PHOSPHORUS FRACTIONS AND ARBUSCULAR MYCORRHIZAL FUNGI AS INFLUENCED BY FOREST CONVERSION IN CENTRAL HIGHLANDS OF KENYA

[FRACCIONES DE FÓSFORO Y HONGOS MICORRÍZICOS ARBUCULARES INFLUENCIADOS POR LA CONVERSIÓN FORESTAL EN LAS TIERRAS ALTAS CENTRALES DE KENIA]

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SUMMARY

Background: Forest conversion to other land use types lead to changes in soil physico-chemical properties and a reduction in soil fertility. Although forest conversion is extensively recognized to alter soil properties, the influence on distribution of soil phosphorus (P) fractions and abundance and diversity of soil biota is not well explored.

Objective: To assess the effects of converting undisturbed natural forest to plantation forest (cypress), grazed pastures and potato fields on soil phosphorus (P) fractions and abundance and diversity of arbuscular mycorrhizal fungi (AMF) in Nyandarua County, Kenya.

Methodology: Transects laid out in triplicate were established in each land use type. In potato fields and grazed pastures, transects measured 50 m with three sampling points established 15 m apart along each transect, each point measuring 1 m² divided into 25 grids with each grid being 400 cm². Transect length was increased to 150 m in forest sites due to relatively larger size of the area studied and sampling points were placed at 50 m apart, each point measuring 25 m² and divided into 1² m grids. Soil and plant samples were randomly collected from sampling points and were used to determine chemical properties, soil P fractions and AMF spore examination. Data were analyzed by analysis of variance (ANOVA).

Results: Soil chemical properties were significantly \((p<0.001)\) higher in natural forest compared to potato fields, grazed pasture and cypress forest. On the contrary, readily labile and moderately labile P was higher in fields cultivated with potato (125.3 mg and 258.2 mg P kg\(^{-1}\), respectively) than in natural forest (51.1 mg and 95.3 mg P kg\(^{-1}\)) and cypress forest (36.4 mg and 82.7 mg P kg\(^{-1}\), respectively). However, non-labile P was higher in natural forest (599.1 mg P kg\(^{-1}\)) and lower in cypress forest (251.3 mg P kg\(^{-1}\)). Of the ten AMF genera identified, only *Glomus* and *Acaulospora* were significantly \((p<0.01)\) affected by land use change, where they were more abundant in fields cultivated with potato than the other three land use types. Land use type did not significantly influence diversity and richness in AMF. However, AMF composition varied across the land use types.

Implication: Land use change may negatively affect soil chemical properties, enhance redistribution of soil P, and change AMF community composition, and this could have long-term implications on soil fertility. Conclusion: Land use change from natural ecosystems to croplands, grazed pastures and tree plantations alter soil chemical properties, AMF composition and spore abundance, and redistribute soil P fractions by increasing labile P and reducing non-labile P fractions showing that the type of land use chosen significantly influence soil physical and chemical properties and soil biodiversity.

Key words: Forest conversion; land use type; spore abundance; soil properties; genera.

RESUMEN

Antecedentes: La conversión de bosques a otros tipos de uso de la tierra provoca cambios en las propiedades fisico-químicas del suelo y una reducción en la fertilidad del suelo. Aunque se reconoce ampliamente que la conversión de bosques altera las propiedades del suelo, la influencia en la distribución de las fracciones de fósforo (P) del suelo y la abundancia y diversidad de la biota del suelo no está bien explorada. Objetivo: Evaluar los efectos de convertir bosques...
INTRODUCTION

Maintenance of soil fertility is crucial for ecosystem functioning and crop productivity yet human activities such as forest clearance alter soil properties thus compromise soil fertility (McGrath et al., 2001; Veldkamp et al., 2020). Small-scale subsistence farming has been named among the primary drivers in forest conversion especially in Sub-Saharan Africa owing to the growing human population and reduced crop productivity due to declining soil fertility (Benhin, 2006; Pinho et al., 2012). Cultivated area has risen by 500% globally in the last five decades, with significant proportion of this being in Africa (Banerjee et al., 2019). However, the tendency to exploit more forest lands to increase the overall agricultural production could have negative effects on soil fertility and biodiversity due to alteration of soil properties and reduction in soil organic matter content (de Graaff et al., 2019; Tolimir et al., 2020). Reduction in soil organic matter content, for instance, causes a collapse in soil structure and a weakening of soils’ capacity to retain nutrients (Qiong et al., 2008; Gatiboni et al., 2017).

Soil P is the second most limiting nutrient after N, and it is highly unstable and easily fixed especially in acidic tropical soils. Phosphorus availability and lability is mediated by chemical processes, soil microbial and plant’s roots activities (Betencourt et al., 2012; Srinivasan et al., 2012). The alteration of soil properties and vegetation by forest conversion influence P transformation and distribution within soil compartments (Bayuelo-Jiménez et al., 2020; Zhu et al., 2021). Indeed, researchers have suggested that P may change from one chemical form to another within a shorter period (<50 years) following land use change than thousands of years under natural conditions (Garcia-Montiel et al., 2000; De Schrijver et al., 2012; Chimdi et al., 2014). This accelerated process could be attributed to changes in soil chemical properties such as pH and organic matter content which determine P dynamics in soil (Richardson et al., 2009; Bayuelo-Jiménez et al., 2020). For instance, low soil pH would lead to P binding with Al and Fe forming P compounds that are less labile (Richardson et al., 2009). Soil management practices such as fertilizer application may lead to a short-term increase in labile P but more of this P end up being fixed or lost through soil erosion and harvested crop (Henriquez, 2002; Nunes et al., 2020).

