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Original Research

Effects of Successive Planting of Eucalyptus on Soil Physicochemical Properties 1–3 Generations after Converting Masson Pine Forests into Eucalyptus Plantations

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Abstract

Soil physicochemical properties play a key role in plant growth and development; however, owing to land use change and successive planting, long-term changes in soil physicochemical properties are rarely reported. The objective of this study was to analyze changes in soil physicochemical properties caused by the conversion of Masson pine forests to Eucalyptus plantations and the successive planting of first-, second-, and third-generation Eucalyptus plantations in China using a space-for-time substitution method. The results demonstrated significant differences in soil physicochemical properties between Masson pine forest (MP) and second-generation (G2) and third-generation (G3) Eucalyptus plantations at 0-20 and 40-60 cm soil depths ($p<0.05$). Alkaline hydrolytic nitrogen levels were significantly lower in G3 than in first-generation (G1) Eucalyptus plantations at 0-20 cm soil depth ($p<0.05$). Available phosphorus, available potassium, and organic matter levels were significantly lower in G2 and G3 than in MP at a 0-20 cm soil depth ($p<0.05$). The pH and bulk density were significantly lower in G2 and G3 than in MP at 0-20, 20-40, and 40-60 cm soil depths ($p<0.05$). However, stable isotope ^{15}N abundance was significantly higher in G2 and G3 than in G1 at 0-20 and 20-40 cm soil depths ($p<0.05$). TP was defined as MP<G1<G2<G3 at the 20-40 and 40-60 cm soil depths ($p<0.05$). From the above results, the conversion of Masson pine forest to Eucalyptus plantations and successive planting decreased soil fertility. These findings highlight the advantages of Masson pines for Eucalyptus plantations and successive planting in improving soil fertility and production by mediating the relationships between soil

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physicochemical properties. This study provides a theoretical reference for the sustainable management of Masson pine forest conversion into multi-generation Eucalyptus plantations.

Keywords: soil physicochemical properties, land-use conversion, Eucalyptus plantation, successive planting, Masson pine forests

Introduction

Eucalyptus plantations are one of the main plantation ecosystems, covering an area of approximately 20 million·ha^{-1} worldwide, accounting for 15% of the world's total plantation forest area [1]. An abundant supply of timber guarantees the security of national timber reserves and promotes the economic development of pulp, wood-based panels, furniture, eucalyptus leaf oil, eucalyptus polyphenols, eucalyptus charcoal, and other related economic industries [1, 2]. At the same time, Eucalyptus plantations play a key role in water and soil conservation, as well as in regulating the climate and improving the ecological environment [3, 4]. However, many ecological and environmental issues make Eucalyptus plantations highly controversial worldwide [5]. This includes a decline in soil fertility, biodiversity, and acidification, which seriously endangers soil health and forestry development [6, 7]. Eucalyptus has a chemosensitizing effect, inhibits the growth of other organisms, and reduces understory plant diversity [5]. Eucalyptus grows rapidly and has a relatively short growth cycle, consuming more water and nutrients than other vegetation species, which, combined with the application of chemical fertilizers, makes the soil susceptible to drought slumping and acidification [5-7]. After multiple generations of cultivation, reduced wood quality and eucalyptus yield can typically be seen [8]. Soil fertility is the basis for timber production and related products, such as eucalyptus leaf oil [1, 9]. Therefore, maintaining soil fertility is extremely important to protect the soil environment and maintain the economic profitability of Eucalyptus plantations [10].

The physicochemical properties of soil are crucial indicators of soil fertility [11]. The intensive management of successive planting of Eucalyptus plantations consumes large amounts of soil nutrients and destroys the original vegetation structure and forest microclimate [4, 5, 12]. At the same time, soil fertility affects the increase in individual growth and timber storage accumulation of Eucalyptus, especially the content of available soil nutrients, which have a more direct impact on the yield of successively planted Eucalyptus plantations [13]. For example, it was reported that the content of available soil nutrients, such as available potassium (AK) and available phosphorus (AP), decreased significantly with an increase in successive planting generations in a plantation with four generations of Eucalyptus [14]. Recently, Zhou et al. [15] reported that AP content declined in Eucalyptus plantations and was associated with Eucalyptus monocultures planted over six successive generations. Li et al. [16] also reported that, after converting Chinese fir plantations to two rotations of Eucalyptus plantations, intensive successive rotations caused lower understory vegetation and soil organic matter (SOM) levels in second-generation plantations compared to those in first-generation plantations. Furthermore, Zhang and Wang [17] found that the contents of soil total nitrogen (TN) and total phosphorus (TP) as well as pH significantly decreased after continuous growth of Eucalyptus for 50 years. In a study of land use conversion from weed and shrub forests to four successive generations of Eucalyptus plantations, Tang et al. [18] showed that pH was significantly lower in third-generation plantations than in first- and second-generation plantations, while the second- and third-generation plantations had the highest TN and TP contents, respectively, and the soil alkaline hydrolytic nitrogen (AN), AP, and AK contents in the second- and third-generation plantations were lower than those in the first-generation plantations. Decreased SOM content and soil pH have been observed in Eucalyptus plantations converted from pasture to planting over six generations [8]. The results of study on the dynamic characteristics of soil nutrients with 34 years of successive planting of Eucalyptus plantations showed that the contents of TN, ammonium nitrogen (NH^{+4}-N), and nitrate nitrogen (NO^{-3}-N) decreased with the increase in planting generations [19-21]. Many studies have shown that successive planting of Eucalyptus has no significant effect on the total soil potassium [2, 9, 15, 17, 19]. However, land use conversion may result in significantly different soil nutrient levels because of poor diversity and stability after species change [22-25]. Zhu et al. [26] reported that soil bulk density (BD) increased significantly and TN, TP, and AP levels decreased significantly in third-generation Eucalyptus plantations compared to those in evergreen broad-leaved forests. Additionally, the conversion of grassland or sugarcane fields to Eucalyptus plantations resulted in significant changes in soil physicochemical properties, such as organic carbon content, nitrogen content, and microbial biomass [27, 28]. However, the abovementioned studies mainly focused on the successive planting of Eucalyptus plantations for four generations, six generations, 34 years, and 50 years, without the land use conversion of one to three generations after converting Masson pine forests into Eucalyptus plantations. The prevalence of land use conversion from Masson pine forests to Eucalyptus plantations is increasing [9]. In a study of four successive generations of Eucalyptus plantations after the conversion of Masson pine to Eucalyptus, Zhang et al. [9] found significantly lower TN content in the fourth generation of Eucalyptus than that in Masson pine, and lower AK content in all four generations of Eucalyptus plantations than in Masson pine. However, Chu et al. [24] showed that after the conversion of Masson pine to the first generation of Eucalyptus plantations, there were no significant differences in N, P, or K contents. The phenomenon of soil degradation after multiple generations of Eucalyptus plantations provides a theoretical reference to study whether the soil of the first three generations of Eucalyptus plantations is degraded and to take timely management measures to protect soil fertility. The effect of converting Masson pine secondary forest to the first three generations of Eucalyptus planting has rarely been studied, but this is also important for the evaluation and protection of soil fertility.

