004; Leff *et al*., 2015; Jing *et al*., 2017; Camenzind *et al*., 2018). Initial available P was 43.48, 44.41 and 53.39 mg kg^{-1} for Sikonge, Urambo and Tabora, respectively, which depicted an increasing trend of bacteria (Marschner *et al*., 2004). Similar results of increasing P levels in soils associated with an increase in bacteria population also were reported by Camenzind.*et al*. (2018). Soil bacteria role in the amelioration of soil nutrients depending on the soil fertility status, amelioration found to be at a steady rate for Ca, K, Mg and P as these nutrients were released very slowly through rock weathering for soil development (Vitousek & Sanford, 1986).

The current study identified dominant bacteria phyla through 16S rRNA in maize, tobacco and fallow plots falling under the *Proteobacteria*, *Actinobacteria*, *Bacterioidetes*, *Firmicutes*, *Acidobacteria* and *Chloroflexi* phyla. The Bacteria under *Proteobacteria* and *Firmicutes* phyla have been reported to mobilize K to the tobacco rhizosphere and enhance tobacco growth, yields, quality and are known as KSB-Potassium-solubilizing bacteria (Zeng *et al*., 2012; Subhashini, 2015). Therefore, in fields where maize is planted as subsequent crop after tobacco, it is more likely to be deficient in K following its depletion in the soil (Verma *et al*., 2017). Bacteria under *Proteobacteria* phylum reported to have exceptional abilities in solubilizing P to be in an available form for the tobacco plant. Since tobacco plant uptake more of nutrients including P, then this nutrient was depleted in tobacco soils (Chakraborty, Chakraborty & Chakraborty, 2010). The bacteria phylum *Proteobacteria* which exist in high abundance levels in tobacco soils have also been reported to having abilities in degrading alkaloids/phenolics compound and hence cleaning the soil environment (Jung & Park, 2015; Irankhah *et al*., 2019). The significant decrease of S nutrient in tobacco growing areas, as observed in this study, could be attributed to the role of bacteria group under *Actinobacteria* and *Proteobacteria* phyla. Members of bacteria groups under these phyla have also been reported being involved in reducing sulphate to hydrogen sulphide (H_2S) and therefore reduce S levels in the soils (Alain *et al*., 2002; Zhang *et al*., 2014; Sungthong & Nakaew, 2015; Saha *et al*., 2018).

In tobacco production areas P, K and S macronutrients have also been observed to decrease significantly (Table 18; Farooq *et al*., 2014; Moula *et al*., 2018). Released nicotine in soils was a primary reason given for the decrease of these nutrients. However, this study revealed that the decrease of these nutrients is due to the abundance and diversity of soil bacteria playing a role of solubilisation of P and K nutrients. This study observed abundance of *Serratia nematodiphila, Serratia marcescens* species under *Proteobacteria* phyla in tobacco growing areas to be associated in solubilisation of P and hence quickly taken by tobacco to a great extent to cause its reduction in soil media (Leff *et al*., 2015; Jing *et al*., 2017; Basharat *et al*., 2018). *Enterobacter asburiae* under Proteobacteria phyla commonly identified in tobacco growing plots are reported to have a role in converting insoluble K and P to be in a form a plant can absorb and hence results into depletion of these nutrients in soil after tobacco cultivation due to its high uptake (Zeng *et al*., 2012; Zhang & Kong, 2014; Ahmad, Nadeem, Naveed & Zahir, 2016). *Caminibacter hydrogeniphilus* spp from under *Proteobacteria* phyla commonly found in tobacco plots produces its energy by reducing elemental sulfur or nitrate and therefore reducing S contents in tobacco soil (Alain *et al*., 2002).