Soil biota play a critical role in nutrient cycling and maintenance of ecosystem functioning. Arbuscular mycorrhizal fungi (AMF) which are associated with over 80% of terrestrial plants provide several ecosystem services including enhanced nutrient uptake, protection against diseases and pathogens, increased tolerance to other environmental stresses, mediate nutrient cycling, and improves soil physical properties (Syibi et al., 2013; Chandrasekaran and Mahalingam, 2014; Campos et al., 2018; Chu et al.,
2020). However, forest conversion influence AMF, with a decline or increase in composition and abundance (van der Heyde et al., 2017; Belay et al., 2020). Soil disturbance brought about by tillage may stimulate increased spore production in AMF species like Glomus but cause a reduction in Gigasporaceae species, with fertilizer use also exhibiting similar effects in AMF species (Jefwa et al., 2009; Dobo et al., 2018). Although soil biota may exhibit some level of resilience to land use change as reported by Alele et al. (2014), it is not certain to what extent this resilience will persist and if such resilience is exhibited by AMF. Thus, this study aimed to evaluate the effect of converting natural forest to other land use types on distribution of P fractions and AMF abundance and diversity under four land use types: natural forest, forest plantation, grazed pasture and potato field. We hypothesized that converting natural forest to cultivated croplands, grazed pastures or tree plantations would lead to (1) modify distribution of soil P fractions and (2) a shift in composition, abundance and diversity of AMF.

MATERIALS AND METHODS

Description of study site

The study was carried out in South Kinangop in Nyandarua County in Kenya, which lies at 0.95° S and 36.76° E at an altitude of 2552 m above sea level. The study area has a cool and temperate climate, and has an annual mean rainfall of 1200 mm and annual mean temperature range of 10° C to 15° C. Rainfall is distributed in two seasons; the first season locally called ‘long rains’ occur from March to May, and the second season called ‘short rains’ from October to December (Jaetzold et al., 2006; Haile et al., 2023). Soil sampling was done in February 2021, and monthly mean rainfall and monthly mean temperature was 190 mm and 16° C, respectively, between December 2020 to March 2021 (Haile et al., 2023). Soils are predominantly Planosols (FAO/UNESCO Soil classification system) which are characterized by poor drainage and a low to moderate soil fertility (Jaetzold et al., 2006). The study area was initially a bushland before it was cleared to settle farmers in 1963 (Rachilo, 1978). The increased human population growth in the last four decades (from 233,302 persons in 1979 to 638,289 persons in 2019) has caused fragmentation of farms from an average of 20 ha to less than 0.5 ha (Jaetzold and Schmidt, 1983; KNBS, 2019; Kamau et al., 2019). Majority of the farmers practice mixed farming, with cultivation of crops such as potatoes and vegetables including carrots, kales and cabbages and keeping of local dairy cows and sheep.

Four land use types common within the study area were purposely selected for this study: 1) A natural forest within Aberdare Forest reserve on the Southern Kinangop block was chosen as a reference site and a benchmark area of 9.9 ha was selected. Aberdare forest reserve is situated between longitude 36° 30’ E to 36° 55’ E and latitude 0° 05’ S to 0° 45’ S, and is surrounded by four counties; Kiambu, Nyeri, Murang’a and Nyandarua. The forest reserve is also surrounded by local communities who rely heavily on agriculture (KFS, 2010). The natural forest had Dombeya goetzenii K. Schum as the dominant tree species; 2) Plantation forest constituted a pure Cupressus lusitanica Mill. (cypress) and a benchmark area of 4.9 ha was established for the study; 3) Grazed pastures were dominated by Kikuyu grass (Pennisetum clandestinum Hochst. ex Chiov) and the Kenyan Holstein-Friesian (Bos taurus taurus) dairy cows and Corriedale (Ovis aries) sheep were animals grazed on these pastures; and 4) Cultivated fields were mainly planted with potato for three consecutive growing seasons and Diammonium Phosphate (DAP) and Calcium Ammonium Nitrate (CAN) were used to fertilize the crop.

Soil and litter sample collection

Soils were collected in each land use type following transects, laid out in triplicate in a zigzag manner as illustrated in Figure 1. In potato fields and grazed pastures, transects measured 50 m and three sampling points, each point measuring 1 m², were established 15 m from one sampling point to the other. Each sampling point was divided into 25 grids, where each grid measured 400 cm². However, the length of transects in natural forest and cypress forest was increased since the area studied was relatively larger in size compared to the farms. Therefore, each transect measured 150 m and had sampling points marked at 50 m apart. Each sampling point measured 25 m² and was divided into equivalent number of grids where each grid was 1 m². Soil samples were randomly taken from five grids in each sampling point to a depth of 30 cm using a soil auger. The soils were mixed thoroughly, and a composite soil sample of 2 kg was taken for laboratory analysis. From each field, nine (9) soil samples were collected (3 transects x 3 sampling points per transect). Plant litter on the ground in natural and cypress forests were randomly hand-picked from each sampling point and composited. Soil samples were divided into two portions for chemical analysis and for AMF spore extraction. Soil samples for chemical analysis were ground and passed through 2 mm sieve.
Determination of soil and plant litter chemical properties

