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Planting density affects soil quality in the deep soils of pine plantations

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ABSTRACT

Planting density is one of the important factors affecting soil properties, thereby directly impacting plantation yields. However, the effect of planting density on soil quality and productivity in plantation ecosystem mostly remain unknown. The present study aims to assess soil quality in four mature pine (*Pinus kesiya*) plantations with increasing planting densities i.e., 700 trees ha^{-1} (low planting density, LPD), 1080 trees ha^{-1} (moderate planting density, MPD), 1260 trees ha^{-1} (high planting density, HPD), and 2340 trees ha^{-1} (very high planting density, VHPD) respectively, up to 1 m soil depth. The soil samples collected at 0–20, 20–60, and 60–100 cm depth intervals were analyzed for a total of thirty-nine physical, chemical, and biological properties. The soil quality index (SQI) was calculated using linear scoring and weighted additive method. Total carbon, arbuscular mycorrhizal fungi (AMF) biomass, gram-positive bacteria biomass, soil moisture content, and β-glucosidase enzyme were selected as the important drivers of SQI. Total carbon, AMF, and moisture content had higher contribution to SQI. The results showed that increasing planting density adversely affected soil quality. SQI was higher in the surface (0–20 cm) soil layer, and it decreased with depth. Planting densities, soil depths, and their interaction had a significant effect on SQI. Increasing SQI promoted the stem biomass with a positive linear response. Therefore, maintaining a moderate to low planting density by thinning practices and selective logging could recover natural understory vegetation, acquire high timber yield, and sustain soil quality in pine plantations.

1. Introduction

Forest plantations are established to provide multiple ecosystem services, including timber, carbon sequestration, nutrient cycling, climate change mitigation, soil conservation and restoration of degraded land (Baral et al., 2016). Plantations grow much faster than the natural forests, and thus serve as an important source of timber supply (Zhang and Stanturf, 2008). Increasing demand for timber products led to the conversion of natural forest to monoculture plantations and to a subsequent increase in planting density in order to produce more wood (FAO, 2020). High planting density may yield a quick profit but it affects timber quality, vegetation characteristics, and soil quality (Brockerhoff et al., 2008). In contrast, low planting density leads to under-utilization of land. Thus, management of planting density in the forest plantations is a critical global issue in sustainable forestry (Boyle and Powers, 2013; Malkamaki et al., 2018).

Pinus is the second widespread and fast-growing conifer, cultivated globally for its timber and resin, and often considered a model for growers of other plantation species (Mead, 2013). In general, planting density determines light availability, understory vegetation diversity and regeneration, quantity and quality of litter, and soil quality in forest plantations. Soil properties are strongly associated with changes in planting density, and even minor changes in vegetation influence the soil functions (Schloter et al., 2018). Planting density affects the important soil properties such as moisture, pH, carbon content, enzymatic activities, and microbial community composition. For example, Zhao et al. (2012) showed that *Pinus massoniana* plantations with a planting density of 1500 trees ha^{-1} had higher contents of soil organic matter and macronutrients. Nan et al. (2020) identified that higher planting densities caused soil moisture deficit in *Pinus sylvestris* var. *mongolica* plantation. In contrast, Wang et al. (2021a) showed Chinese fir plantations with a higher planting density of 6667 trees ha^{-1} increased soil carbon fractions. Soil biological properties determine soil biochemical and biophysical transformations and aboveground

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vegetation characteristics due to their rapid responsiveness to changes in soil quality (Van der Heijden et al., 2008; Overby et al., 2015; Díaz Villa et al., 2022). Previous studies have reported that stand density affects soil microbial community composition, enzyme activities, and growth productivity in coniferous plantations (Zhao et al., 2012; Ali et al., 2019; Wang et al., 2021b). Given the importance of soil microbes, biological properties should be considered as significant integrals to the soil–plant system for better plantation management.

Changes in soil properties vary based on environments and management practices, thus making their selection challenging and complex in soil quality assessment (Griffiths et al., 2010). Also, soil quality cannot be evaluated using specific soil indicators as soil properties are interlinked (Burger and Kelting, 1999). Therefore, a minimum data set (MDS) is used to identify representative and measurable soil indicators to assess soil quality (Andrews and Carroll, 2001). Studies on soil quality assessment using SQI in the plantation ecosystems such as rubber, Chinese fir, *Camelia* sp., and *Larix* sp. are increasing on a national scale (Liu et al., 2017; Zhijun et al., 2018; Qiu et al., 2019; Hemati et al., 2020). Most of the previous soil quality assessments in the plantations have focused only on physical (bulk density, porosity, water holding capacity) and chemical properties (soil carbon, total and available form of soil macronutrients) because of low measurement costs and simple analyzing techniques (Tu et al., 2019; Zou et al., 2021). Nevertheless, soil biological indicators are still discounted and were missing from the MDS of 40 % of the published studies on soil quality assessment (Lal, 2016; Bunemann et al., 2018). Soil quality assessment on pine plantation soils, using a multivariate ordination approach, is limited so far. Previous studies have also suggested a management approach to improve soil fertility but failed to estimate MDS and the relationship between productivity and SQI in the pine plantations (Noh et al., 2013; Overby et al., 2015; Martín-Peinado et al., 2016; Zhao et al., 2021). Therefore, inclusion of novel, sensitive, and relevant soil biological properties is important for the assessment of SQI in sustainable plantation forests and soil management.

The changes in soil properties observed within the soil profile might influence soil quality (Eilers et al., 2012; Gonzaga et al., 2016; Vasu et al., 2020). Previous studies on soil quality mainly focused on the surface soil only (≤30 cm) (Raiesi and Salek-Gilani, 2020; Gruss et al., 2021; Zou et al., 2021) as major biological activities occur within the surface soil and are easily accessible. However, such soil sampling has not promoted an understanding of the dynamics of whole soil profiles and estimates of soil quality. To our knowledge, this is the first study to assess soil quality along a soil profile (1 m) and to identify the relationship between SQI and stem biomass of pine plantations.