The increase of soil total N after tobacco production (Table 18; Farooq *et al*., 2014) is due to the N fixation at the tobacco rhizosphere following abundance levels of bacteria under the Proteobacteria phylum. Identified bacteria species in tobacco plots under *Proteobacteria* such as *Herbaspirillum seropedicae, Massilia aurea* and *Enterobacter asburiae* are reported to fix N in the soils and hence increasing soil total N after tobacco (Trovero *et al*., 2018; Balsanelli, Serrato, Pedrosa, Souza & Monteiro, 2015; Zúñiga-Feest *et al*., 2018). *Chloroflexi* another dominant phylum identified in tobacco growing area was reported to catalase Mn (Baginski & Sommerhalter, 2017), oxidizes CO aerobically in hotter areas (Wu *et al*., 2009) and having a role for nitrification in soil (Sorokin *et al*., 2012).

Another nutrient reported being increased in the soil after tobacco cultivation is Ca (Table 18; Farooq *et al*., 2014). Astonishingly, Ca^{2+} levels increased drastically in tobacco soils, the increase of Ca^{2+} level is evident due to the abundances of diversified bacteria in tobacco rhizosphere of which their surfaces were negatively charged. As a result of their negative charge, subjected them to be an attracting zone for Ca and Mg divalent cations; therefore, increases their levels in soils (Ferris, Stehmeier, Kantzas & Mourits, 1996; Stocks-Fischer, Galinat & Bang, 1999; Ramachandran *et al*., 2001). However, Ca^{2+} ions bind more frequently

into the negatively charged cell surface of bacteria than Mg^{2+} due to its higher power for ionic selectivity (Wold, 1994; Sanchez-Roman, Rivadeneyra, Vasconcelos & McKenzie, 2007).

4.7.9 Soil bacteria mechanisms for mineral solubilisation and increase of micronutrients in soil

The mechanism behind the ability of bacteria in solubilizing minerals in tobacco rhizosphere is through the production of organic acids (acidolysis) which solubilize minerals for easy uptake by plant (Uroz, Calvaruso, Turpault & Frey-Klett, 2009; Basak & Biswas 2012; Parmar & Sindhu 2013; Zarjani, Aliasgharzad, Oustan, Emadi & Ahmadi, 2013). Soil bacteria take advantage of acidolysis and complexolysis mediated by organic acids interchangeably for the transformation of insoluble minerals into soluble minerals form (Zeng *et al*., 2012). Soluble minerals then become available and enhance more uptake of nutrients, crop growth and productivity (Basak & Biswas 2012). The acidification in the rhizosphere mediated by bacteria also producing H^+ and thus facilitates in the ion-exchange process. Therefore, levels of micronutrients such as Cu^{2+}, Fe^{2+} Zn^{2+} and Mn^{2+} (Table 21) also increases in excess in the soil solution and become available to the plants.

Therefore, this metagenomics study through soi bacterial 16S rRNA gene revealed *Proteobacteria* and *Actinobacteria* phyla to be mostly soil dominant in tobacco, maize and fallow plots, but with different bacteria species having different roles in the rhizosphere. Bacteria species identified in tobacco rhizosphere, found to have a potential role of solubilizing insoluble K and P into available forms of which tobacco plants can absorb and hence reduce their levels in tobacco soil. These phyla also play a role of reducing sulphate to hydrogen sulphide S and fixing N in soils and increase N levels in tobacco soils. The negatively charged bacteria was revealed to bind more Ca^{2+} ions and increase more their concentrations in soils. Through the bacteria activities in the rhizosphere, resulted into producing H^+ which increasing acidity in the soils. Acidity in soils influence solubility and increases levels of Cu^{2+}, Fe^{2+} Zn^{2+} and Mn^{2+} in the soils, as the tobacco plant require trace amount of these micronutrients.

4.7.10 Summary results on effects of nicotine on the diversity of bacteria in soils

The results showed that bacterial species in tobacco soils under *Proteobacteria* phyla, namely *Serratia nematodiphila* and *Serratia marcescens* were found to be associated with

