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Dynamics of nicotine across the soil–tobacco plant interface is dependent on agro-ecology, nitrogen source, and rooting depth

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1. Introduction

Tobacco (*Nicotiana tabacum* L.) is the primary nicotine producing plant through its root system (Steppuhn et al., 2004; Shoji et al., 2008). It produces nicotine to as high as 4% of its leaf content compared with potato, tomato, eggplant, pepper, tea, cauliflower, and wild mushrooms (Davis et al., 1991; Siegmund et al., 1999; Moldoveanu et al., 2016; Ikka et al., 2018), which produce negligible amounts ranging from 0.00001 to 0.00000038% of their leaf contents (Siegmund et al., 1999; Moldoveanu et al., 2016).

Tobacco plants transfer nicotine via the xylem to leaf vacuoles for storage (Shitan et al., 2009), where it provides defensive functions against predators (Ballaré, 2011). Previous studies have shown that some synthesized nicotine is passively released from the meristematic root regions to protect against harmful soil microorganisms, hence increasing the plant's competitive advantage for soil nutrients (Darwent et al., 2003; Walker et al., 2003). The levels of nicotine released to the rhizosphere have been reported to negatively affect the proliferation of bacteria and availability of K and P in soils (Adediran et al., 2004; Moula et al., 2018). Thus, nicotine may affect the productivity of crops planted subsequently in the same field.

Cheng et al. (2018) demonstrated that higher atmospheric temperatures resulted in the accumulation of nicotine in roots, consequently increasing the amount of nicotine released to the rhizosphere. Nicotine dynamics in the rhizosphere are also associated with soil moisture (SM) (Hsiao and Xu, 2000). The influence of SM and soil temperature (T) on nicotine concentrations in tobacco leaves has been well documented (Parups et al., 1960; Benowitz et al., 2006; Bilalis et al., 2009; Cakir and Cebu, 2010; Malik et al., 2013; Cheng et al., 2018). However, these effects are not directly linked with the dynamics of nicotine released from roots in different depth (Hsiao and Xu, 2000; Cakir and Cebu, 2010).

The main objective of the present study was to investigate the dynamics of nicotine released by tobacco roots under fertilization, and to assess the influence of rooting depth on soil parameters (pH, organic carbon (OC), SM, and T). Our findings will help tobacco growers make informed decisions on the suitability of crops for planting subsequent to tobacco based on rooting depth.

2. Materials and methods

2.1. Site description

Field experiments were conducted in the 2017–2018 cropping season in three sites in Tabora region, Tanzania, East Africa, namely, Sikonge (Ulyanyama), Tabora (Tumbi), and Urambo (Mbaoni). Ulyanyama (05° 31′ 47.4″ S, 032° 50′ 03.2″ E; 1191 m a.s.l.) had annual mean rainfall and air temperature of 1050 mm and 29 °C, respectively. Tumbi (05° 03′ 44.4″ S, 032° 40′ 07.4″ E; 1160 m a.s.l.) had annual mean rainfall and air temperature of 950 mm and 27 °C, respectively. Mbaoni (05° 04′ 33.5″ S, 032° 00′ 09.8″ E; 1108 m a.s.l.) had annual mean rainfall and air temperature of 890 mm and 25 °C, respectively. Soils for Sikonge and Tabora characterised as eluvial, catenary association-dominant and illuvia soils, while for Urambo characterised as black or grey clay plateau and plain soils.