Soils were analyzed for pH, total organic C, N, Ca, K, Mg and CEC. Soil pH was measured in deionized water-soil suspension (1:2.5 soil: water) using a pH meter (Anderson and Ingram, 1993). Total organic C was determined by Walkley-Black method (Nelson and Sommers, 1996). Total N was determined by Kjedhal method (Hinds and Lowe, 1980). Exchangeable Ca, K and Mg were extracted using ammonium acetate method and determined by atomic absorption spectrophotometer as described by Anderson and Ingram (1993). Plant litter was analyzed for total N, P, Ca, Mg, K, organic matter and lignin content. Total N, P, Ca, Mg and K were extracted using mixed reagent (selenium powder, lithium sulphate, hydrogen peroxide and concentrated sulphuric acid), after which determination of total N was done according to Kjedhal method (Hinds and Lowe, 1980), total P according to Murphy and Riley (1962) method and Ca, Mg and K using atomic absorbance spectrophotometer. Organic matter (OM) content was determined by complete combustion method (Carter and Gregorich, 2007).

Determination of soil P fractions using Hedley sequential fractionation method

Hedley sequential fractionation method (Hedley et al., 1982) uses a sequence of chemical solutions that increase in dissolution strength which first removes labile inorganic and organic P, and then non-labile P. Soil P fractions were quantified and presented as resin-P, NaHCO₃-P, NaHCO₃-Po, NaOH-P, NaOH-Po, sonicate NaOH-P, sonicate NaOH-Po, HCl-Pi and residual P. The fractions were further grouped into readily labile P (resin-Pi + NaHCO₃ Pi and Po), moderately labile P (NaOH Pi and Po) and non-labile P (sonicate NaOH Pi and Po + HCl-Pi + residual P). One gram of air-dried soil was repeatedly extracted starting with dilute alkali and acid and eventually with concentrated acid in the following sequential order: Resin-P was extracted by first saturating resin strips (1.5 cm × 5 cm) with 1 M NaHCO₃ for 1 hour and rinsed with distilled water. The saturated resin strips were put in 50 ml centrifuge tubes containing soil and to which 30 ml of distilled water was added. The contents were shaken for 16 hours, and resin strips were removed using tweezers and washed off using distilled water into the centrifuge tubes. To remove P adsorbed on the exchange sites of resin strips, resin strips were transferred into centrifuge tubes containing 20 ml of 0.5 M L⁻¹ HCl and shaken for 1 hour after which resin strips were removed. The extract was used to determine the content of P (resin-Pi). The centrifuge tubes containing soil were centrifuged at 5000 rpm for 10 minutes and water decanted and discarded. NaHCO₃-P was determined by dispensing 30 ml of 0.5 M L⁻¹ NaHCO₃ into the centrifuge tubes containing soil and was shaken for 16 hours. The soil suspension was centrifuged at 5000 rpm for 10 minutes and...
NaHCO₃ extract was carefully decanted into new sets of centrifuge tubes to be used for P determination. NaOH-P was determined by dispensing 30 ml of 0.1 M L⁻¹ NaOH into the centrifuge tubes containing soil and shaken for 16 hours. The soil suspension was centrifuged at 5000 rpm for 10 minutes and the extract kept for P determination. Sonicate NaOH-P was determined by extracting soil in the centrifuge tubes with 20 ml of 0.1 M L⁻¹ NaOH. The soil suspension was first sonicated for 15 seconds and then shaken for 16 hours, after which it was centrifuged at 5000 rpm for 10 minutes. Sonicate NaOH extract was decanted into new sets of centrifuge tubes and kept for P determination. HCl-P was determined by extracting the soil in the centrifuge tubes with 30 ml of 1 M L⁻¹ HCl. The soil suspension was shaken for 16 hours and thereafter centrifuged at 5000 rpm for 10 minutes, and HCl extract was collected in new sets of centrifuge tubes and kept for P determination. The soil in centrifuge tubes was quantitatively transferred into 75 ml digestion tubes with distilled water and put in the oven at 70° C to evaporate excess water. The soil was added with 5 ml of mixed digestion reagent and heated at 350° C for 3 hours. The soil digest was filled up to 75 ml with distilled water and transferred to storage bottles and used for P determination. Inorganic P (Pᵢ) in extracts of resin, NaHCO₃, NaOH, sonicate NaOH, HCl and residual P was determined directly by taking an aliquot of 6 ml and P concentration measured according to Murphy and Riley method (Murphy and Riley, 1962). To determine organic P (Pₒ) in NaHCO₃, NaOH and sonicate NaOH extracts, a separate aliquot (6 ml) was taken from their extracts and digested with 6 ml of acidified ammonium persulfate at 15 psi, 120° C for 1 hour to convert all the dissolved organic P into inorganic P, and the content of P determined in the digest was quantified as total P (tP). Organic P was then computed as the difference between tP and P. The pH for the final extracts was first adjusted using either an acid or alkali and P was determined according to Murphy and Riley method (Murphy and Riley, 1962) and absorbance taken at 880 nm using UV Spectrophotometer. Phosphorus concentration was calculated based on soil dry weight (mg P kg⁻¹).