Stable isotope ^{15}N ($\delta^{15}N$) is an important indicator for evaluating the degree of soil nitrogen mineralization in forests, grasslands, agriculture, and other ecosystems [8, 29]. Ectomycorrhizal-secreting isotopically enriched compounds transfer isotopically light N (^{14}N) to plants, and the absorption of ^{15}N-depleted inorganic nitrogen derived from SOM mineralization (N isotope fractionation) causes the $\delta^{15}N$ enrichment [30, 31]. This indicates that the degree of SOM mineralization can be analyzed by $\delta^{15}N$ abundance and that soil nitrogen mineralization is enhanced as the $\delta^{15}N$ value increases [32]. Teixeira et al. [33] showed that soil $\delta^{15}N$ levels increased with increasing Eucalyptus growth time, which was related to SOM mineralization in Brazil [34]. However, there have been few reports on the characteristics of changes in $\delta^{15}N$ after the conversion of Masson pine forest to Eucalyptus plantations and successive planting [30, 35]. Therefore, it is important to evaluate soil fertility using $\delta^{15}N$ as a tool to determine the degree of soil nitrogen mineralization after converting Masson pine into Eucalyptus plantations.

In recent years, Eucalyptus plantations in China have reached 5.46 million·ha^{-1} in China in area [6]. Owing to the multiple impacts of long-term successive planting and excessive production after land use conversion, the soil fertility of the Chinese Eucalyptus plantation ecosystem has significantly decreased [9]. The decrease in soil fertility caused by land use change seriously restricts the sustainable development of the forest economy and the ecological environment in the Eucalyptus plantation area [36]. There is an urgent need to assess the impact of successive planting of Eucalyptus on soil fertility, as it plays a key role in sustaining intensive management and soil fertility in plantations.

In the present study, in was hypothesized that (a) the soil physicochemical property values would decrease significantly after converting Masson pine to Eucalyptus plantations, and (b) the soil physicochemical property values of Eucalyptus plantations would decrease with planting generations. To test these hypotheses, this study used Masson pine forests and *Eucalyptus urophylla* × *Eucalyptus grandis* (Eucalyptus) plantations as research objects and discussed the changes in soil physicochemical properties of the soil profile (0-20, 20-40, and 40-60cm) caused by converting Masson pine to Eucalyptus and subsequent Eucalyptus planting (first, second, and third generations of Eucalyptus). Finally, this study provides a theoretical basis for the successive planting of Eucalyptus plantation ecosystems to maintain soil fertility and sustainably produce Eucalyptus plantation resources.

Materials and Methods

Study Site

This study was conducted on forest hillslopes in the Leika Branch of Dongmen Forest Farm, Dongmen Town, Fusui County, Guangxi Zhuang autonomous region, China (22°17′-22°30′N, 107°14′-108°00′E). The area is dominated by terrace hills, the altitude is 82-95 m, and the slope direction is southeast with a slope of 7-10°. Before planting the Eucalyptus plantation, the hillsides were covered with natural secondary Masson pine forests. In addition, they experienced controlled burning before planting the Eucalyptus plantations. No stand fertilization was applied during the growth of the Masson pine forest. The Eucalyptus plantation was adjacent to an existing 26-year-old natural secondary mature Masson pine forest. The amount of base fertilizer applied to Eucalyptus plantations was 0.5 kg/plant twice a year 2 years after afforestation. The total amount of fertilizer applied was N (nitrogen fertilizer) 200 kg·hm^{-2}, P_2O_5 (phosphorus fertilizer) 150 kg·hm^{-2}, and K_2O (potassium fertilizer) 100 kg·hm^{-2}. The study region has a typical subtropical monsoon climate, with a mild climate, sufficient light and rainfall, and the annual mean air temperature and cumulative rainfall values are 18.9°C and 1,958 mm, respectively. The precipitation is mainly concentrated in May to September, and the annual accumulated temperature of >10ºC is 7,190-7,762ºC, with an annual sunshine duration of 1,634-1,739 h. The annual frost-free period is 320 days and the area has a short, severe winter period. The highest and lowest temperature is –4ºC and 41ºC, respectively, and the relative humidity is greater than 74%. The soil in the area is classified as lateritic red soil, which is derived from parent materials such as sand shale; the pH value is 4.2-4.9; and the soil thickness is more than 80 cm. The characteristics of the Masson pine forest and Eucalyptus plantations of different generations are presented in Table 1.

Experimental Design

The sample plots were located in the Leika Branch of Dongmen Forest Farm. In April 2014, a stand survey was conducted at the study site; four stands of Eucalyptus plantations of the first, second, and third generations with similar stand characteristics and adjacent Masson pine forest were selected, and a standard sample plot of 20 × 20 m was laid out in each stand. The average

Table 1. The characteristics of Masson pine forest and Eucalyptus plantations with different planting generations.