2.2. Experimental design and transplanting of seedlings

We used a randomized complete block design with treatments replicated three times. The treatments involved different levels of fertilizers, $N_{10}P_{18}K_{24}$ (N, P, and K combination) and calcium ammonium nitrate (CAN; 27% N), checked against a control with no fertilizer added. Parallel experiments were installed in the three sites on fallowed fields with no tobacco cultivation background. The sites had grass vegetation with few shrubs and farmers used to cut grass for roofing their houses. Tobacco variety K326 was obtained from the Tobacco Research Institute of Tanzania (TORITA) based in Tabora. A seedbed nursery measuring 20 m × 1.5 m was established in each site, and 3 g of K326 tobacco seed were sown with 5 kg of $N_{10}P_{18}K_{24}$ fertilizer as a booster. Four weeks after sowing, the seedlings were transferred to a mother seedbed and managed for 2 weeks, and then clipped and hardened off for a further 2 weeks before being transplanted in the experimental fields. Tobacco seedlings were transplanted to six plots, each measuring 6 m × 6 m with 1.2 m between ridges and 0.50 m between plants.

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Basal $N_{10}P_{18}K_{24}$ fertilizer was manually applied at a rate of 30 g per plant per hill, which was equivalent to the recommended rates of 50 kg N ha^{-1}, 90 kg P ha^{-1}, and 120 kg K ha^{-1} as recommended by the Tobacco Research Institute of Tanzania (TORITA) based on soil fertility evaluation (Kuboja et al., 2012). Fertilizers were applied 7 days after transplanting the seedlings. Two weeks after basal application of $N_{10}P_{18}K_{24}$ fertilizer, a top dressing with CAN 27% was manually applied at 8 g per plant, which is equivalent to the recommended rate of 33.75 kg N ha^{-1} as recommended by TORITA (Kuboja et al., 2012). Other agronomic management practices such as weeding, hand removal of suckers and earthing-up were performed throughout the plant growth period.

2.3. Data collection

2.3.1. Determination of nicotine concentration in leaves

Tobacco leaves were sampled at 10 weeks after transplantation. One well-matured leaf was sampled from the upper, middle, and lower parts of the tobacco plant in a row from each of the inner three out of five rows, totalling nine leaves per plot. All leaf samples were initially air dried under shade around 25 °C to reduce moisture content and then oven-dried at 65 °C to a constant weight. The dry samples were chopped and ground to pass through a 2-mm sieve and for nicotine concentration measurements (Figueiredo et al., 2009). All results (leaves, roots, and soil) were converted to mg kg^{-1} to ensure consistency and comparability.

2.3.2. Determination of nicotine concentration, moisture, rooting depth, temperature, pH, and organic carbon

In each plot, SM and T were determined once using an SM probe series 2900 F and soil thermometer, respectively, at different depths (0–10 cm, 10–20 cm, and 30–50 cm) immediately after harvesting the tobacco. Three samples were collected from each depth making six composite samples per experimental site. The composite soil samples were air-dried and sieved through a set of 2 mm, 1 mm, and 0.5 mm in order to remove fine root tips (Guo et al., 2004), then pH was measured using a soil:water ratio of 1:2.5 extractant (Moberg, 2001). About 10 g of soil was transferred into a 100-ml plastic bottle followed by 25 ml of the extractant. The mixture was shaken for 30 min and allowed to settle for 5 min, and the supernatant solution was read using an electrode pH meter. Organic carbon was determined by the Walkley Black method as modified by Moberg (2001). Briefly, 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 and left for 30 min to cool. The indicator diphenylamine was added and the mixture titrated using ferrous sulfate ($FeSO_4$). The OC was determined using the same amount of dichromate used in the oxidation.

Nicotine content was determined by spectrophotometric analysis using a UV visible single beam fixed at 602 nm. Previous research has indicated no significant difference at 95% confidence level according to the Student's t – test when comparing spectrophotometric and HPLC method (Figueiredo et al., 2009). Standard L-nicotine of 99% purity was purchased from Merck (Germany), NaOH, $(CH_3COO)_2Zn$, $K_4Fe(CN)_6.3H_2O$, charcoal, and methanol were purchased from BDH Chemicals Ltd., East Yorkshire, UK through Sokoine University of Agriculture, Morogoro Tanzania. About 0.3 g of powdered sample 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 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. Total nicotine content was determined using a calibration curve concentration of 0.06–3 mgL^{-1}. For our 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.