solubilization of the insoluble P. *Enterobacter asburiae* was observed to be responsible in solubilisation of the insoluble K and *Caminibacter hydrogeniphilus* to be responsible in solubilization of sulphate to H_2S. The solubilization of P, K and S in soils resulted into readily available by the tobacco heavy feeder crop leaving low levels of these nutrients in the soils and/or to the subsequent crop. The bacteria employ solubilisation mechanisms of these macronutrients through producing H^+, which also increases the solubility of the micronutrients Cu^{2+}, Fe^{2+}, Zn^{2+}, Mn^{2+} and their levels in the soils. *Enterobacter asburiae, Herbaspirillum seropedicae and Massilia aurea* were found to increase soil N through fixation. Levels of Ca^{2+} increased in the soil through attraction forces towards bacteria abundances with the negatively charged surface.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The present study revealed that nicotine released into the soil is twice in NPK + CAN fertilized tobacco (10.27 mg kg^{-1}) compared with the unfertilized tobacco plants (3.07 mg kg^{-1}) indicating that N fertilized tobacco releases more nicotine to the soil than unfertilized tobacco. The dynamics of nicotine released in the rhizosphere is mainly dependent on the soil moisture and the rooting depth. Higher nicotine levels (7.59 mg kg^{-1}) seemed to be at a depth of 30-50 cm and lower nicotine levels (5.50 mg kg^{-1}) at a depth of 0-10 cm. The maximum nicotine adsorption and desorption in soils observed to be 4.61 and 2.21 mg kg^{-1}, respectively. Such observation indicates that nicotine persists in soils after its release. Since higher nicotine is observed at a depth of 30 – 50 cm, shallow-rooted crops such as lettuce, potato etc. recommended to be planted as a subsequent crop avoid strong interaction with high levels of nicotine at the depth beyond 20 cm.

Maize roots absorb residual nicotine from the soils; the levels of nicotine reaching in maize grain is negligible (0.001%). However, reduced maize grain yields by 0.33 t ha^{-1} and attain 3.53 t ha^{-1} in comparison to the maize planted not after tobacco which had higher grain yields of 3.86 t ha^{-1}. Nicotine levels in the soil affect the soil chemistry of nutrients by decreasing levels of P. K and S, explaining to why a subsequent maize crop after tobacco has reduced grain yields, as P and K are essentials to impart grain fillings in maize cobs.

Furthermore, tobacco rhizosphere has been linked in this study with abundancies of bacteria-loving nicotine from the *Proteobacteria* phylum. Bacteria under this phylum can solubilize insoluble K and P into an available form that is quickly taken up by the tobacco heavy feeder plant, leaving little or nothing to the subsequent crop. However, bacteria under *Proteobacteria* phylum such as *Enterobacter aburiae* and *Massilia aurea* as identified in this study are involved in N fixation in the tobacco rhizosphere and hence increases N levels in the tobacco soil. In connection to this, negatively charged bacteria surface, can attract more Ca^{2+} (ionic selectivity) than Mg^{2+} in their surface and increase significantly Ca^{2+} levels in the tobacco soils. The released nicotine in the tobacco rhizosphere increases soil acidity (H^{+}) resulting into increases in the solubilities of Cu^{2+}, Fe^{2+}, Zn^{2+} and Mn^{2+} and hence increases their levels in the soils, as these nutrients are required by tobacco in trace amount.

5.2 Recommendations

(i) Soil moisture influenced the dynamic of nicotine in soils, and higher levels of nicotine observed to the depth of 30 – 50 cm. Therefore, planting of shallow-rooted crops such as lettuce, groundnuts, beans, potatoes and even some maize varieties with shallow roots is recommended to avoid strong interaction with high levels of nicotine at deeper depths.

(ii) For increased grain yields to 3.86 t ha^{-1} and above, farmers should plant maize in the land, not after tobacco. However, since land is scarce to the majority of farmers planting maize after tobacco, the grain yields expected to be reduced by 0.33 t ha^{-1}. Therefore, supplementing P and K fertilizers beyond the current recommended rate of 50 kg P ha^{-1} and 50 kg K ha^{-1} may increase the yields.

(iii) To the areas where cultivable land is not a problem, as nicotine observed to persist in the next cropping season, it is recommended for the fastest growing inedible leguminous crops such as sunhemp (*Crotalaria juncea* L.) to be planted soon after harvesting the tobacco leaves to intercede the tobacco crop and the intended maize food crop. This practice would improve soil fertility and reduces residual nicotine levels before planting maize.