Forest type	Tree age/(year)	Average DBH/(cm)	Average tree height/(m)	Forest density/ (plant · hm^{-2})	Main undergrowth vegetation
Masson pine	26	22.7	17.2	640	*Rhodomyrtus tomentosa*, *Mallotus barbatus*, *Ficus hirta, Heteropogon contortus*, *Microstegium vagans*, *etc.* (The coverage is 90%, and the thickness of litter layer is about 2.5 cm)
First generation Eucalyptus	7	14.8	17.4	1320	*Sageretia theezans*, *Litsea pungens*, *Rhodomyrtus tomentosa*, *Litsea glutinosa*, *Euodia lepta*, *Miscanthus floridulus*, *Microstegium vagans*, *etc.* (The coverage is 85%, and the thickness of litter layer is about 2.0 cm)
Second generation Eucalyptus	7	14.3	17.2	1380	*Litsea glutinosa*, *Rhus chinensis*, *Litsea pungens*, *Mallotus repandus*, *Macaranga denticulate*, *Miscanthus floridulus*, *Eupatorium odoratum*, *etc.* (The coverage is 75%, and the thickness of litter layer is about 1.7 cm)
Third generation Eucalyptus	7	14.5	17.0	1350	*Rhus chinensis*, *Litsea pungens*, *Geum aleppicum*, *Litsea glutinosa*, *Heteropogon contortus*, *Miscanthus floridulus*, *Eupatorium odoratum*, *etc.* (The coverage is 70%, and the thickness of litter layer is about 1.7 cm)

diameter at breast height (DBH) and height in the sample plot were determined by measuring the DBH and height of each tree and observing its growth condition. Three trees close to the average DBH and height with good growth condition were identified as standard trees in each sample plot and marked. Masson pine and three generations of Eucalyptus plantations were the research objects: 26-year-old Masson pine forests (MP), 7-year-old first-generation Eucalyptus plantations (G1), second-generation Eucalyptus plantations (G2), and third-generation Eucalyptus plantations (G3). Before afforestation, the Eucalyptus plantations of all three generations underwent controlled burning, land preparation and base fertilizer application. *E. urophylla* × *E. grandis* (clone DH32-29) tissue culture seedlings were used for the afforestation of all planting generations, and the spacing between rows was 2 × 3 m.

Soil Sampling

Soil samples collected in the four seasons of spring, summer, autumn and winter of 2014 were brought back to the laboratory, processed and stored indoors. Soil samples were collected from four 20 × 20 m plots, and three representative sampling points were set in each standard sample plot at a distance 80–100 cm from each standard tree. A German STEPS soil sampler was used to collect soil profile samples at three depths of 0-20, 20-40 and 40-60 cm, respectively, after removing the surface litter at the sampling points. The soil samples collected from the three sampling points were mixed thoroughly by soil layer, and approximately 1 kg of soil was placed in sealed bags using the quadrat method, labelled, and brought back to the laboratory. Roots, gravel, plants, and animal debris were removed from the collected soil samples and passed through a 0.15 mm sieve. Each soil sample was divided into two parts. One fresh soil sample was used to determine AN, AP, and AK contents. The other sample was dried naturally, and the air-dried soil samples were prepared for SOM, TN, TP, $\delta^{15}N$, and pH analyses. The undisturbed soil at the three soil depths was collected using a stainless-steel ring knife (100 cm^3).

Soil Sampling and Physicochemical Analysis

AN was determined using the Kjeldahl method. AP was determined by diacid extraction spectrophotometric colorimetry, and AK was determined by ammonium acetate extraction using a flame photometer. SOM content was determined using the Walkley method (1935). The micro-Kjeldahl method and a fully-automatic Kjeldahl Nitrogen meter (Foss Kjeltec8420) were used to determine TN. $\delta^{15}N$ was determined using a MAT 253 Isotope Ratio Mass Spectrometer and Flash 2000 HT Elemental Analyzer. The sample was burned at a high temperature in the element analyzer to generate N_2, which detects the ^{15}N to ^{14}N ratio of N_2, compared with the international standard (atmospheric N_2), and N isotopic abundances were calculated as $\delta^{15}N$ (‰) using the following formula:

$$\delta^{15}N\ (‰) = (R_{sample} / R_{standard} - 1) \times 1000$$

where R_{sample} is the stable isotopic ratio in the samples and $R_{standard}$ is the ratio in the standard. The TP was measured using a Prodigy High Dispersion ICP-OES (Teledyne Technologies Inc., USA). Soil pH was determined using a potentiometric method (soil:water ratio of 1:2.5), and BD was determined using the cutting ring method.

Statistical Analysis

All data were checked and collated using Microsoft Office Excel 2019, and data analysis and image rendering were performed using SPSS 26.0 and Origin 2021. Principal component analysis (PCA) was used to compare the differences in soil physicochemical properties among MP, G1, G2, and G3 at different soil depths (0-20, 20-40, and 40-60 cm) ($p<0.05$). A one-way ANOVA was performed with Duncan's multiple range test to show significant differences in soil physicochemical properties between MP, G1, G2, and G3 at different soil depths (0-20, 20-40, and 40-60 cm) ($p<0.05$). Pearson's correlation analysis was used to analyze the correlation between the soil physicochemical properties.

Results and Discussion

Effect of Land Use Conversion and Successive Planting on Available Soil Nutrients