2.4. Statistical analyses

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 30–50 cm), 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.

A multiple linear regression analysis was performed such that nicotine was regressed as a response variate (Y) and the fitted terms were the SM, OC, soil pH, T and generated the following regression model:

$$\text{Nicotine (Y)} = m_1X_i + m_2X_{ii} + m_3X_{iii} + m_4X_{iv} + C$$

where X_i to X_{iv} stand for parameters SM, OC, soil pH and T.

m_1 to m_4 represent coefficients of the parameters.

C is the constant.

Nicotine was further subjected to the correlation against the SM, OC, soil pH and T in order to measure the strength and direction of multiple linear relationship. The value of correlation 'r' was between +1 (positive correlation) and −1 (negative correlation) by comparing the p-value of significance level (0.05).

3. Results

3.1. Effect of fertilizer treatments on nicotine concentrations at different sites

Site effects were highly significant ($P \leq 0.001$) on nicotine concentration in the roots, soil, and leaves, and total nicotine (Table 1). Sikonge had the highest soil nicotine concentration (9.55 mg kg^{-1}) followed by Tabora (6.04 mg kg^{-1}) and Urambo (4.42 mg kg^{-1}). Fertilizer addition significantly ($P \leq 0.001$) influenced nicotine concentration in the soil, leaves, and total nicotine, but did not in the roots (7.29 mg kg^{-1}) compared with unfertilized plants (7.12 mg kg^{-1}). Nicotine was higher in the soil of fertilized plants (10.27 mg kg^{-1}) than unfertilized plants (3.07 mg kg^{-1}). The interactions between sites and fertilizer treatments significantly ($P \leq 0.001$) increased nicotine in the soil, and the highest levels of nicotine were observed in the fertilized plots in Sikonge (8.98 mg kg^{-1}), while the lowest was observed in Urambo (5.72 mg kg^{-1}; Fig. 1a).

Table 1
Nicotine concentrations in soils and different tobacco parts as affected by heterogeneity in three site.

	Interfaces evaluated for tobacco nicotine			
	Leaves	Roots	Soils	Total (leaves, roots, and soils)
	(mg kg^{-1})			
Site				
Sikonge	30.98 ± 2.38a	8.98 ± 0.21a	9.55 ± 2.12a	49.51 ± 4.30a
Tabora	23.53 ± 2.26 b	6.92 ± 0.07 b	6.04 ± 1.52 b	36.48 ± 3.82 b
Urambo	20.91 ± 2.33c	5.72 ± 0.21c	4.42 ± 1.20c	31.04 ± 3.54c
Fertilizer				
Fertilized	29.99 ± 1.45a	7.29 ± 0.49a	10.27 ± 1.06a	47.54 ± 2.98a
Unfertilized	20.29 ± 1.82 b	7.12 ± 0.49a	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

Key: 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.

3.2. Effects of site, fertilizer, and soil depth on nicotine release and soil properties

Soil OC, pH, T, SM, and nicotine content were assessed under different levels of fertilizer application and at different soil depths (Table 2). Nicotine content at 0–10 cm, 10–30 cm, and 30–50 cm was 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. 1b) and SM (Fig. 1c). 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%).

Sikonge had the highest SM (13.37%) and Urambo the lowest (9.66%). Soil temperature decreased in fertilized soils (Fig. 1d); 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. 2a). The nicotine content also increased with an increase in soil depth (Fig. 2b). 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. 2c). 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