Spore extraction and identification

Spores were extracted using wet sieving and sucrose (50 %) density centrifugation method. A 100 g of air-dried soil was washed through 710 µm sieve into 45 µm sieve, repeatedly. The soil remaining on 45 µm sieve was gently washed into 50 ml centrifuge tubes and centrifuged at 1500 revolutions per minute (rpm) for 5 minutes. The supernatant was carefully poured out and 50 % of sucrose solution was added onto the tubes containing the soil and filled up to the 50 mark and centrifuged again at 1500 rpm for 1 minute. The supernatant was collected on 50 ml plastic beaker. The supernatant was transferred to a petri dish with grid lines in order to count, collect and separate spores under a dissecting microscope. Spores were separated based on morphological characteristics such as spore color using Edinburgh Botanic Gardens color chart, size, shape, presence or absence of hyphae attachment and surface appearance. The spores were mounted on glass slides using polyvinyl lacto glycerol (PVLG), in which the spores were maintained whole, and Meltzer’s reagent, in which the spores were slightly crushed for finer details. To identify spore morphotypes, a compound microscope was used to examine spores for more details such as spore wall, number of spore walls, wall ornamentation, type of hyphae, germination characteristics and reaction to Meltzer reagent. The morphological spore characteristics were recorded and matched with species characteristics described by International Collection of (Vesicular) Arbuscular Mycorrhizal Fungi (INVAM) database available online (https://invam.ku.edu/) and Schenck and Perez (1990). The spore morphotypes were identified to family and genus levels. The AMF spore specimens were preserved and deposited at the Mycology laboratory at the Department of Botany of the National Museums of Kenya.

Statistical analysis

Data on soil chemical properties, P fractions and plant litter properties were first converted into standard measurement units. Spore abundance was calculated from direct count of spores per 100 g of air-dried soil. Vegan package in R statistical software was used to determined species richness and Shannon-Weiner diversity indices. The effect of land use type on soil chemical properties and P fractions were determined using generalized linear models (GLMs) using lme4 package. Data on spore count, species richness and diversity were analyzed by generalized linear mixed models (GLMMs), and since the data contained significant number of zero values, negative binomial regression was chosen as an extension of Poisson distribution as described by Kamau et al. (2020), and thus the models were fitted using glmer.nb function in R statistical software. Sampling site was fitted in the models as a random factor while land use type as a fixed factor. Estimation of model parameters was done using maximum likelihood (ML), and Akaike Information Criterion (AIC) was used to select models, where models with least values of AIC were chosen to be the best models. Where analysis of variance (ANOVA) showed significant effect of land use type, means were separated by Tukey’s Honest Significant Difference (HSD) test at α=0.05. All data analysis was done in R statistical software version 4.0.4 (R Core Team, 2021).
RESULTS

Soil chemical properties

Land use type had significant ($p<0.001$) effect on soil chemical properties measured (Table 1). Generally, natural forest had higher contents of total organic C, N, exchangeable Ca and Mg than other land use types. However, soil K was higher in grazed pastures. Soil pH was significantly higher in natural forest (4.9) compared to that in potato fields (3.8), but it was not significantly different between cypress forest and grazed pastures. Natural forest also had higher CEC (25.3 cmol kg$^{-1}$) than cypress forest (14.6 cmol kg$^{-1}$), potato fields (9.9 cmol kg$^{-1}$) and grazed pastures (11.7 cmol kg$^{-1}$). Other soil chemical properties showed similar differences across grazed pastures, potato fields and cypress forest.

Chemical characteristics of plant litter

Chemical properties of litter were greatly affected by land-use type in which they were collected (Table 2). Litter from natural forest had significantly ($p<0.01$) higher total N, P, Ca and Mg than in cypress plantation. However, total K and OM were similar between the two forests. On the other hand, lignin content was high in litter collected from natural forest (447.4 g kg$^{-1}$) compared those from cypress plantation (356.5 g kg$^{-1}$).

Distribution of soil P fractions

Soil P fractions were significantly affected by land-use type with varying magnitudes (Table 3). For example, resin-P was significantly ($p<0.05$) higher in soils collected from potato growing fields (34.9 mg P kg$^{-1}$) compared to cypress forest (3.9 mg P kg$^{-1}$). Similar differences were observed for NaHCO$_3$-P, NaHCO$_3$-P$_{oc}$ and NaOH-P$_{oc}$. On the other hand, residual P and sonicate NaOH-P$_{oc}$ fractions were higher ($p<0.001$) in

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**Table 1. Soil chemical properties as affected by land use types in the study site.**

<table>
<thead>
<tr>
<th>Soil properties</th>
<th>Natural forest</th>
<th>Cypress forest</th>
<th>Grazed pastures</th>
<th>Potato fields</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH$_{\text{(water)}}$</td>
<td>4.9 (0.2)$^a$</td>
<td>4.3 (0.1)$^b$</td>
<td>4.2 (0.1)$^b$</td>
<td>3.8 (0.1)$^c$</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total C (g kg$^{-1}$)</td>
<td>69.2 (5.3)$^a$</td>
<td>47.2 (3.2)$^b$</td>
<td>38.0 (2.8)$^b$</td>
<td>35.3 (2.3)$^b$</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total N (g kg$^{-1}$)</td>
<td>7.8 (0.6)$^a$</td>
<td>3.8 (0.2)$^b$</td>
<td>4.9 (0.3)$^b$</td>
<td>4.0 (0.2)$^b$</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CEC (cmol kg$^{-1}$)</td>
<td>25.3 (1.3)$^a$</td>
<td>14.6 (1.4)$^b$</td>
<td>11.7 (0.9)$^bc$</td>
<td>9.9 (0.4)$^c$</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>K (g kg$^{-1}$)</td>
<td>0.4 (0.1)$^{ab}$</td>
<td>0.3 (0)$^{ab}$</td>
<td>0.5 (0)$^a$</td>
<td>0.2 (0)$^b$</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ca (g kg$^{-1}$)</td>
<td>4.0 (0.3)$^a$</td>
<td>2.3 (0.2)$^b$</td>
<td>1.6 (0.1)$^c$</td>
<td>1.4 (0.1)$^c$</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mg (g kg$^{-1}$)</td>
<td>0.5 (0)$^a$</td>
<td>0.2 (0)$^b$</td>
<td>0.2 (0)$^b$</td>
<td>0.2 (0)$^b$</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Abbreviations: C = carbon, N = nitrogen, CEC = cation exchange capacity, K = potassium, Ca = calcium, Mg = magnesium. Within rows, means followed by different letters in superscript are significantly different at $p<0.05$, and mean standard deviations are in parenthesis. Means were separated by Tukey’s Honest Significant Difference (HSD) test.