The specific objectives were (i) to select potential soil quality indicators as an MDS by principal component analysis, (ii) to determine soil quality under different planting densities and soil depths (up to 1 m) using SQI, and (iii) to evaluate the relationship between SQI and stem biomass. We hypothesized that (i) low planting density has enhanced soil properties and soil quality than very high planting density of pine trees, (ii) planting density and soil depth interactions significantly affect soil quality index, and (iii) stem biomass increases with SQI.

2. Materials and methods

2.1. Study area

The study was conducted in the Ailaoshan mountains (24°32′N, 101°01′E), situated in the central Yunnan province, southwest China (Fig. 1). Ailaoshan mountain has the largest evergreen broad-leaved reserve forest in China, with an area of 677 km^2 (Zhu and Yan, 2009). The forest has various land-use types (tea, walnut, tobacco) including pine plantations. The altitude varies from 1500 to 2460 m above mean sea level. The mean annual temperature is 11.8 °C with a maximum (15.3 °C) in July and a minimum (5.3 °C) in January. The mean annual precipitation is 1799 mm, with the most precipitation occurring from May to October (Qi et al., 2021). The soil texture is loamy, and the soil type is Alfisol, classified based on USDA Soil Taxonomy (Soil Survey Staff, 2014).

2.2. Site selection

The plantations in Ailaoshan were established in 1980 by converting natural broadleaf forests to different densities of *P. kesiya* seedlings for

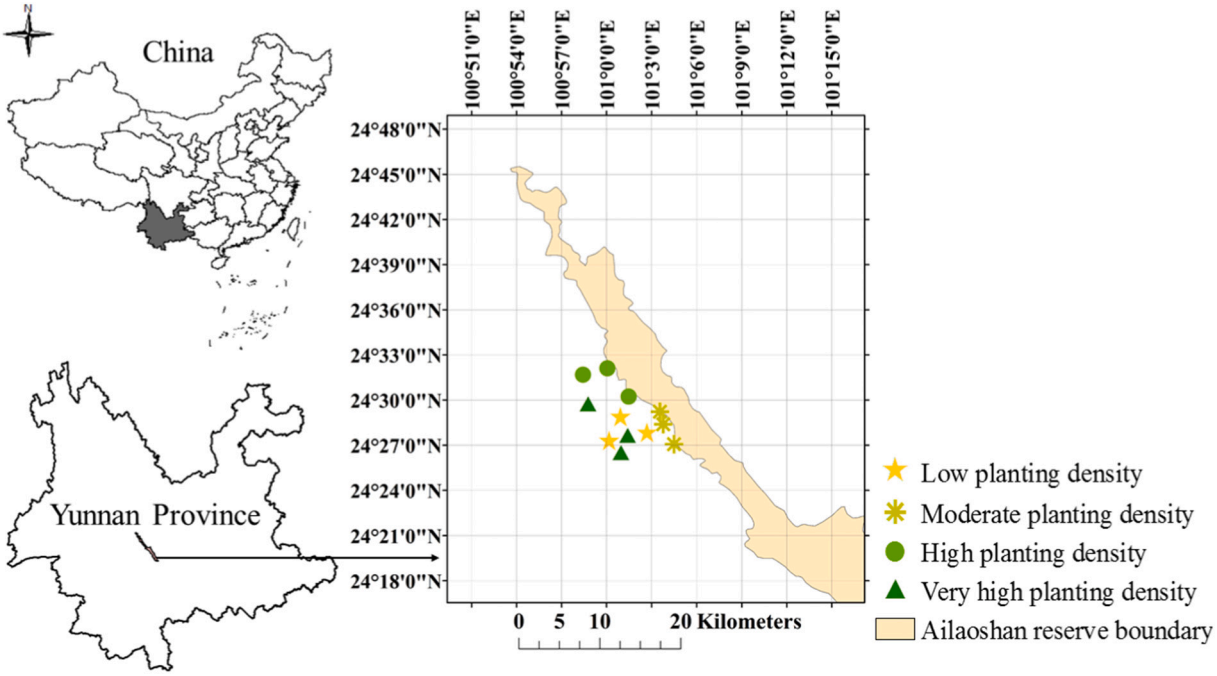

Fig. 1. Locations of the study sites. Low planting density (700 trees ha^{-1}), moderate planting density (1080 trees ha^{-1}), high planting density (1260 trees ha^{-1}), Very high planting density (2340 trees ha^{-1}).

Table 1
Site, soil, and stand characteristics of different *P. kesiya* planting densities in Ailaoshan.

Site and stand characteristics	Low planting density (LPD)	Moderate planting density (MPD)	High planting density (HPD)	Very high planting density (VHPD)
Mean elevation (m)	2169	2012	1917	1979
Slope (°)	45 ± 0.6	42 ± 0.5	45 ± 0.5	40 ± 1.2
Aspect	East-West	East-West	East-West	East-West
Soil type	Alfisol	Alfisol	Alfisol	Alfisol
Stand age (years)	~35	~40	~40	~40
Pine canopy cover (%)	10 ± 2^{a}	47 ± 4^{b}	75 ± 7^{c}	97 ± 2^{d}
Pine diameter at breast height (cm)	18.9 ± 3.7^{a}	16.9 ± 4.1^{ab}	15.8 ± 2.9^{b}	13.5 ± 5.4^{b}
Pine tree height (m)	9.2 ± 0.5^{a}	9.8 ± 1.0^{a}	9.7 ± 2.7^{a}	9.2 ± 2.5^{a}
Pine tree stem biomass (kg ha^{-1})	7477 ± 140^{a}	4152 ± 33^{b}	2807 ± 145^{c}	2081 ± 34^{c}
Planting density (trees ha^{-1})	700 ± 10^{a}	1080 ± 28^{b}	1260 ± 25^{c}	2340 ± 38^{d}
Understory vegetation cover (%)	90.4 ± 3.45^{a}	47.6 ± 1.16^{b}	11.8 ± 0.16^{c}	0.06 ± 0.18^{d}
Understory plant biomass (kg ha^{-1})	1328 ± 213^{a}	856 ± 59^{b}	330 ± 12^{c}	64 ± 34^{d}
Root biomass (kg ha^{-1})	5294 ± 271^{a}	4642 ± 134^{b}	2554 ± 117^{c}	1021 ± 45^{d}