PCA ordination indicated that the physicochemical properties of MP were significantly different from those of G2 and G3 at the 0-20 cm soil depth ($p<0.05$) (Fig. 1a). AP and AK at a 0-20 cm soil depth contributed to the significant differences in the PCA. AP and AK were significantly lower in G2 and G3 than in MP at 0-20 cm soil depth ($p<0.05$) (Fig. 2b-c). The rate of nutrient uptake was faster in Eucalyptus than in Masson pine; therefore, the variation in tree species may be the reason for the lower AP and AK contents in G2 and G3 than in MP [14, 15]. The AN content of G3 was significantly lower than that of G1 at a 0-20 cm soil depth ($p<0.05$) (Fig. 2a). AK was significantly lower in G2 and G3 than in G1 at all three soil depths ($p<0.05$) (Fig. 2c). This may be a result of nutrient leaching due to the destruction of the soil structure caused by artificial soil tilling, which may also be lost to gas by nitrification after soil tilling [37]. The decrease in available soil nutrient levels may be the result of a decrease in SOM, which leads to a decrease in the ability of the soil to adsorb nutrients, thereby decreasing available soil nutrients levels. Our correlation analysis showed that SOM was significantly and positively correlated with AN, AP, and AK ($p<0.05$) (Fig. 4). This was consistent with the fact that the levels of AN, AP, and AK of Eucalyptus plantations were significantly lower than those of natural and secondary forests, as reported in many studies [9,38,39]. However, in our study, the AK content was significantly higher in G1 than in MP at a 40-60 cm soil depths ($p<0.05$) (Fig. 2c). This may be related to the control of burning and fertilization of Eucalyptus plantations. Control burning converts organic matter, such as Masson pine residues and understory vegetation, into inorganic matter, resulting in increased AK content. The application of potassium fertilizer to Eucalyptus plantations also contributes to increased AK content. In a study by Devin et al. [40] on successive rotations of Eucalyptus plantations in Brazil, soil nutrients were not significantly depleted over time and there was a tendency for available soil nutrient levels to increase, contrary to the results of our study. This difference may be because the number of planting years for Eucalyptus in the present study had a 7-year difference to the 12 years in Devin's study. Different planting and fertilizer management patterns also contributed to an increase in soil nutrient contents in Eucalyptus plantations in a study by Devin et al. [40].

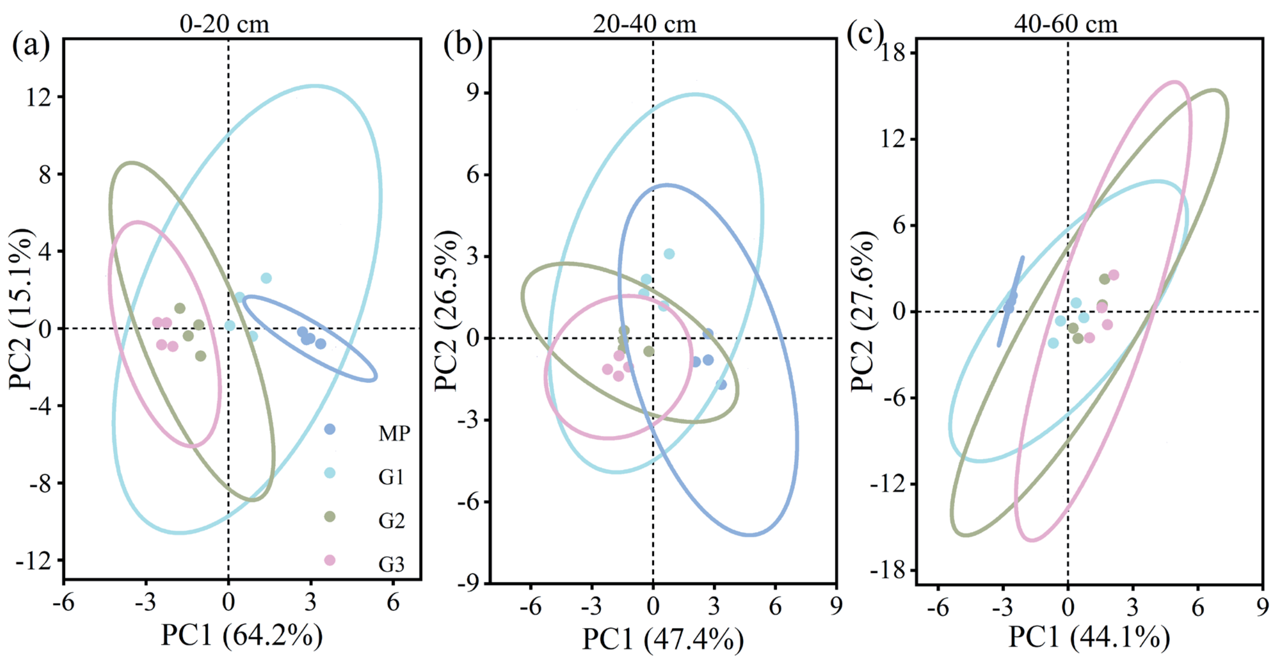

Fig. 1. Principal component analysis (PCA) of soil physicochemical properties among different forest stand types. Ellipses: 95% confidence areas of the different forest stand types. MP, G1, G2, and G3 refer to the Masson pine forest and the first-, second-, and third-generation Eucalyptus plantations, respectively.