**Table 2. Chemical characteristics of litter collected from the floor of the two forests.**

<table>
<thead>
<tr>
<th>Litter characteristics</th>
<th>Natural forest</th>
<th>Cypress forest</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total N (g kg$^{-1}$)</td>
<td>37.7 (5.7)$^a$</td>
<td>12.7 (1.2)$^b$</td>
<td>0.0027</td>
</tr>
<tr>
<td>Total P (g kg$^{-1}$)</td>
<td>4.1 (0.4)$^a$</td>
<td>1.8 (0.1)$^b$</td>
<td>0.0014</td>
</tr>
<tr>
<td>OM (g kg$^{-1}$)</td>
<td>810.6 (139.2)$^a$</td>
<td>928.3 (21.8)$^b$</td>
<td>0.1120</td>
</tr>
<tr>
<td>K (g kg$^{-1}$)</td>
<td>12.9 (3.5)$^a$</td>
<td>12.0 (0.9)$^a$</td>
<td>0.5868</td>
</tr>
<tr>
<td>Ca (g kg$^{-1}$)</td>
<td>45.9 (11.6)$^a$</td>
<td>11.4 (0.4)$^b$</td>
<td>0.0056</td>
</tr>
<tr>
<td>Mg (g kg$^{-1}$)</td>
<td>16.8 (5.3)$^a$</td>
<td>2.9 (0.2)$^b$</td>
<td>0.0076</td>
</tr>
<tr>
<td>Lignin (g kg$^{-1}$)</td>
<td>447.4 (17.9)$^a$</td>
<td>356.5 (6.1)$^b$</td>
<td>0.0020</td>
</tr>
</tbody>
</table>

Abbreviations: N = nitrogen, P = Phosphorus, C = carbon, K = potassium, Ca = calcium, Mg = magnesium, OM = organic matter. Within rows, means followed by different letters in superscript are significantly different at $p<0.05$, and mean standard deviations are in parenthesis. Means were separated by Tukey’s Honest Significant Difference (HSD) test.
Table 3. Distribution of soil P fractions as affected by land use types in the study site.

<table>
<thead>
<tr>
<th>P-fractions (mg kg⁻¹)</th>
<th>Land-use type</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Natural forest</td>
<td>Cypress forest</td>
</tr>
<tr>
<td>Resin P i</td>
<td>13.7 (2.4)ab</td>
<td>3.9 (1.2)b</td>
</tr>
<tr>
<td>NaHCO₃ P₁</td>
<td>10.7 (2.2)ab</td>
<td>2.9 (0.5)b</td>
</tr>
<tr>
<td>NaHCO₃ P₀</td>
<td>26.7 (2.9)b</td>
<td>29.6 (3.0)b</td>
</tr>
<tr>
<td>NaOH P₁</td>
<td>53.5 (5.5)b</td>
<td>59.3 (3.1)b</td>
</tr>
<tr>
<td>NaOH P₀</td>
<td>41.9 (5.2)a</td>
<td>23.3 (3.6)a</td>
</tr>
<tr>
<td>Sonicate NaOH P₁</td>
<td>15.7 (2.3)a</td>
<td>21.9 (2.5)a</td>
</tr>
<tr>
<td>Sonicate NaOH P₀</td>
<td>75.3 (6.3)a</td>
<td>51.2 (4.2)b</td>
</tr>
<tr>
<td>HCl P₁</td>
<td>24.5 (7.8)a</td>
<td>10.2 (1.1)a</td>
</tr>
<tr>
<td>Residual P</td>
<td>574.7 (53.2)a</td>
<td>241.2 (16.5)b</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Readily labile P</th>
<th>Moderately labile P</th>
<th>Non-labile P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>51.1 (5.5)bc</td>
<td>36.4 (3.4)c</td>
<td>690.1 (54.5)a</td>
</tr>
<tr>
<td></td>
<td>95.3 (9.9)b</td>
<td>82.7 (4.1)b</td>
<td>324.5 (15.7)c</td>
</tr>
<tr>
<td></td>
<td>690.1 (54.5)a</td>
<td>324.5 (15.7)c</td>
<td>396.4 (21.2)bc</td>
</tr>
</tbody>
</table>

Abbreviations: P i = inorganic phosphorus, P₀ = organic phosphorus, NaOH = sodium hydroxide, HCl=hydrochloric acid, P=phosphorus. Within rows, means followed by different letters in superscript are significantly different at p < 0.05, and mean standard deviations are in parenthesis. Means were separated by Tukey’s Honest Significant Difference (HSD) test.

natural forest (574.7 P mg and 75.3 mg P kg⁻¹, respectively) compared to the other land use types. When the fractions were grouped based on availability, all the three groups were significantly affected by land-use type. Readily and moderately labile P were highest in potato growing fields (125.1 P mg and 186.1 mg P kg⁻¹, respectively) and lowest in cypress forest (36.4 P mg and 82.7 P mg kg⁻¹, respectively). However, non-labile P was highest (p<0.001) in natural forest (690.1 P mg kg⁻¹) and lowest in cypress forest (324.5 P mg kg⁻¹).