timber production. During this study, over 85 % of the pine plantations were cultivated with various ages and planting densities. Four planting densities viz., low planting density (LPD, 700 trees ha^{-1} with 3.80 × 3.80 m spacing), moderate planting density (MPD, 1080 trees ha^{-1} with 3.05 × 3.05 m spacing), high planting density (HPD, 1260 trees ha^{-1} with 2.04 × 2.04 m spacing), and very high planting density (VHPD, 2340 trees ha^{-1} with 1.83 × 1.83 m spacing) were selected in January 2020. We established twelve plots (20 m × 30 m) with similar topographical features and soil types. The study was conducted with three replications for each planting density. The distance between any two replicates of the same planting density was maintained within 2 km to avoid site variation.

2.3. Measurement of stand characteristics

Plantation characteristics (planting density, tree height, diameter at breast height of pine trees, overstory pine cover, and understory vegetation cover) were measured in each plot. The height and diameter at breast height (>5 cm) of all pine trees were measured to calculate the stem biomass using allometric equations (Miksys et al., 2007) The overstory pine cover was measured using a spherical densiometer. Understory vegetation cover was calculated according to Wang and Wang (2019). The plant biomass of understory vegetation was determined by harvesting aboveground plants at ground level, oven-dried at 65 °C and then weighed. In each plot, three soil cores with an inner diameter of 5 cm were used to collect soil from 0–20, 20–60, to 60–100 cm depths to measure root biomass. The root biomass was measured after rinsing under running water in a 1-mm screen nylon bag and oven-dried at 80 °C to a constant weight. Dominant understory species in the low and moderate planting densities include *Vaccinium duclouxii*, *Lyonia ovalifolia*, *Stewartia pteropetiolata*, *Ilex corallina*, *Eriobotrya bengalensis*, *Lithocarpus xylocarpus*, *Castanopsis rufescens*, *Ternstroemia gymnanthera*, and *Rhododendron leptothrium* in LPD and MPD. The natural understory vegetation (shrubs and herbs) is cleared manually in HPD and VHPD. Thinning and pruning activities were done at the mid stand age of ~15–20 years. The experimental sites had no external fertilizer inputs. The site characteristics and detailed descriptions of sampling sites in the pine plantations are given in Table 1.

2.4. Soil sampling

After removing the litter horizons, soil samples were collected in three replicates at 0–20, 20–60, and 60–100 cm depths, respectively. For this, three soil pits, each approximately 90-cm wide and 120-cm deep were excavated in a diagonal direction in each plot and three soil cores from each depth were collected by using a core sampler (5 cm depth and 5 cm diameter). Soil samples obtained at the same soil depth were mixed and homogenized to a single composite sample. In addition, three soil cores from each depth were collected using metal cores (100 cm^3) to determine soil bulk density and moisture content. Thus, a total of 108 soil samples (four planting densities × three replicate stands × three plots × three soil depths) were collected. Coarse fragments such as stones, rocks, and roots measuring over 2 mm in size were removed manually, and the soil volumes were measured.

2.5. Soil processing and analysis

Field-moist soils were dried at room temperature and sieved through a 2-mm mesh to remove roots and gravel. Each soil sample was divided into three parts: (1) air-dried samples to measure soil physical and chemical properties, (2) fresh soil stored at −20 °C for microbial community analysis, and (3) fresh soil stored at 4 °C to determine microbial biomass carbon (MBC) and enzyme activities.

2.5.1. Analysis of soil physical–chemical properties

Soil bulk density (BD) was determined by the soil core method (Blake and Hartge, 1986). Soil moisture content (MC) was determined by overnight drying at 105 °C (Gardner, 1986). Soil pH was measured in a soil:water suspension (1:2.5) with a digital pH meter (Mettler-Toledo GmbH, Switzerland). Electrical conductivity (EC) was measured from the soil extract (1:5 soil–water ratio) using an electrical conductivity meter (Shanghai Precision Scientific Instrument Company, Shanghai). Soil total carbon (TC) and total nitrogen (TN) concentrations were determined using an elemental analyzer (VarioMAX CN, Elementar Analysensysteme, Germany). Total phosphorus (TP) was analyzed by wet digestion coupled with a spectrophotometric method (Plasma atomic emission spectrometer, IRIS ADVANTAGE iCAP7000, USA). Soil C:N, C:P, and N:P ratios were expressed as mass ratios.

Soil organic carbon (SOC) was determined by acid dichromate wet oxidation (Nelson and Sommers, 1982). Microbial biomass carbon (MBC) was measured by a fumigation-extraction method (Vance et al., 1987). Dissolved organic carbon (DOC) was estimated by adding 0.5 M K_2SO_4 (1:5 m/v) to the soil sample, and determined using a TOC-2000 analyzer (Shimadzu, Japan; McGill et al., 1986). Particulate organic carbon (POC) and mineral-associated organic carbon (MOC) were determined using a modified approach adapted from the study by Cambardella and Elliott (1992). Briefly, 30 ml $(NaPO_3)_6$ solution (5 g L^{-1}) was added to 10 g soil sample and mixed for 18 h at 100 rpm. Soil suspension was sieved using a 53 μm sieve. The fractions recovered on the sieve (POC; >53 μm) and recovered from rinsing with deionized water (MOC; <53 μm) were oven-dried at 60 °C for 48 h. The carbon fraction stocks in the bulk soil were estimated using an equivalent soil mass correction (Ellert and Bettany, 1995). Potassium permanganate oxidizable carbon (POXC), a labile form of soil carbon fraction, was determined by oxidation with 330 mM of $KMnO_4$ solution (Blair et al., 1995). Soil recalcitrance carbon (RC) was measured by acid hydrolysis by refluxing 2 g of soil in 6 M HCl for 18 h. The refluxed samples were washed three times with deionized water, dried at 55 °C and sieved through a 0.25-mmmesh, and determined using $K_2Cr_2O_7$ oxidation method (Nelson and Sommers, 1982).