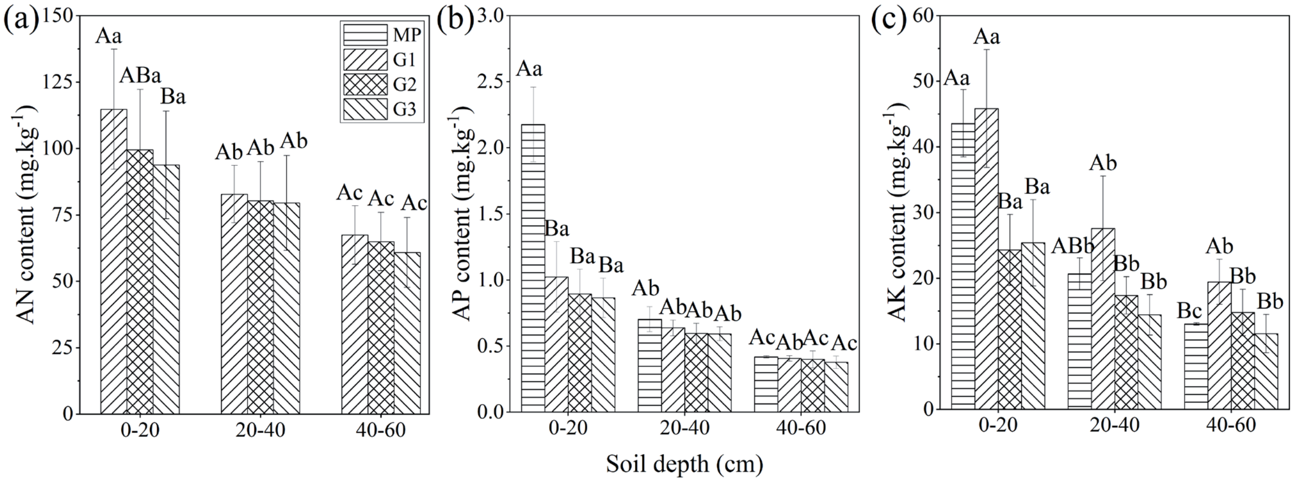

Fig 2. Soil available nutrient properties in the Masson pine forest and successive Eucalyptus plantations. MP, G1, G2, and G3 refer to the Masson pine forest and the first-, second- and third-generation Eucalyptus plantations, respectively. AN, alkaline hydrolytic nitrogen; AP, available phosphorus and AK, available potassium. Different uppercase and lowercase letters denote significant differences at p<0.05 across different generations and soil depths in soil available nutrient properties, respectively.

In addition, Eucalyptus grows rapidly and has a strong root system that absorbs nutrients effectively and stores them in biomass, thus, reducing the nutrient content of the soil [2, 5, 9]. This was also the reason for the decrease in effective nutrient levels with increasing generations in the Eucalyptus plantations.

The results showed that the available soil nutrients levels decreased significantly with increasing soil depth. AN content all showed as 0-20 cm>20-40 cm >40-60 cm in G1, G2, and G3 (p<0.05) (Fig. 2a). The AP content decreased significantly with increasing soil depth in MP, G2, and G3, respectively (p<0.05) (Fig. 2b). The AK content was significantly higher in the 0–20 cm soil depth than in the 20-40 and 40-60 cm soil depths in G1, G2, and G3 (p<0.05) (Fig. 2c). This significant decrease in available soil nutrient levels with increasing soil depth may be because surface soils have more litter and humus than deeper soils, and have more suitable temperature conditions and aeration for microbial survival; therefore, more inorganic nutrients are produced with a faster rate of soil mineralization and a higher level of available soil nutrients in the surface layers [35, 40]. Yoshinori et al. [19] showed that N, P, and K levels decreased with increasing soil depth, as Eucalyptus exhibits a deep root system that mainly absorbs deep nutrients. Zhu et al. [26] found that AN, AP and AK decreased with increasing soil depth, which was caused by retaining or burning harvest residues, which increased plant residues and mineralized inorganic nutrients in the surface soil. This was consistent with the decrease in available soil nutrients with increasing soil depth due to the burning of harvest residues prior to planting Eucalyptus in the present study. In addition, surface fertilization was responsible for the significantly higher available nutrient levels in surface soils than in deeper soils.

Effects of Land Use Conversion and Successive Planting on Soil Total Nutrients, pH and BD

The PCA results showed that approximately 79%, 74% and 72% of the total variability could be explained by the first two principal coordinate axes for soil physicochemical properties at the three soil depths, respectively (Fig. 1). The physicochemical properties of MP were significantly different from those of G2 and G3 at 0-20 and 40-60 cm soil depths, respectively (p<0.05) (Fig. 1a-c). SOM, TP, pH, and BD at 0-20 and 40–60 cm soil depths contributed to significant differences in the PCA. SOM content was significantly lower in G2 (25.97 $g\cdot kg^{-1}$) and G3 (24.84 $g\cdot kg^{-1}$) than in MP (31.94 $g\cdot kg^{-1}$) at a 0-20 cm soil depth (p<0.05) (Fig. 3a). This decrease was due to the Eucalyptus plantation consisting of a single species and having less plant litter than the Masson pine forest (Table. 1), whereas SOM mainly originated from the aboveground vegetation litter in the plantation [41]. The results of Ashagrie et al. [42] support those of the present study, as they found that SOM content declined during the transformation of a Podocarpus falcatus dominated natural forest into a monoculture Eucalyptus globulus plantation in Munesa, Ethiopia, because of clearing and burning before forest land conversion. However, SOM content of MP was significantly lower than that of the Eucalyptus plantations at 20-40 and 40-60 cm soil depths (p<0.05) (Fig. 3a). This may be because the fact that the felling of Masson pine prior to the planting of Eucalyptus plantations incompletely burned litter, which was present at the deeper soil depths due to tilling, resulting in higher SOM levels in Eucalyptus plantations than in Masson pine forests. The SOM content of G2 and G3 was significantly lower than that of G1 at 0-20 and 20-40 cm soil depths (p<0.05) (Fig. 3a). The transfer