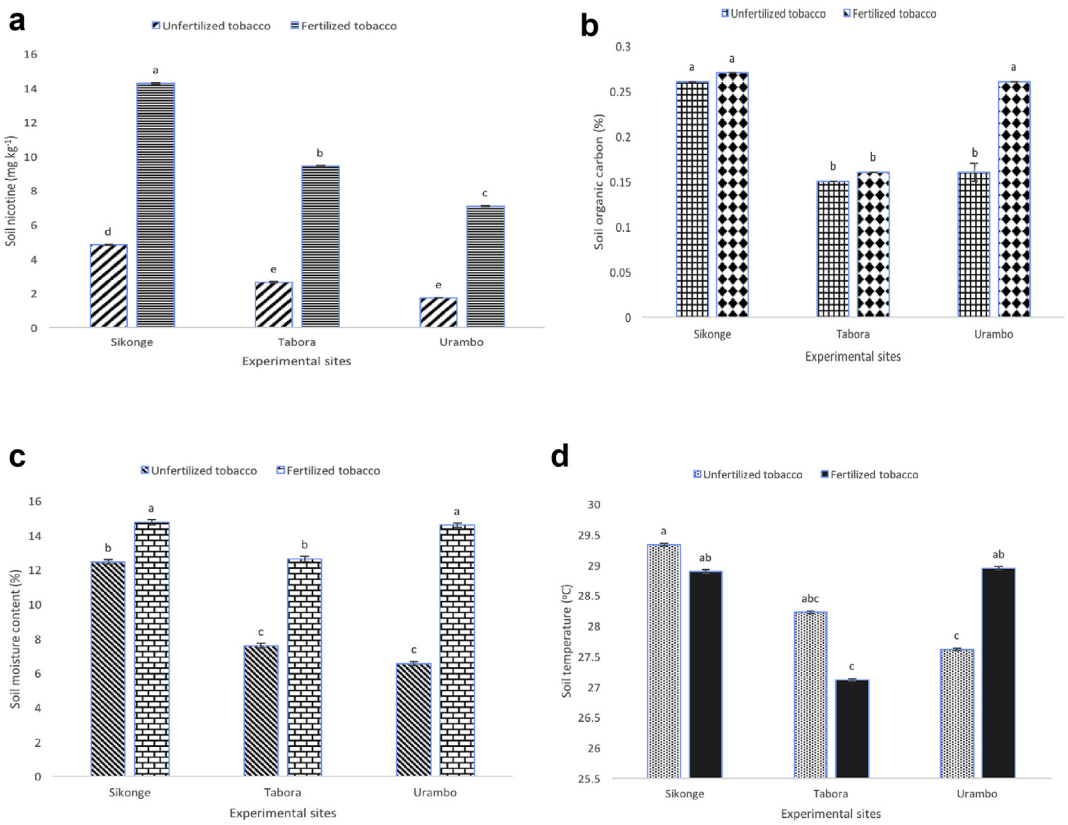

Fig. 1. a. Interaction of sites and fertilizer on soil nicotine b. Interaction of sites and fertilizer on organic carbon. c. Interaction of sites and fertilizer on soil moisture d. Interaction of sites and fertilizer on soil temperature.

Table 2
Effects of the selected soil properties on nicotine retained in Sikonge, Tabora and Urambo agro-ecologies.

	Measured variables in soils				
	pH	OC (%)	Nicotine (mg kg^{-1})	Temperature (°C)	Moisture (%)
Site					
Sikonge	5.33 ± 0.05c	0.27 ± 0.01a	9.55 ± 1.16a	29.11 ± 1.04a	13.37 ± 1.75a
Tabora	5.50 ± 0.02 b	0.15 ± 0.00c	6.04 ± 0.84 b	27.66 ± 1.13 b	11.51 ± 0.84 b
Urambo	5.69 ± 0.02a	0.21 ± 0.01 b	4.42 ± 0.82c	28.27 ± 0.63 ab	9.66 ± 1.27c
Fertilizer					
Fertilized	5.49 ± 0.04a	0.23 ± 0.01a	10.27 ± 0.69a	28.31 ± 0.78a	12.62 ± 1.19a
Unfertilized	5.42 ± 0.03a	0.19 ± 0.01 b	3.07 ± 0.27 b	28.39 ± 0.78a	10.39 ± 1.16 b
Depth (cm)					
0–10	5.59 ± 0.05a	0.22 ± 0.01a	5.50 ± 1.00c	33.14 ± 0.47a	4.95 ± 0.65c
10–30	5.48 ± 0.05 ab	0.23 ± 0.02a	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 ± 1.13a	24.53 ± 0.43c	16.98 ± 1.01a
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