2.5.2. Analysis of soil biological properties

Phospholipid fatty acid (PLFA) analysis was used to determine microbial community composition using methyl nonadecanoate (19:0) as an internal standard (Frostegard and Baath, 1996). The total microbial concentration was determined according to Fichtner et al. (2014). PLFA biomarkers used to determine functional microbial groups are listed in Table S1. A total of 114 PLFAs were extracted, and the total of all microbial concentrations was considered total PLFA. Actinomycetes (ACT), arbuscular mycorrhizal fungi (AMF), anaerobes (ANA), eukaryotes (EUK), gram-negative bacteria (GN), fungi (FUN), gram-positive bacteria (GP), and unspecific microbes (USM) were identified using their specific biomarkers (Veum et al., 2019). The concentration ratios of bacterial to fungal lipids (B:F) and GP:GN were also included. Relative abundance (hereafter referred to as 'abundance') was calculated by the number of individuals in each microbial community.

The activities of carbon (β-Glucosidase, BG; phenol oxidase, PPO; peroxidase, POD), nitrogen (*N*-acetyl glucosidase, NAG) and phosphorus (acid phosphatase, AP) degrading enzymes were measured. A 0.75 ml aliquot of a 5 mM standard solution pNP – β – glucopyranoside for BG assay, pNP– β–N acetylglucosaminide for NAG assay, pNP–phosphate for AP assay, respectively was dispensed into microplate wells (Allison and Vitousek, 2005). Soil samples were suspended in sodium acetate buffer (pH 5.0) (1:15 soil: buffer), and 0.75 ml of extract buffer was mixed with 0.75 ml of substrate buffer (5 mM) and dispensed into 96-well microplates. The wells consist of three replicates of the substrate control (substrate buffer and sodium acetate buffer), sample control (sample and sodium acetate buffer), and sample (sample and substrate buffer). The prepared plates were incubated in the dark at 20 °C for up to 4 h following substrate addition with constant shaking (LRH-250-GSI, Zhujiang, China). The activities of PPO and POD were measured spectrophotometrically (450 nm) after a 2 h incubation using L-3, 4 – dihydroxyphenylalanine as the substrate (Freeman et al., 1995). The reaction was stopped by adding 75 μl aliquot of 1 M NaOH to each well.

2.6. Statistical analysis

2.6.1. Univariate analysis

Normality (Kolmogorov-Smirnov) and homoscedasticity (Levene's test) were tested for soil properties among planting densities. Variables without normal distribution and equal variance were subjected to logarithmic transformation to obtain normal distribution and stabilize the variances. We selected thirty-nine soil properties and estimated the significant difference between the mean values of each of the soil properties corresponding to the respective planting densities by one-way analysis of variance (ANOVA) and the least significant difference using OriginPro 2020b software (Origin Lab Corporation, Massachusetts, USA). Two-way ANOVA was performed to test the effect of significant interactions between the planting densities and soil depths on soil properties using SPSS 19.0 software (SPSS Inc., Chicago, IL, USA). Only significant soil variables were included in the total dataset (Gong et al., 2015; Qiu et al., 2019). The linear relationship between stem biomass and SQI values was determined by linear regression.

2.6.2. Soil quality index assessment

Principal component analysis (PCA) is a data reduction technique used to select key soil indicators of the MDS from the total dataset. The principal components (PCs) explain combinations of variables having maximum variance in a dataset. A varimax rotation was performed on selected PCs in order to enhance interpretability of the uncorrelated components (Askari and Holden, 2014). For each PC, variables with eigenvalues ≥1 were chosen, and indicators with an absolute loading value within 10 % of the highest loading factor were retained (Andrews et al., 2002). When more than one indicator was retained under a single factor, Spearman correlation analysis was used to determine the correlation between the variables, and the well-correlated variables were considered redundant ($r > 0.70$). From the correlated variables, only one variable with the highest loading factor within each PC was considered for inclusion in the MDS (Andrews et al., 2002). If the highly weighted variables were not correlated ($r < 0.70$), each was considered important and selected in the MDS (Andrews and Carroll, 2001). Communality describes the proportion of variance in each of the soil properties explained. Larger communality values represent the higher proportion of indicator's variance (Brejda et al., 2000).

After determining the variables of MDS, each soil indicator with different units was transformed and normalized into a unitless score ranging between 0 and 1 using a linear scoring function (Askari and Holden, 2014). The indicator scores of selected soil indicators were ranked as 'more is better', 'less is better', and 'optimum' approaches based on the contribution of each indicator to soil quality (Table S2). The 'optimum' function was applied to MC as its optimum value has negligible impact on soil function (Oladele, 2019). 'More is better' was assigned to soil quality indicators such as total carbon, biomass of AMF and GP, and BG based on their role in soil fertility (Monkai et al., 2018). For linear scoring, "more is better" (Eq. (1)) and "less is better" (Eq. (2)) functions were used:

$$S = \frac{x}{x_{max}} \tag{1}$$

$$S = \frac{x_{min}}{x} \tag{2}$$

where, S is the linear score of the soil indicators, x is the soil indicator's value, and x_{max} and x_{min} are the maximum and minimum values of each soil indicator observed under the four planting densities (Askari and Holden, 2014; Raiesi, 2017). Weightage for MDS indicators was calculated by percentage variation of each PC divided by the total percentage of variation explained by all PCs with eigenvectors >1.