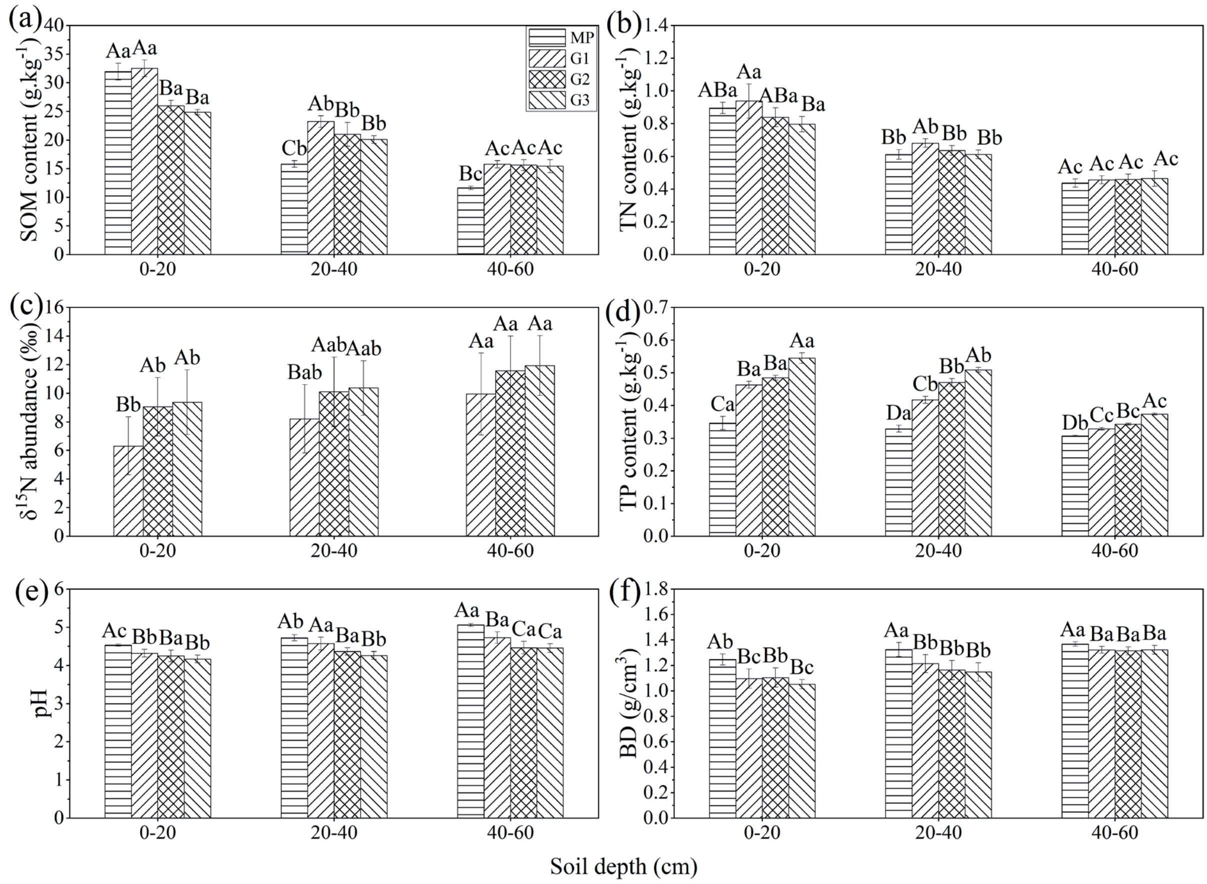

Fig. 3. Soil total nutrients, pH, and BD in the Masson pine forest and the successive Eucalyptus plantations. MP, G1, G2, and G3 refer to the Masson pine plantation and the first-, second- and third-generation Eucalyptus plantations, respectively. SOM, soil organic matter; TN, total nitrogen; $\delta^{15}N$, stable isotope ^{15}N; TP, total phosphorus; pH, soil degree of acid or alkali; BD, soil bulk density. Different uppercase and lowercase letters denote significant differences at $p<0.05$ across different generations and soil depths for soil physicochemical properties, respectively.

of above-ground biomass during the felling of mature Eucalyptus plantations is an important reason for the significant decline in SOM content across generations. Insufficient nutrient levels from surface fertilizers and litter decomposition to overcome the depletion of soil nutrients by evapotranspiration can also contribute to the decline of SOM content [1, 14]. Augusto et al. [43] showed that SOM reserves change significantly after land-use change, and SOM content decreased with the extension of Eucalyptus cultivation time. The TN content in G3 (0.80 $g\cdot kg^{-1}$) was 14.9% significantly lower than G1 (0.94 $g\cdot kg^{-1}$) at 0-20 cm soil depth, and the percentages of TN content was 5.9% and 10.3% significantly lower in G2 (0.64 $g\cdot kg^{-1}$) and G3 (0.61 $g\cdot kg^{-1}$) than in G1 (0.68 $g\cdot kg^{-1}$) at 20-40 cm soil depth, respectively ($p<0.05$) (Fig. 3b). This may be due to the reduction in SOM content due to the reduction in plant litter levels in the Eucalyptus plantation (Table 1), which leads to the reduction of organic nitrogen levels in the SOM, and finally, the reduction of TN levels. The correlation analysis in the present study showed that SOM content and TN levels were significantly positively correlated ($p<0.05$) (Fig. 4). Bargali et al. [38] showed that TN, TP, and TK levels decreased with increasing planting age, thus, affected the soil chemical properties of Eucalyptus reforestation plantations. Dan et al. [44] showed that, under an intensive management system of Eucalyptus in Hawai, nitrogen fertilizer was overconsumed and not supplemented in a timely manner, and the TN level declined. However, in the present study, the TN level was lower in MP than in G1 at 20-40 cm soil depth ($p<0.05$) (Fig. 3b). This may be because the Masson pine forest clear-cutting and residue burning caused a significant increase in the TN content of G1, owing to the transformation of a large amount of aboveground organic matter into underground inorganic matter (inorganic nitrogen). $\delta^{15}N$ abundance of G2 (9.05-10.10 ‰) and G3 (9.37-10.36 ‰) at 0-20 and 20-40 cm soil depths was 23.0-43.2% and 26.2-48.3% significantly higher than G1 (6.32-8.21‰), respectively ($p<0.05$) (Fig. 3c). Ectomycorrhiza-secreting isotopically enriched compounds transfer isotopically light N to plants, causing $\delta^{15}N$ enrichment [30,31]. The aboveground biomass was transferred during logging, resulting in the loss of light N from available nutrients and the retention of heavy N-$\delta^{15}N$