Key: Values presented are means ± $SE_{\bar{x}}$ (Standard error of means); *** = significant at $P \leq 0.001$; ** = significant at $P \leq 0.01$; 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.

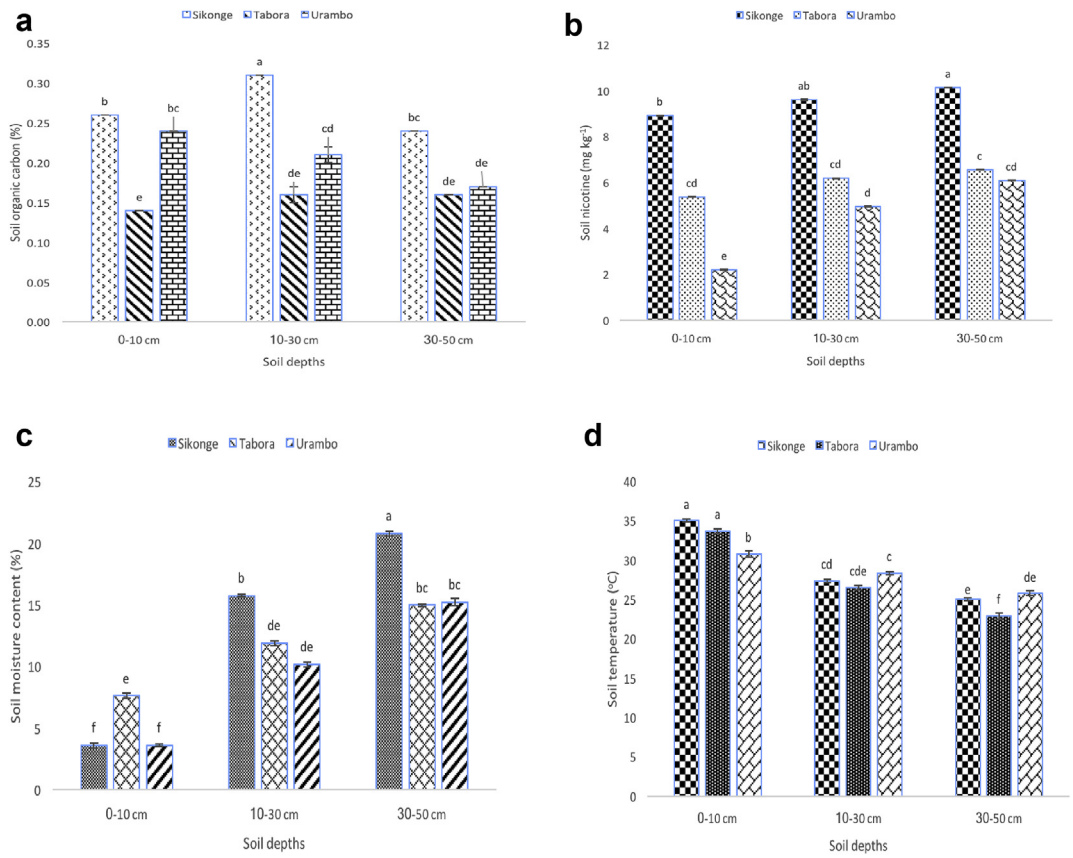

Fig. 2. a. Interaction of sites and soil depths on soil organic carbon b. Interaction of sites and soil depths on soil nicotine c. Interaction of sites and soil depths on soil moisture d. Interaction of sites and soil depths on soil temperature.

depth increased (Fig. 2d). 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 30–50cm.