The mean SQI for each soil layer was calculated using the Integrated Quality Index equation after the selected indicators were scored and weighted (Doran and Parkin, 1996).

$$SQI = \sum_{1}^{n} W_i S_i \tag{3}$$

where n is the number of soil indicators in the MDS, S_i is the indicator score, and W_i is the weight of the MDS indicator. Finally, the equation was normalized to yield a maximum SQI of 1. Higher SQI values represent better soil quality. The percentage contribution of each MDS indicator (scored and weighted) towards soil quality was calculated (Raiesi and Kabiri, 2016). SQI gains importance in the general assessment of soil quality, reflecting the role of planting density in maintaining soil function. The integrated value of SQI will be most relevant for evaluating management-associated effects for comparing planting density in different regions because of the differences in scoring curves based on inherent soil properties in the plantation ecosystem.

3. Results

3.1. Effect of planting densities and soil depth on soil properties

Two-way ANOVA results showed no significant effect of planting density, soil depth, and their interaction on GP:GN, bacteria:fungi, POD, and NAG (Table S3), and so these soil properties not included in the total dataset. The soil properties included in the total dataset were strongly

influenced by planting density ($P < 0.0001$; Table S3). The soil properties TC, TN, C:P, N:P ratios, DOC, MOC, and the biomass of all the microbial communities were found to significantly decrease in the order of LPD > MPD > HPD > VHPD (Table S4). Also, soil depth significantly affected the values of EC, TC, TN, C:N,C:P, N:P ratios, organic carbon fractions, biomass of all microbial communities, and the abundance of ACT, GP, GN, and USM (Table S3) and decreased with increasing soil depth (Table S4). The strong interaction between the planting density and soil depth had a significant effect on these properties MC, pH, EC, RC, POXC, the biomass of all microbial communities, and the abundance of ACT, FUN, GP, and GN (Table S3). Soil pH increased with increasing soil depth and planting density, whereas the biomass of all microbial communities decreased (Table S4).

3.2. Effect of planting densities on soil quality index

The PCA produced five PCs with eigenvalues >1 and explained 85.65 % of the variation in soil attributes (Table 2). The close placing of most of the soil properties in the same quadrant of the PCA variability plot indicates a strong and positive relationship between the soil physical, chemical, and biological properties (Fig. 2).

PC1 accounted for maximum variance (53.80 %), comprising pH, EC, TC, TN, C:N, C:P ratios, DOC, MOC, POXC, MBC, and RC with high factor loadings (Table 2). Soil pH was negatively loaded (−0.738), whereas other soil properties were positively loaded in PC1. However, properties in PC1 were highly correlated with each other (Table S5). Hence, only total carbon with the highest factor loading was selected in the MDS. PC2 accounted for 13.91 % of the total variance, and the microbial community biomass was considered for MDS due to the high positive loadings (0.761–0.829). Among them, AMF biomass had the maximum loading factor (0.829) and strongly correlated with other highly loaded soil properties (biomass of AMF, EUK, FUN, GN and Total PLFA; Table S5), therefore, only the AMF was retained in MDS. Four highly weighted variables namely moisture content, bulk density, the abundance of AMF and GN in PC3 explained 8.50 % of the variation (Table 2) and strongly correlated with each other (Table S5). Based on factor loading, only the moisture content was included in the MDS. PC4 and PC5 explained 6.07 and 3.32 % of the variation, respectively. In PC4, the concentrations of ANA and GP were the highly loaded properties, and they were highly correlated ($r > 0.85$; Table S5). Thus, GP biomass was selected for the MDS since it had the highest loading value (0.772). From PC5, BG was selected in the MDS based on a high loading factor (0.931). Finally, TC, AMF biomass, MC, GP biomass, and BG were selected as the MDS. The commonality values for most of the soil properties were found to be >0.80 (Table 2).

The weight for each PC was calculated by dividing the variance

Table 2
Rotated factor loading matrix, eigenvalue, variance explained, and commonality of principal component analysis at 1-m soil depth. Bold values indicate highly weighted loading factor. Bold and underlined values indicate soil quality indicators included in the minimum dataset.

Principal components	PC1	PC2	PC3	PC4	PC5	Commonality
Eigen value	18.833	4.870	2.978	2.125	1.174	
Variance %	53.089	13.914	8.509	6.072	3.354	
Cumulative variance %	91 %	67.723	76.232	82.305	85.659	
Weighted factor	0.628	0.162	0.099	0.070	0.039	
Factor loadings						
Moisture content	−0.096	0.209	**0.910**	0.036	0.02	0.899
Bulk density	0.128	−0.171	**−0.892**	−0.131	−0.023	0.895
pH	**−0.738**	−0.533	−0.031	−0.025	0.131	0.847
Electrical conductivity	**0.781**	0.477	0.072	0.021	0.045	0.845
Total carbon	**0.919**	0.29	0.06	0.121	0.087	0.954
Total nitrogen	**0.821**	0.439	0.091	0.132	0.099	0.902
Total phosphorus	0.473	0.046	−0.47	−0.342	−0.187	0.598
C:N ratio	**0.904**	0.043	−0.004	0.096	0.022	0.828
C:P ratio	**0.812**	0.212	0.25	0.299	0.164	0.884
N:P ratio	0.618	0.416	0.335	0.279	0.283	0.825
Dissolved organic carbon	**0.848**	0.289	0.164	0.118	−0.007	0.843
Mineral associated organic carbon	**0.888**	0.359	0.079	0.182	−0.034	0.959
Recalcitrant carbon	**0.865**	0.32	0.089	0.206	0.168	0.929
POXC	**0.879**	0.369	−0.039	0.221	−0.032	0.961
Microbial biomass carbon	**0.879**	0.184	0.088	0.089	0.041	0.823
PLFA concentration						
Actinomycetes	0.286	0.682	0.234	0.577	0.079	0.941
Arbuscular mycorrhizal fungi	0.420	**0.829**	0.169	0.220	0.078	0.947
Anaerobes	0.215	0.417	0.344	**0.760**	0.054	0.920
Eukaryotes	0.354	**0.761**	0.307	0.239	0.028	0.856
Fungi	0.547	**0.794**	0.132	0.057	0.058	0.953
Gram-negative bacteria	0.491	**0.783**	0.067	0.258	0.122	0.940
Gram-positive bacteria	0.296	0.482	0.110	**0.772**	0.106	0.939
Unspecific microbes	0.416	0.611	0.136	0.605	0.053	0.935
Total PLFA	0.477	**0.758**	0.155	0.397	0.077	0.990
β-glucosidase	0.046	0.039	−0.08	0.057	**0.931**	0.880
Polyphenol oxidase	0.063	0.011	0.192	0.06	**0.869**	0.800
Acid phosphatase	0.055	0.152	−0.292	−0.013	**0.871**	0.871
Relative abundance of microbes						
Actinomycetes	0.024	0.201	0.561	0.689	−0.162	0.856
Arbuscular mycorrhizal fungi	0.326	0.267	**0.713**	0.238	0.069	0.748
Anaerobes	0.454	0.07	0.335	0.587	0.168	0.695
Eukaryotes	0.44	0.148	0.563	0.293	−0.122	0.633
Gram-negative bacteria	0.247	−0.187	**0.858**	0.161	−0.12	0.872
Gram-positive bacteria	0.611	0.132	0.444	0.297	0.039	0.677
Unspecific microbes	0.632	0.172	0.51	0.218	−0.062	0.741
Fungi	0.466	0.29	0.665	0.098	−0.2	0.793