Spore abundance, AMF richness and diversity

Of the ten AMF genera identified, only *Glomus* and *Acaulospora* were significantly (p<0.01) affected by land-use type. The highest spore abundance was found in potato growing fields (81.1 spores 100 g⁻¹ of soil) compared to grazed pastures (46.5 spores 100 g⁻¹ of soil), cypress forest (20.0 spores 100 g⁻¹ of soil) and natural forest (27.6 spores 100 g⁻¹ of soil) (Table 4). All the other AMF genera were not significantly affected by land-use type. Similarly, AMF taxonomic richness and diversity was not significantly different across the four land use types.

Correlation between AMF abundance, soil chemical properties and P fractions

The Canonical Correspondence Analysis (CCA) plot between soil chemical properties and AMF abundance and diversity showed strong but negative relationship between soil pH and two AMF genera; *Glomus* and *Acaulospora* as well as the total spore count (Figure 2). In this case, the two AMF genera and the total spore count were found to be abundant in acidic soils, a characteristic which was predominant in potato fields. There was also a negative but a weaker relationship between genera *Ambispora*, *Funneliformis*, *Septoglomus*, and *Scutellospora* with NaOH-P₀. There were no unique patterns that could be drawn for the other AMF genera. In addition, the variation explained by the two axes (axis 1 and 2) was very low, with axis 1 explaining about 20% of the variation and axis 2 about 12%, for a combined value of just above 30%.

DISCUSSION

Soil chemical properties

Forest conversion to other land use types had strong significant effect on soil chemical properties. Soil organic C, N, K, Ca and Mg were relatively lower in potato fields than in natural forest suggesting that converting natural forest for agricultural crop production result to degradation of the soil. Removal of vegetation, which characterize forest conversion lead to changes in litter quantity and quality hence negatively influencing soil physical, chemical and biological properties (Pérez-Bejarano et al., 2010). Litter input and quality affect the build-up of soil organic matter and key functions such as nutrient cycling, nutrient retention capacity of soil and soil aggregation. Thus, lack of permanent vegetation cover in cultivated agricultural fields due to removal of...
residues from the farms for other competitive use like animal feed result to reduction in litter input to soil, and consequently reduced soil chemical properties as found in potato fields. Furthermore, conventional crop management practices such as continued application of inorganic fertilizers, especially DAP, which is commonly used by potato farmers has been shown to further lower soil pH (Muthoni and Nyamongo, 2009; Banerjee et al., 2019). Frequent tilling of soil also exposes soil to higher temperatures and soil organic matter to microbial attack hence accelerates decomposition and mineralization processes, thus a reduction in soil nutrient content (Awiti et al., 2008; Groppo et al., 2015). In addition, cultivation of potato has been associated with declining soil fertility due to sub-optimal fertilizer application and lack of fallow periods between growing seasons leading to nutrient mining (Muthoni and Nyamongo, 2009; Muthoni, 2016). The cumulative effect of these agronomic practices in potato fields could therefore explain the lower soil chemical properties found in this study, and that these practices in potato cropping systems may be slowly degrading soil fertility. Soils under cypress forest were also relatively acidic and had the lowest soil chemical properties which may be attributed, but not limited, to the chemical composition of cypress litter. The dominant tree species exert significant influence on soil chemical properties of the upper soil layer through litter quality which determines soil nutrient availability and decomposition rate (Iwashima et al., 2012). For example, coniferous litter contain low concentrations of basic cations and produce considerable amounts of organic acids hence causing soil acidification (Iwashima et al., 2012; Zajícová and Chuman, 2019), implying that soils under coniferous trees could exhibit low nutrient content due to poor nutrient replenishment through litter input. Cypress litter had lower chemical (N, P, Ca and Mg) properties than litter from natural forest hence may also be contributing to the lower soil chemical properties found in cypress forest plantation. Chen et al. (2000) also reported a reduction in soil C, N and P under pine forest plantation. Similar observations in the decline of soil N and organic C content following deforestation for crop cultivation and grazed pastures have also been reported (Jafarian and Kavian, 2013; Kamau et al., 2022). Soils under natural forest exhibited significantly higher nutrient content than soils under grazed pastures, cypress forest plantation and potato fields possibly from sustained build-up of soil organic matter, which play a key role in maintaining soil’s capacity to retain nutrients. The perennial vegetation cover in natural forests also allow closed nutrient cycling leading to minimal nutrient loss from the system which commonly does not happen in agroecosystems due to crop harvests and accelerated soil erosion. Trees are well known to pump nutrients from the subsoil layers and redistribute them on the surface soil via throughfall and litterfall (Qiong et al., 2008). In addition, the nutrient-rich litter, i.e., higher total N, P, Ca and Mg, from the common tree species (Dombeya goetzenii K.) in natural forest could.
Figure 2. Canonical Correspondence Analysis (CCA) of AMF genera and soil chemical properties and soil P fractions in South Kinangop Sub-county, Nyandarua County. Abbreviations for soil chemical properties: C-organic carbon, N-nitrogen, CEC- cation exchange capacity, Mg-magnesium, Ca-calcium and K-potassium. Abbreviations for soil P fractions: BiPi-NaHCO$_3$ inorganic phosphorus (P$_i$), BiPo-NaHCO$_3$ organic phosphorus (P$_o$), NaPi-NaOH inorganic phosphorus (P$_i$), NaPo-NaOH organic phosphorus (P$_o$), SNaPi-sonicate NaOH inorganic phosphorus (P$_i$), SNaPo-sonicate NaOH organic phosphorus (P$_o$), HCL-HCl inorganic phosphorus (P$_i$), Res-residual phosphorus, LabP-labile phosphorus, ModLabP-moderately labile phosphorus and Nonlab-non labile phosphorus. Abbreviations for AMF genera: Ac.sp-Acaulospora, Gl.sp-Glomus, Sp.sp-Septoglomus, Pa.sp-Paraglomus, Gi.sp-Gigaspora, Sc.sp-Scutellospora, Fu.sp-Funneliformis, En.sp-Enterophospora, Ar.sp-Archeospora, Am.sp-Ambispora and Count-Total spore count.