The interactions among sites, fertilizer treatments, and soil depths significantly affected the nicotine in the soil (Fig. 3a). 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.

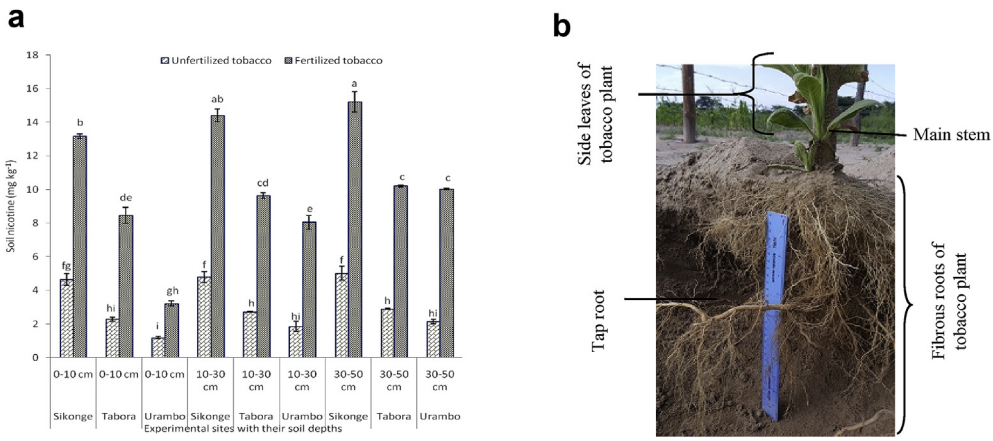

Fig. 3. a. Relationship between interaction of sites, fertilizer b. Root architecture and penetrating depths of a treatments and soil depths on nicotine. Tobacco plant. Take note of the extensive root system and distribution of roots.

3.3. Association between soil nicotine content and soil moisture, organic carbon, temperature, and pH

Multiple linear regression results (Table 3) were generated based on a regression model with nicotine as a response variable (Y) and the fitted terms constant (C), SM, OC, soil pH, and T such that:

$$Y = 76.1 + 0.024SM + 6.19OC + 0.042\,T - 13.13pH$$

The coefficient of determination (R^2) was 84.0 and the standard error of observation was estimated to be ± 1.01. This model indicated that for every unit increase in SM, OC, and T the amount of nicotine produced was expected to increase by 0.024, 6.19, and 0.042%, respectively. However, with the same unit increase in soil reaction the amount of nicotine would decrease by 13.13%.

Further correlation analysis (Table 4), however, clearly indicated that SM (r = 0.57) and OC (r = 0.45) had positive but not significant relationships with nicotine retained in the soil. In addition, there was negative correlation between soil nicotine and pH (r = −0.95; P = 0.0001) and T (r = −0.17).

Table 4
Correlations between nicotine and moisture, organic carbon, pH and temperature in soils.

		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 of measured variables.

4. Discussion

4.1. Effect of agro-ecology and fertilizer treatment on nicotine concentration

The results of present study revealed that fertilizer addition to tobacco soils significantly increased the nicotine content transferred to leaves as well as that released into the soil via roots. Leaf and root nicotine concentrations differed among sites in the following order: Sikonge > Tabora > Urambo. However, there was an inconsistent trend with respect to the tobacco nicotine released in soils, whereby 9.55 mg kg^{-1} was found in the loamy sand of Sikonge, which was 19.29% of the total nicotine produced. The amount of nicotine found in sand of Tabora, was 6.04 mg kg^{-1} equivalent to 16.56% of the total nicotine produced. This was higher than that recorded in the sandy loam of Urambo, which was 4.42 mg kg^{-1} equivalent to 14.24% of the total nicotine produced.