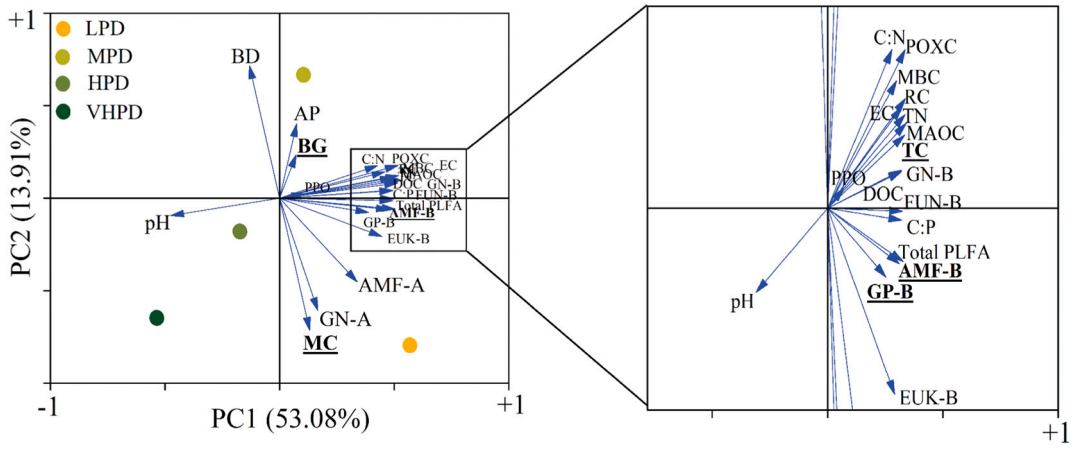

Fig. 2. The ordination biplot of the soil physical, chemical, and biological properties by principal component analysis. LPD, Low planting density; MPD, Moderate planting density; HPD, High planting density; VHPD, Very high planting density.

accounted for by each PC by the total variance. Thereby, TC had the maximum weightage (0.628) followed by AMF biomass (0.162), MC (0.099), GP biomass (0.070), and BG (0.039) had the lowest weight and the SQI was calculated using Eq. (4).

$$SQI = (0.628 \times TC) + (0.162 \times AMF) + (0.099 \times MC) + (0.070 \times GP) + (0.039 \times BG) \quad (4)$$

TC contributed the highest to the SQI followed by MC and AMF, and the lowest contribution was by BG at 1-m soil depth (Fig. 3a). The contribution of soil quality indicators namely TC, AMF, and GP decreased with soil depth (Fig. S1 a, b, d). Among the four planting densities examined, the SQI value ranged from 0.161 to 0.907. SQIs significantly decreased in the order of LPD > MPD > HPD > VHPD in all the soil depths (Fig. 3b). Increase in planting density have negative effect on SQI ($P < 0.001$) and reduced soil quality in all the depth intervals, thus showing a strong interaction between these two factors (Fig. 3b). A significant positive correlation was observed between the SQI and stem biomass with a linear response (Fig. 4). The regression Eq. (5) for this relationship is given below:

$$\text{Stem biomass } \left(\text{Kg ha}^{-1}\right) = 0.514\text{SQI} + 3.32; \text{R}^2 = 0.731; P < 0.001 \quad (5)$$

4. Discussion

4.1. Soil quality indicators

Soil quality assessment involves a comprehensive set of soil properties as indicators because any one or two properties cannot adequately represent overall changes in soil quality (Karlen et al., 2003). However, soil biological properties as soil quality indicators remained elusive in most of the earlier studies (Lal, 2016; Bunemann et al., 2018). This study filled that gap by including sensitive soil indicators such as soil carbon fractions, microbial community composition, and enzyme activities in the MDS.

The MDS selected in this study includes physical (moisture content), chemical (total carbon), and biological (arbuscular mycorrhizal fungal biomass, gram-positive bacterial biomass, β-glucosidase) properties as soil quality indicators. Few previous studies have advocated the use of soil organic carbon, microbial communities (fungal biomass, GP:GN), and soil enzymes (catalase) as soil quality indicators in different plantation ecosystems (Liu et al., 2017; Boafo et al., 2019; Qiu et al., 2019). In the present study, the majority of the soil variables have commonality values >0.85 (Table 2), indicating the significant contribution of each soil attribute to all the extracted principal components.