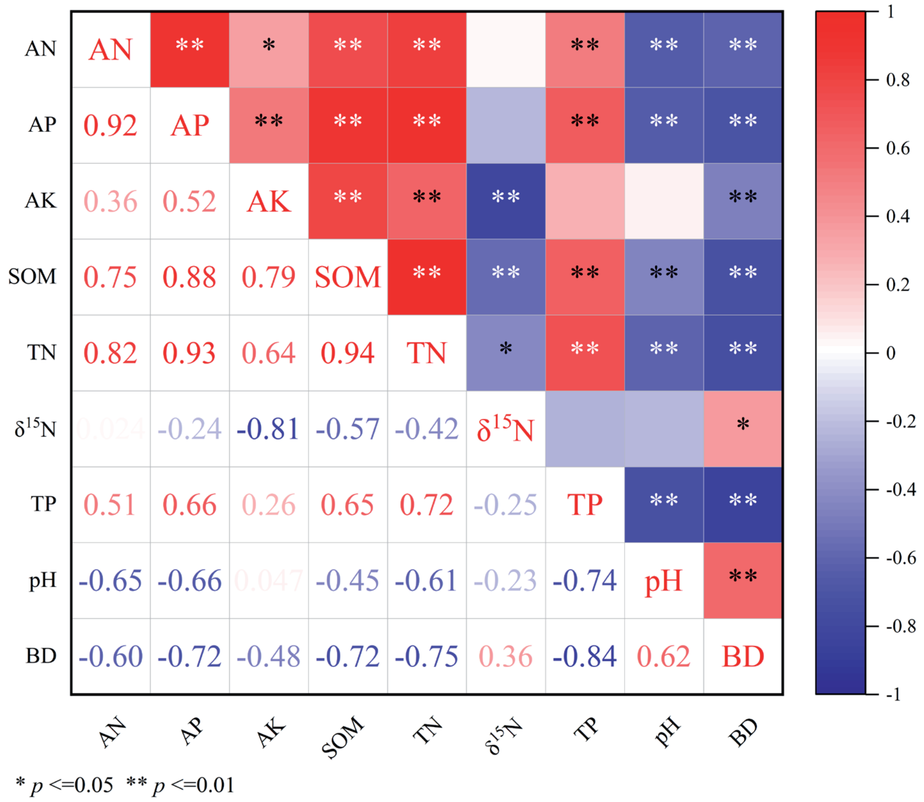

Fig. 4. The correlation heatmap reflects the correlation among soil physiochemical properties with different letters indicating significantly different properties. AN, alkaline hydrolytic nitrogen; AP, available phosphorus; AK, available potassium. SOM, soil organic matter; TN, total nitrogen; $\delta^{15}N$, stable isotope nitrogen 15; TP, total phosphorus; pH, soil degree of acid or alkali; BD, soil bulk density. The positive and negative correlations between them are represented by red and blue rectangles, respectively, and the asterisk indicates significant correlation: * $p<0.05$, * * $p<0.01$.

after converting Masson pine forest to Eucalyptus plantations. Therefore, it is important to consider the increase in $\delta^{15}N$ abundance. Plant absorption of ^{15}N-depleted inorganic nitrogen derived from SOM mineralization (N isotope fractionation) causes $\delta^{15}N$ enrichment [30,31]. Therefore, it was considered that the abundance of $\delta^{15}N$, like SOM content, was largely affected by the branch and leaf litter of Eucalyptus plantations, and the coupling relationship between them supported the increase in $\delta^{15}N$ abundance with a decrease in SOM and TN contents (Fig. 3a-c) [45]. The correlation analysis of the present study showed that $\delta^{15}N$ was significantly and negatively correlated ($p<0.05$) with TN and SOM contents (Fig. 4). Consequently, $\delta^{15}N$ was identified as an important indicator for evaluating the degree of soil nitrogen mineralization, and $\delta^{15}N$ abundance indicated that G2 and G3 were significantly more mineralized than G1 at 0-20 and 20-40 cm soil depths ($p<0.05$) (Fig. 3c). Boone et al. [12] showed that partially decomposed plant residues represent the light part (^{14}N) of SOM, whereas mineralization-related degradation products represent the heavy part (^{15}N) of SOM. The light component of SOM is generally considered to be more unstable. Thus, $\delta^{15}N$ abundance increases because of microbial isotope fractionation when nitrogen and other nutrients are converted from plant residues to the heavy part (^{15}N) through the light component (^{14}N) in the SOM during SOM development and formation [46]. Ngaba et al. [47] also showed that Eucalyptus intensified the mineralization of SOM to meet the demand for inorganic nutrients after the conversion of natural forests to plantation forests in eastern China. Finally, Pamela et al. [48] showed that the $\delta^{15}N$ value of SOM was significantly and positively correlated with mineralization and nitrification rates [49]. These findings suggest that $\delta^{15}N$ can be used to evaluate the degree of soil mineralization. In this study, the TP level increased significantly after converting Masson pine forest to Eucalyptus plantations, and the TP level of Eucalyptus plantations increased with planting generations ($p<0.05$) (Fig. 3d). The TP level increased because of the low mobility of phosphorus and fertilization in Eucalyptus plantations [9]. Part of the aboveground phosphorus biomass was added to the soil after the burning of the Masson pine forests, which also increased the TP level. The results indicated that the pH decreased significantly after converting Masson pine forest to Eucalyptus plantations, and the pH of the Eucalyptus plantations decreased with planting generations. The pH of the Eucalyptus plantations was significantly lower than that of MP at 0-20 and 40-60 cm soil depths ($p<0.05$). The pH values of G2 and G3 were significantly lower than those of G1 at 20-40 and 40-60 cm soil depths ($p<0.05$) (Fig. 3e). This decrease may have been caused by the excessive use of chemical fertilizers in successive multi-generational Eucalyptus plantations. Physiological acidic fertilizers such as potassium chloride and ammonium sulfate, aggravate soil acidification. The secretion of organic acids by soil microorganisms and Eucalyptus roots also lowers pH [14, 38]. In the present study, this phenomenon was observed in BD, where it was significantly lower in Eucalyptus plantations (1.05-1.32 g/cm^3) than in MP (1.25-1.37 g/cm^3) across the three soil depths ($p<0.05$) (Fig. 3f). The decrease in BD observed in this study may be due to soil loosening caused by fertilization and reclamation. Eucalyptus usually produces litter with low nutrient concentrations, and litter decomposition is slow in the early stages; therefore, litter accumulation is also the reason for the reduction in BD [50]. However, Navarrete et al. [51] found that conversion from forests to pastures in the Colombian Amazon resulted in an increase in BD. In addition, the characteristics of soil acidification in Eucalyptus plantations may affect the microbial activity of decomposed SOM, eventually resulting in an increased in SOM content but a decreased in BD. The correlation analysis in this study showed that pH and BD were significantly positively correlated ($p<0.05$), whereas the pH and BD were significantly negatively correlated with SOM content ($p<0.05$) (Fig. 4). Finally, Yihenew et al. [52] showed that the transformation of land use systems from natural forests to other systems (cultivated or grassland) had a negative impact on the physicochemical properties of soil. Therefore, the monitored soil physicochemical properties were especially important in the 1-3 generations of Eucalyptus plantations after converting Masson pine forest into Eucalyptus to supplement soil fertility over time. However, the PCA results showed that, unlike the 0-20 and 40-60 cm soil depths, there was no significant difference between the soil physicochemical properties of MP, G2, and G3 at 20-40 cm soil depth ($p>0.05$) (Fig. 1). The soil physicochemical properties were significantly different at 40-60 cm soil depth, but not at 20-40 cm soil depth, and there were presumably some factors that differed significantly at 40-60 cm soil depth, causing significant differences in soil physicochemical properties. This impact factor may be pH. The pH difference between MP, G2, and G3 was greater at 40-60 cm soil depth than at 20-40 cm soil depth (Fig. 3e). The distribution of roots in Masson pine forest and Eucalyptus plantations was lower at the 20-40 cm soil depth, and more roots secreted more organic acids at 40-60 cm soil depth [14, 36, 38, 47], making the pH difference between MP, and G2 and G3, greater at 40-60 cm soil depth than at 20-40 cm soil depth.