The present study revealed that, regardless of agro-ecological differences, fertilized tobacco plants released twice the amount of total nicotine into the soils than unfertilized plants (21.60% and 10.07%, respectively). This indicates that tobacco plants had the minimum required nicotine level in the leaves, and thus the unfertilized plants released less nicotine to the soil at 20.29 mg kg^{-1}, which was equivalent to 2% of the nicotine in leaves. Nicotine levels in tobacco plants can reach around 4% in leaf depending on the variety of tobacco (Moldoveanu et al., 2016). Nagarajan and Prasadrao (2004) suggested that the nicotine in tobacco leaves should be limited to 1.75–2.00% as a safe threshold for the nicotine content. Nicotine safe threshold level is influenced by agronomic trait, climate conditions and pests and diseases (Xie et al., 2017).

Our findings suggest that if fertilizers are not used in tobacco cultivating systems, then soil nicotine concentration is likely to decrease and have less of an effect on subsequently planted crops. However, the quality of the tobacco in terms of the amount of nicotine in the leaves also decreases. Therefore, further investigation is required of mechanisms that transfer more nicotine to the leaves while maintaining low levels in the soil.

Table 3
A multiple linear regression analysis of nicotine as a response variate and the measured variables in soil such as moisture, organic carbon, pH and temperature.

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 – 13.13** pH			

4.2. Effects of interactions among site, fertilizer, and soil depth on nicotine release into different soil types

The interactions among sites and fertilizer treatments showed that OC and SM increased more in fertilized soils than in unfertilized soils, indicating that these soil properties increased the amount of nicotine released into the soil. Although OC, SM, and T positively influenced tobacco nicotine released into the soils, the influence of T was not as pronounced as that of OC and SM. This interaction significantly affected N mineralization in tobacco cultivated soils (Hu et al., 2018).

The results of the present study indicated that, at shallow depths (0–10 cm), soils contained more fresh organic materials, which were not completely decomposed into soil organic matter. The highest OC at all sites was recorded at 10–30 cm, and the highest overall OC was observed in the loamy sand of Sikonge. The OC correlated positively with nicotine released into fertilized soils.

Nicotine increased as the soil depth increased, and the highest nicotine of 10.12 mg kg^{-1} was recorded at 30–50 cm in the fertilized loamy sand of Sikonge, and the lowest was 6.09 mg kg^{-1} recorded at the same depth and site but unfertilized. This highlights the importance of mineral N in nicotine production and its distribution into various sinks (Xi et al., 2008). Soil moisture increased at all sites with increasing soil depth, while T decreased. However, changes depended on soil type because they differed in texture.

Nicotine dynamics are also linked with SM as roots penetrate deeper to access underground water (Hsiao and Xu, 2000). The increase in nicotine concentrations with increased soil depths may be attributed to an increase in N released by the soil for tobacco plant utilization (Chen et al., 2005), and the effect is stronger as the tobacco plant roots grows in deeper soils searching for moisture and needs more N for leaf formation, strengthening and plant photosynthesis (Fig. 3b). Our study did not measure root biomass in fertilized and unfertilized plots, however, soil samples were taken from both plots in order to measure the residual nicotine levels in soils. Roots penetrated deep to the soils for either fertilized or unfertilized tobacco and released nicotine was significantly ($p = 0.001$) higher in fertilized (10.27 mg kg^{-1}) than unfertilized (3.07 mg kg^{-1}) tobacco. Zou (2015) also reported that tobacco root systems are extensively branched and exhibit developmental plasticity to penetrate different soil layers. Our findings revealed that, with increasing soil depth, nicotine released into soils increased as the rooting depth increased, and that the increase was determined by the levels of SM. Thus, the effect of SM on nicotine dynamics far exceeds the likely negative effect of T.