Total carbon has been widely used as a soil quality indicator because of its key role in carbon cycling and microbial processes in the soil (Noh et al., 2013). The TC was significantly increased with decreasing planting density (Table S4). The positive correlation between TC and TN (Table S5), showed that an acceleration of carbon sequestration by nitrogen deposition in forest soils (Lu et al., 2021). TC can be used to assess soil microbial composition, as microbial biomass is a direct measure of soil carbon (Singh and Gupta, 2018). Similar to our results, TC was identified as an important and sensitive soil quality indicator in other plantations (Delelegn et al., 2017; Hemati et al., 2020; Zhang et al., 2021).

Soil microorganisms are effective indicators due to their sensitivity and relative convenience in assessing changes in the soil environment (Hodge and Fitter, 2010; Delgado-Baquerizo et al., 2016). AMF determines ecological processes such as nutrient cycling and soil fertility by maintaining symbiotic relationships with plants and bacteria (Klironomos et al., 2000). Increasing planting density and canopy cover leads to decreasing understory vegetation and diminished inputs of organic matter from plant biomass, root exudation, and labile carbon inputs, which decrease microbial composition and carbon metabolism in HPD and VHPD (Murugan et al., 2014; Lange et al., 2015; Wang et al., 2021b). The positive and significant correlation of TC and TN with AMF and GP biomass (Table S5) indicates that changes in soil carbon and nitrogen concentrations influence the soil bacterial and fungal composition by providing a substrate source for their growth and activity (Murugan et al., 2014; Trentini et al., 2020).

Soil moisture controls microbial community structure and regulates the soil carbon transformation catalyzed by BG (Brockett et al., 2012). In this study, soil moisture decreases significantly with increasing pine planting densities and are consistent with previous studies (Yi et al., 2006; Andrews et al., 2020). Moist soils can hold more diverse microbial communities, but excessive MC limits oxygen diffusion and restricts the growth of GP bacteria and mycorrhizal fungi (Shukla et al., 2012). The significant effect of MC on TC may result from an increase of labile inputs to the soil, thus indirectly stimulating the growth of AMF and GP bacteria.

β-Glucosidase, a hydrolase enzyme produced by soil microorganisms, is involved in the depolymerization of cellulose to oligomers and monomers (Tomme et al., 1995). BG activity plays a significant role in carbon cycling (Luo et al., 2019). Contrary to the study reported by Wang et al. (2021a), BG activity in our study was found to be increasing with increasing planting density (Table S4). This could be because of the utilization of abundant energy substrates such as labile carbon fractions (DOC, MOC, and POXC) by soil microorganisms (Esen, 1993; Wang et al., 2021a).

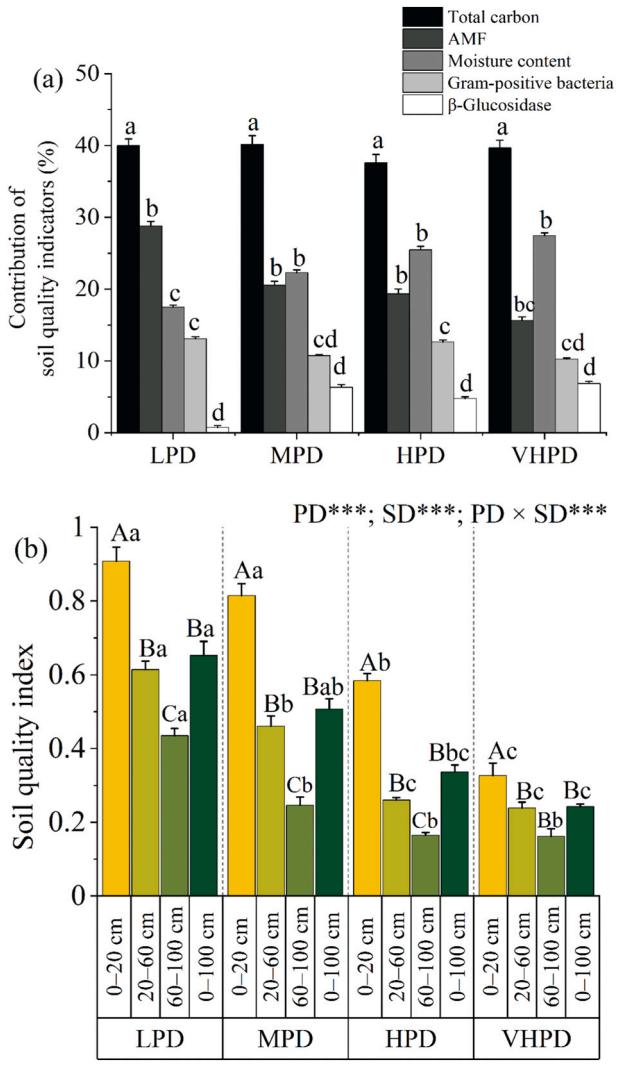

Fig. 3. (a) Percentage contribution of soil quality indicators to SQI in different planting densities at 1 m soil depth. Values are means ± standard deviation. Error bars with different lowercase letters indicate significant differences among the soil quality indicators in the same planting density (b) SQI computed under different planting densities of *P. kesiya* plantation (n = 3). Error bars with different lowercase letters indicate significant differences among the planting densities. Different uppercase letters indicate significant differences among soil depth with the same planting densities ($P < 0.05$). F values of two-way ANOVA for the effects of planting density (F = 69.38) and soil depth (F = 84.00), planting density × soil depth (F = 9.054) on SQI. LPD, Low planting density; MPD, Moderate planting density; HPD, High planting density; VHPD, Very high planting density; PD, Planting density; SD, Soil depth.