have partly contributed to enrichment of soils. Iwashima et al. (2012) observed that soils under broadleaved vegetation dominated by Japanese oak (Quercus crispula Blume) had higher pH, inorganic N, Ca and Mg than those under conifers, Picea jezoensis (Siebold and Zucc.) Mast, and they attributed this to litter characteristics between the Japanese oak and conifers where the former had higher N, Ca, Mg and K than the latter. Contrary to the findings of this study, Muchane et al. (2012) found higher soil nutrient concentrations (N, P and K) in agricultural ecosystems than in natural ecosystems which they attributed to increased use of fertilizers. However, such changes are not sustainable in the long term, because of the constant nutrient mining and export from agroecosystems with inadequate nutrient replenishment. In addition, the gradual decline in soil organic matter means that these nutrients are less protected and retained thus are more susceptible to loss through soil erosion, volatilization or leaching. Therefore, cultivated agricultural lands would continue to be deficient in nutrients even though they are supplemented with mineral fertilizers as is clearly shown by the current study.

Changes in soil P fractions

Labile P fractions (resin, NaHCO$_3$ and NaOH) were relatively low in natural forest and increased in cultivated potato fields. Agronomic practices in cultivated croplands significantly influence P transformation and distribution in soil. For instance, McLaren et al. (2015), when assessing fertilized and unfertilized pastures, found that fertilizer application led to an increase in P in NaHCO$_3$ and NaOH fractions, and observed that applied P may accumulate in less
labile fractions (e.g., NaOH) than in more labile fractions (NaHCO₃). The results of this study are consistent with those of McLaren et al. (2015) since NaHCO₃-P and NaOH-P were higher in cultivated potato fields receiving fertilizer. Henriquez (2002) also reported an increased P in labile (NaHCO₃-P and NaOH-P) fractions after fertilizer application, and concluded that these fractions may be important sinks for applied P. The results of this study and those reported (e.g., Henriquez, 2002; McLaren et al., 2015) show that the use of inorganic fertilizers greatly influence labile P pool more than non-labile P pool. The use of phosphate fertilizers like DAP for long durations further lower soil pH, indirectly influencing sorption-desorption processes governing P availability. Low soil pH increases P sorption by soil minerals such as Al and Fe oxides hence the lower soil pH in potato fields could have also influenced P partitioning, especially in NaOH fraction which is associated with Al and Fe oxides leading to higher moderately labile P (Richardson et al., 2009; Bayuelo-Jiménez et al., 2020). Grazed pastures as well exhibited higher content of labile and moderately labile P which may be partly due to animal grazing which has been shown to exert significant influence on labile P (Chirino-Valle et al., 2016). It has been reported that 60-95% of P used by plants and taken up by animals through grazing is returned to the soil in form of litter and animal excreta (Condron et al., 2005). In addition, secretion of P mobilizing organic acids by grasses and accumulation of SOM via root residues and litter recycles P from non-labile pool into labile pool (Almeida et al., 2018). For example, Almeida et al. (2018) found that growing Ruzi grass after soya bean crop led to an increase in labile P (resin, NaHCO₃ and NaOH-P) which they attributed to higher SOM deposition and secretion of low molecular weight organic acids by Ruzi grass. On the other hand, non-labile P (sonicate NaOH P, & Pₒ, HCI-P and residual-P) in natural forest was nearly twice that found in other land uses and this could be attributed in part to greater accumulation of SOM. The absence of soil disturbance through tillage means that natural forest soils are less exposed to external forces such as surface runoff, heating and disintegration of soil aggregates that would accelerate SOM P mineralization and loss. The higher content of sonicate NaOH-P, in natural forest, for instance, reveals that soils under natural forest have stable aggregates since P bound in this fraction is considered to be physically protected by soil aggregation. It has also been found that majority of P in natural forest soils tend to accumulate in non-labile forms (Garcia-Montiel et al., 2000), indicating that natural forests may be good in conserving soil P. However, cypress forest showed an overall lower P content in labile and non-labile fractions which could be attributed to tree characteristics such as fast growth rate requiring higher nutrient uptake. In assessing the role of soil P fractions to tree nutrition in a short-term experiment, Niederberger et al. (2017) found poplar trees to decrease readily and moderately labile P fractions, thus showing the importance of these fractions in tree nutrition. It has also been shown that pine, eucalyptus and cypress trees decrease organic P content and, utilize even non-labile P due to enhanced enzymatic activities, mycorrhizal associations as well as changes in soil pH, which enhances P mineralization (Chen et al., 2008; Gatiboni et al., 2017). Phosphorus is mainly recycled into the soil through litterfall in forests (Qiong et al., 2008) hence litter input and quality greatly influence P concentration in forest soils. Cypress litter had low nutrient (N, P, Ca and Mg) content compared to litter from natural forest which may have partly led to lower P concentration in cypress forest soils. Therefore, continuous cultivation of cypress trees may decreasing P stocks in the long-term, and thus may not be a desirable land use system wherein the ultimate goal is to restore and maintain high fertility of P.