Soil pH did not significantly influence levels of nicotine retained in the soil, but it is also likely that acidity of the soil is increased by the higher nicotine retained in soils (Rakić et al., 2010). Atmospheric temperature was similar in Sikonge (29 °C), Tabora (27 °C), and Urambo (25 °C) but had a strong influence on the biosynthesis of nicotine in roots, and thus, that released into soils, which was similar to the results reported by Cheng et al. (2018). However, owing to the higher rains in Sikonge (1050 mm), significantly higher nicotine content was observed in deeper (30–50 cm) tobacco root zones relative to shallow depths (0–30 cm), followed by Tabora (950 mm) and Urambo (890 mm).

In all sites, higher soil nicotine was observed in the deeper soil layers, and soil nicotine levels may be even higher than those reported herein when tobacco roots can penetrate beyond 50 cm. Therefore, based on our results, soil nicotine may have a significant residual effect on subsequent crops planted immediately after tobacco and if they are deeply rooted. This is because tobacco is deep-rooted crop, which produces a dense fibrous root system in the lower horizon (Zou, 2015) with reasonable amounts of nicotine being released into the rhizosphere. Thus, shallow-rooted crops would be more suitable in the rotation sequence with tobacco because the lower levels of nicotine in the shallow root zones cannot significantly disrupt the availability of macronutrients such as P and K or the proliferation of soil bacteria (Adediran et al., 2004; Moula et al., 2018).

Nicotine in soils is reported to persist longer than a year and this differs with soil types such as sandy loam, silty loam and clayey soil (Farooq et al., 2014; Yazdani, 2014; Niu et al., 2017). For instance, in such duration, the persistence of nicotine in the soil may negatively affect the germination of grain legumes and cereals in the rotation sequence through the allelopathic effects of this compound (Yazdani and Bagheri, 2011; Baek et al., 2017). In silty loam soils, Yazdani (2014) showed that, the persistence of nicotine decrease seedlings emergence rate, seedling weight, vigour and chlorophyll content in the subsequent cereal crops. Thus, nicotine residue in soils such as those reported in this study could have negative effects on the subsequent crops. Despite nicotine persistence in soils, its contents in soils could be lowered as a result of soil bacteria activities in utilizing nicotine as sole carbon and degrade nicotine (Chen et al., 2008; Xia et al., 2019). Therefore, any cultural activities supporting the proliferation of nicotine biodegrading microorganisms should be promoted in areas where tobacco is grown and rotated with other crops.

The dynamics of nicotine and their persistence in different soil types requires further investigation. This is due to the fact that the exact duration of nicotine persistence on various soil types is still not clearly known and hence opens up a window for further study (Lisuma et al., 2019).

5. Conclusion

The results of the present study revealed that nicotine produced by tobacco roots is distributed between the soil and plant organs. Leaves had higher nicotine levels than roots, and, of the total produced nicotine, a relatively small proportion is released into the soil. Of the studied agro-ecologies, Sikonge provided increasing nicotine content produced by tobacco roots, which was followed by Tabora and Urambo. The amount of nicotine released to the rhizosphere was largely dependent on SM and rooting depth. The nicotine released into the soil also increased as the roots penetrated into deeper soils. Nicotine released into the soil was twice as much in fertilized plants compared with unfertilized. However, the nicotine in tobacco roots for both unfertilized and fertilized tobacco plants did not significantly differ.

Nicotine levels in the soil may have residual effects on immediate subsequently cultivated crops, and the higher content of nicotine in deeper soils indicated that shallow rooted crops would be more suitable. Most shallow rooted crops have rooting depths of 0–20 cm where the level of nicotine is low and cannot limit the availability of macronutrients such as P and K or the proliferation of soil bacteria. Degradation of nicotine through soil bacteria could also result in decreasing significantly nicotine levels in soils and cause less effects. Therefore, evaluating the dynamics of nicotine and its persistence across different soil types, soil-plant interface considering root depths exceeding 50 cm and different nutrient sources and levels remains a pertinent area for further investigation.

Declaration of Competing Interest

The authors declare to have no conflict of interests regarding this paper publication.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rhisph.2019.100175.

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