(iv) Regardless of the cultivable land scarcity or availability, tobacco stalks must be uprooted with great care immediately after harvesting leaves when there is still adequate moisture in the soil to allow easy removal of roots and hence reduce nicotine residues in the soil and to the subsequent crop.

(v) Solubilization of P and K by soil microbes provide an avenue for exploring possibilities of developing inoculant to improve P and K solubilities in soils and/or organic fertilizers such as Minjingu for maize or other crops production.

(vi) Further studies are required to quantify the amount of P and K extracted by tobacco plant from the soil and re-calibration of new recommendations for P and K on maize as a subsequent crop after tobacco.

(vii) Further studies are recommended to establish the critical nicotine levels in different soil textures, and nicotine absorbed levels by the maize cultivars. Thus will enable recommending tobacco cultivars for production with good leaf quality but also

releasing low levels of nicotine in the soils. The studies should go concurrently with intensive research at the molecular level to explore the role of other bacteria species in the tobacco soils of the studied sites, as this study revealed large per cent of unknown bacteria species yet to be identified.

(viii) Since the current study observed tobacco plant to have influenced increases of micronutrient Cu^{2+}, Fe^{2+}, Mn^{2+} and Zn^{2+} in soils and their leaf concentrations; there could also be higher possibilities in increasing heavy metals (Cd, Pb, Cr) and non-essential elements (Na, Si, Al, Sr, V). Therefore, further studies are required to determine levels of heavy metals and non-essential elements which were not determined in the current study to both soils and tobacco leaf.

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APPENDICES

Appendix 1: Supplementary data on biochemical data related to soil fertility

Results indicated that soil measured parameters were correlated with bacteria diversity (Table 32). Soil pH along with N, S, P, Ca, K and soil pH along with Cu, Fe, Mn and Zn significantly improved the R2 values from 94.88 to 96.05%, respectively, in predicting bacteria diversity (Table 33).

Table 32: Correlations between soil parameters and bacterial diversity indices ($p<0.05$)

Parameters	Chao1	SDI	pH	Cu (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})	Fe (mg kg^{-1})	N (%)	OC (%)	S (mg kg^{-1})	P (mg kg^{-1})	Ca (cmol (+) kg^{-1})	K (cmol (+) kg^{-1})	Nicotine (mg kg^{-1})
1. Chao1	1													
2. SDI	0.44	1												
3. pH	0.57	0.28	1											
4. Cu (mg kg^{-1})	-0.80	-0.23	-0.1	1										
5. Zn (mg kg^{-1})	-0.09	-0.48	0.28	0.42	1									
6. Mn (mg kg^{-1})	0.02	0.14	0.27	0.39	0.75	1								
7. Fe (mg kg^{-1})	-0.59	0.01	-0.34	0.69	0.39	0.64	1							
8. N (%)	0.06	-0.44	-0.25	-0.10	0.61	0.49	0.31	1						
9. OC (%)	0.58	-0.21	0.55	-0.26	0.73	0.54	-0.13	0.61	1					
10. S (mg kg^{-1})	0.63	-0.14	0.39	-0.70	-0.22	-0.56	-0.98	-0.16	0.31	1				
11. P (mg kg^{-1})	0.67	-0.02	0.16	-0.89	-0.35	-0.52	-0.89	0.04	0.26	0.89	1			
12. Ca (cmol (+) kg^{-1})	-0.10	-0.08	0.09	0.41	0.76	0.85	0.75	0.55	0.50	-0.62	-0.63	1		
13. K (cmol (+) kg^{-1})	0.38	-0.53	0.02	-0.39	0.57	0.22	-0.11	0.81	0.80	0.30	0.37	0.39	1	
14. Nicotine (mg kg^{-1})	-0.56	-0.4	-0.55	0.40	0.46	0.49	0.77	0.70	0.05	-0.70	-0.48	0.58	0.30	1

SDI = Shannon Diversity Index

Table 33: Stepwise multiple regression between bacterial diversity and soil parameters