**Response of AMF to change in land use type**

Spores mostly dominated cultivated potato fields than natural forest which could be attributed to frequent soil disturbance from agronomic practices such as tillage, application of mineral fertilizers and pesticides and monocropping. These agronomic practices have been shown to influence AMF spore and hyphal abundance and consequently alter AMF community composition (Muchane et al., 2012; Campos et al., 2018; Dobó et al., 2018). Tilling the land, for instance, may cause a positive or negative effect on AMF depending on how AMF species resident in that particular ecosystem respond to stress. Tilling soil essentially destroy spores and hyphae reducing spore abundance and AMF’s potential for new colonization in soils (Soka et al., 2015). However, some AMF species which are considered ‘generalists’ (i.e. found in most soils) may respond to stress associated with land use change by increased sporulation. Thus it is possible that the common AMF species, e.g., *Glomus, Acaulospora*, in soils of the study area were being stimulated by stress to sporulate. *Acaulospora* and *Glomus* species are reported to be common in most soils in the tropics (Campos et al., 2018; Dobó et al., 2018). The application of sub-optimal fertilizers by farmers in growing potato may have also contributed to high spore abundance in potato fields since the soluble P added through mineral fertilizers to the soil may not be sufficient enough to suppress AMF spore production and colonization. This is in line with other studies that have found low input farming systems to maintain high AMF abundance and diversity (Muchane et al., 2012; Belay et al., 2015). This is because the use of optimum P-based fertilizers in crop cultivation increase P availability for plant uptake reducing the need to establish association with AMF, hence suppressing
root colonization and spore production (Sharma et al., 2013). While some AMF species may be suppressed in soils receiving fertilization, others like Ambispora are reported to be stimulated in such soils (Wang et al., 2011), which was what was found in this study where the genus was found only in potato fields receiving mineral fertilizers. Grazed pastures likewise exhibited high spore abundance, richness and diversity which could be attributed to soil disturbance through animal trampling which cause AMF to produce spores in response to stress. Defoliation through grazing may limit C allocation to the roots of the host plant reducing AMF root colonization, and instead triggers AMF to produce spores (Soka and Ritchie, 2018). The abundance of spores in pastures has also been associated with greater root density in grasses which improve availability of resources for AMF colonization, and produce high mycelial biomass for abundant spore production (Picone, 2000). Forests recorded low AMF spore abundance and richness and diversity, with cypress forest plantation having the lowest values. Soil acidity, and reduced undergrowth in forests especially in coniferous forest plantations limit substrate availability to AMF hence suppress their growth and abundance (Moora et al., 2014). The low abundance of spores in forests has also been attributed to feeding by soil arthropods which are more abundant in forests than other land use types (Picone, 2000). Soil physical and chemical properties also influence AMF community structure leading to changes in spore abundance (Dobo et al., 2018). More importantly, soil pH, organic matter content, P and N influence AMF, implying that any alteration in these soil properties may significantly alter AMF community structure. For example, Glomus hoi, Septoglomus constrictum, Funneliformis coronatum are stimulated in soils by high available P while Scutellospora projecturata, Rhizophagus clarus, R. irregularis, R. intraradice thrive well in low available P, whereas Funneliformis geosporum, F. mosseae, F. fragilistratum, F. verruculosum tolerate both extremes (Wang et al., 2015). It is also reported that Glomus accumulate P and K into their spores and hyphae when they experience carbon starvation (Hammer et al., 2011), pointing out to a possible limitation in spore development and hyphal growth in soils with low content of P and K. Based on canonical correspondence analysis (CCA), there was generally a weak relationship between AMF parameters and soil chemical properties and P fractions showing that soil properties and P availability were not the primary factors influencing AMF composition and abundance. Converting natural forests may not only influence AMF abundance and diversity but can also lead to changes in ecosystem functions provided by AMF such as improved soil texture and structure, mobilization of soil nutrients and water relations as well as important interactions with other soil microorganisms thus affecting the overall ecosystem functioning and sustainability.

CONCLUSION

Forest conversion to other land use types led to lower soil chemical properties showing that soil nutrient availability may not be maintained after forest conversion. This may be associated with gradual loss of soil organic matter, which is key in nutrient cycling, partly caused by reduced litter input and soil management practices that accelerate decomposition and mineralization of soil organic matter. The results of this study also demonstrate that forest conversion alter P distribution and lead to greater accumulation of labile P than non-labile P. The elevated labile P may present a short-term P availability for plant use but may not be sustainable in the long run due to losses through soil erosion and crop harvests. In addition, land uses particularly those of conifer tree plantations may deplete all P fractions as the stand ages hence may not be a desirable land use system in restoration and maintenance of soil P.

A shift in land use from natural forest to agricultural use led to higher spore abundance and AMF species richness and diversity. In particular, potato fields had the highest spore abundance and number of AMF genera which may imply that farming practices induce stress on AMF causing them to sporulate, and may also be stimulating AMF species which are present but dormant in the soils of the study area. Therefore, a change in land use from natural forest lead to soil degradation, and the extent to which soil is degraded may be determined by the type of land use and present management practices.

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