The results showed that SOM, TN, and TP contents decreased with increasing soil depth. The SOM and TN contents all showed as 0-20 cm>20-40 cm>40-60 cm in MP, G1, G2, and G3 ($p<0.05$) (Fig. 2a-b). The TP content was 0-20 cm>20-40 cm>40-60 cm in G1, G2, and G3 ($p<0.05$) (Fig. 2d). This is because plant litter is an important source of SOM, TN, and TP contents, and plant litter from Masson pine and Eucalyptus is mainly distributed in the soil surface layer; therefore, SOM, TN, and TP contents decreased with an increasing soil depth [16, 35]. Surface fertilization of Eucalyptus plantations was also an essential factor for the significant decrease in TN and TP contents with increasing soil depth. Nutrients from the decomposition of plant litter were mainly retained above a 0-20 cm soil depth, and nutrients absorbed mainly by Masson pine and Eucalyptus through the deeper root system were also factors considered for TN and TP levels reduction [25, 28, 35]. In present study, in all Eucalyptus generations, $\delta^{15}N$ abundance was significantly enriched at 40-60 cm soil depth compared to 0-20 cm soil depth ($p<0.05$) (Fig. 2c). Pamela et al. [48] showed an increase in $\delta^{15}N$ abundance from the soil litter surface layer to the organic soil layer and deep soil layer, which is similar to the results of the present study. Because the root systems of mature Masson pine forests and Eucalyptus plantations were relatively less distributed at 20-40 cm soil depth, the deeper root systems absorbed light N and transferred it to the plant, whereas the remaining heavy N at 40-60 cm resulted in increased $\delta^{15}N$ abundance under the effect of isotope-enriched compounds secreted by ectomycorrhizal mycorrhizae [36, 47]. Mineralization of SOM increase $\delta^{15}N$ abundance, which eventually leads to the deposition of $\delta^{15}N$ in deeper soils via rainfall leaching and gravity [45, 49]. It is worth noting that fertilization also affected the $\delta^{15}N$ abundance increase in the present study [49]. The results showed that the pH increased significantly with increasing soil depth. The pH values in the MP were all showed as 0-20 cm<20-40 cm<40-60 cm ($p<0.05$). The pH value at 20-40 and 40-60 cm soil depths were significantly higher than 0-20 cm soil depth in G1 ($p<0.05$). In G3, the pH value at 0-20 and 20-40 cm soil depths was significantly lower than that at the 40-60 cm soil depth ($p<0.05$) (Fig. 2e). This phenomenon may be due to surface soil acidification caused by chemical fertilizer application in the Eucalyptus plantations, whereas deeper soils are less disturbed with a higher pH [6, 7]. The secretion of acidic substances by soil microorganisms to degrade organic matter on the soil surface is also responsible for the low pH of surface soil [14, 38]. The results showed that the BD increased significantly with increasing soil depth. In the MP, the BD of the 0-20 cm soil depth was significantly lower than that of the 20-40 and 40-60 cm soil depths ($p<0.05$). BD showed as 0-20 cm<20-40 cm <40-60 cm in G1 and G3 ($p<0.05$). Finally, the 40-60 cm soil depth BD was significantly higher than the 0–20 and 20-40 cm soil depths BD in G2 ($p<0.05$) (Fig. 2f). This was because the upper layer of soil was affected by human factors, such as fertilization and weeding; therefore, the BD was lower, whereas the lower layer of soil experienced less disturbance and was more stable, thus, the BD was higher. Therefore, the BD increased with increasing soil depth. In addition, in our study, SOM was mainly distributed on the soil surface, and the SOM content was lower in the upper soil layer than in the lower soil layer [25, 26]. The BD decreased as SOM content increased. The correlation analysis of this study showed that BD and SOM content were significantly negatively correlated ($p<0.05$) (Fig. 3).

Conclusions

Findings from our study suggest that, the physicochemical properties of MP were significantly different from those of G2 and G3 at 0-20 and 40-60 cm soil depths, respectively. AP and AK levels decreased significantly in G2 and G3 compared to those in MP at 0-20 cm soil depth. SOM content, TP level, pH, and BD were significantly different between MP, G2, and G3 at 0-20 and 40-60 cm soil depths. Soil mineralization ($\delta^{15}N$) was enhanced after converting Masson pine forest to Eucalyptus plantations. Although TP content and $\delta^{15}N$ abundance increased with planting generations, the available nutrients, SOM content, TN level, pH and BD generally decreased with planting generations. In summary, the physicochemical properties of the first generation were the best, those of the third generation were the worst, and those of the second generation were moderate. Our research results emphasize that long-term successive planting of Eucalyptus leads to a decline in soil physicochemical properties in forest areas. We should pay attention to the health of soil during the long-term operation of Eucalyptus plantations and to improve management measures. This study provides a theoretical reference for the sustainable management of Masson pine forest conversion into multi-generation Eucalyptus plantations.

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Conflict of Interest

The authors declare no conflict of interest.

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