	Variables	R^2	R^2 adjusted	R^2 predicted
1.	BD: soil pH	56.57%	27.76%	32.00%
2.	BD: soil pH, OC	65.40%	35.14%	42.77%
3.	BD: soil pH, Ca, N, P, K, S	94.88%	83.03%	90.02%
4.	BD: soil pH, Cu, Fe, Mn, Zn	96.05%	89.04%	92.26%

BD = Bacterial Diversity

Appendix 2: Supplementary data on leaf nicotine, green and dry leaf yield in Sikonge, Tabora and Urambo

Results indicated that leaf nicotine concentration and dry leaf yield decreased significantly with experimental sites in the order Sikonge > Tabora > Urambo. The green leaf yield followed a decreasing trend of Sikonge > Tabora = Urambo. Fertilization resulted in a significant increase in leaf nicotine concentration and tobacco dry and green leaf yields. Interactions between experimental sites and fertilization or unfertilized conditions were significant (Table 34).

Table 34: Leaf nicotine and tobacco leaf yield from Sikonge, Tabora and Urambo site

Descriptions	Leaf nicotine (%)	Green leaf yield (kg ha^{-1})	Dry leaf yield (kg ha^{-1})
Sites:			
Sikonge	2.85 ± 0.36 a	10522.92 ± 2996.12 a	1117.11 ± 287.95 a
Tabora	2.36 ± 0.23 b	7060.65 ± 1873.49 b	749.07 ± 208.64 b
Urambo	2.10 ± 0.24 c	6287.73 ± 1994.81 b	614.58 ± 201.38 c
Treatments:			
Unfertilized tobacco	1.82 ± 0.07 b	2892.59 ± 360.76 b	311.39 ± 51.89 b
Fertilized tobacco	3.05 ± 0.16 a	13021.60 ± 1079.90 a	1342.45 ± 108.29 a
2-WAY ANOVA F-statistics			
Site (S)	129.55***	45.95***	55.61***
Treatment (T)	979.48***	695.05***	655.09***
S × T	26.07***	18.31***	9.48**

Means in each column with different letter(s) differ significantly.

Fertilizers applications significantly increased leaf nicotine concentrations and tobacco green and dry leaf yields in Sikonge site (Table 35).

Table 35: Leaf nicotine concentrations and tobacco leaf yields of Sikonge site

Cultivation type	Leaf nicotine (%)	Green leaf yield (kg ha^{-1})	Dry leaf yield (kg ha^{-1})
Unfertilized tobacco	2.04 ± 0.02 d	3826.85 ± 184.96 c	477.69 ± 72.28 c
Fertilized tobacco	3.67 ± 0.01 a	17218.98 ± 109.96 a	1756.53 ± 22.36 a

Means in each column with different letter(s) differ significantly.

Fertilization resulted in a significant increase in leaf nicotine concentrations and tobacco green and dry leaf yields in Tabora site (Table 36).

Table 36: Leaf nicotine concentrations and tobacco leaf yields of Tabora site

Cultivation type	Leaf nicotine (%)	Green leaf yield (kg ha^{-1})	Dry leaf yield (kg ha^{-1})
Unfertilized tobacco	1.85 ± 0.03 e	2986.11 ± 564.34 cd	289.82 ± 27.61 d
Fertilized tobacco	2.87 ± 0.03 b	11135.19 ± 793.48 b	1208.33 ± 77.33 b

Means in each column with different letter(s) differ significantly.

Application of fertilizers significantly increased leaf nicotine concentrations and tobacco green and dry leaf yields in Urambo site (Table 37).

Table 37: Leaf nicotine concentrations and tobacco leaf green and dry yields of Urambo site

Cultivation type	Leaf nicotine (%)	Green leaf yield (kg ha^{-1})	Dry leaf yield (kg ha^{-1})
Unfertilized tobacco	1.57 ± 0.04 f	1864.81 ± 490.00 d	166.67 ± 42.43 d
Fertilized tobacco	2.62 ± 0.09 c	10710.65 ± 306.59 b	1062.50 ± 18.37 b

Means in each column with different letter(s) differ significantly