4.2. Impact of planting density on soil quality index

Increase in planting density negatively impacts soil quality, thus supporting our first hypothesis (Fig. 3b). The significant contributions of soil quality indicators to the SQI indicate a higher nutrient level in LPD (Table S4) which could be attributed to a higher understory vegetation cover (98.2 %; Table 1). Higher plant and root biomass from the understory vegetation (Table 1) accumulated more soil nutrients and significantly influenced total carbon and microbial activity (Murugan et al., 2014; Fang et al., 2021). An increased understory vegetation cover increases the input of soil substrates from root exudates, dead fine roots, and leaf and litter leachates (Chen et al., 2015; Fang et al., 2021). In general, pine needles are low in litter quality (high lignin and

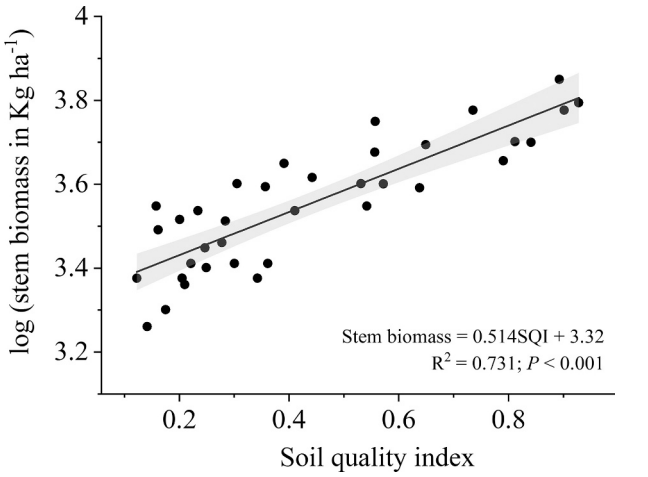

Fig. 4. Linear regression between the stem biomass and the soil quality index (SQI). Regression equation, line of best fit, coefficient of determination (R^2), and statistical significance (*P* value) are provided.

polyphenols) and associated with a slow litter decomposition rate and less nutrient transfer to soil (Thomas, 1968; Díaz Villa et al., 2022). Concurrently, in this study, a higher pine canopy cover in VHPD (97 %; Table 1) accumulates more pine needle litter on the forest floor resulting in less carbon content and microbial composition. This impedes the growth of understory vegetation, which becomes the limiting factor for soil quality (Dang et al., 2018; Ali et al., 2019; Li et al., 2020). Planting fewer trees increases canopy openness and light penetration, and allows understory vegetation to establish itself (Delgado-Baquerizo et al., 2019; Zhao et al., 2021). Previous studies have also reported that reducing the planting density can help to recover the understory vegetation in pine plantations (Laughlin et al., 2007; Chen and Cao, 2014; Overby et al., 2015).

Soil quality decreased with increasing soil depth (Fig. 3b) due to the lower values of soil properties in the subsurface layers than those of the surface layers (Table S4), hence, their contribution to the SQI is less (Fig. S1). The higher abundance and composition of soil microbes in the surface soil has direct interaction with plant biomass from the natural understory vegetation in the LPD and MPD plantations (Table 1), leading to 'surface-aggregation' (Prommer et al., 2019; Ma et al., 2020). Lower contribution of total carbon, microbial biomass and abundance (Fig. S1), lead to an insufficient supply of carbon and lesser activity of microbes, and subsequently declining SQI in deeper soil layers (Fontaine et al., 2007). Significant interactions between planting densities and soil depth were strong and corroborated our second hypothesis that changes in the selected MDS observed in the deep soil profile influence soil quality. Soil depth have higher F values than planting density, which indicated that the effects of soil depth on SQI was greater than planting density (Fig. 3b), and thus soil depth should be considered while evaluating the SQI.

The correlation between SQI and stem biomass indicates that soil quality increased the stem biomass. The regression model showed that up to 73 % of the variance in the computed SQI (Fig. 4) could be explained by productivity, which supports the positive impact of soil quality on the stem biomass. Studies have demonstrated that SQI has a strong relationship with the yield of oil plantation crops (Liu et al., 2017; Boafo et al., 2019). Zhang et al. (2019) demonstrated that soil quality is closely linked to vegetation restoration in the degraded karst landscapes of southwest China. Therefore, planting trees at low density may enhance soil properties through understory vegetation growth which could be the main driving force of the enhanced soil quality in pine plantations.

Similar to our study, previous studies highlighted that the increase in

pine stand densities declined the soil properties in different viewpoints. For example, Overby et al. (2015) showed that clear-cut openings of *Pinus ponderosa* enhanced AMF and bacterial growth. Chen and Cao (2014) recommended planting *P. tabulaeformis* stands below 1875 trees ha^{-1} for increasing the soil organic matter and moisture content. Li et al. (2020) concluded that HPD affects soil microbial diversity and community composition in *Pinus yunnanensis* plantations. The reasonable planting density of the *Pinus* sp. proposed in these studies was different due to the site conditions, stand age, and management objectives, but the effects of density on soil properties were similar. Planting density estimation is an important management practice to regulate timber yield and soil quality. Although this study indicates that plant biomass influences soil quality indicators directly or indirectly, it is still unclear whether the extent of such influences depends on understory vegetation composition (Raiesi and Salek-Gilani, 2020). This is an important aspect to consider because stand density has been shown to alter vegetation composition and in turn soil quality in pine plantations (Chen and Cao, 2014; Zhang et al., 2021).

5. Conclusion

This study evaluated the effect of planting density of *P. kesiya* plantations on soil quality up to 1 m soil depth using a SQI derived from soil quality indicators which are selected by PCA. TC, AMF biomass, GP biomass, MC, and BG were selected as key soil indicators. The higher contribution of biological properties to the SQI indicate their importance in soil quality assessment. The significant decrease in SQI with increasing planting density showed that high planting densities in subtropical plantations have led to soil degradation, especially in the deep soils. The positive relationship between stem biomass and SQI indicates that soil quality increases yields. The higher soil quality in the LPD characterized by a higher understory vegetation cover, demonstrating that restoration of understory vegetation can recover soil quality in HPD and VHPD. Thus, soil quality pine plantations with high planting densities can be improved by thinning and maintaining a moderate number of pine trees through selective logging. This can help recover the natural understory vegetation, acquire a high timber yield thereby ensuring economic benefits, and maintaining the balance of soil ecosystem.

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Declaration of competing interest

Authors declare there is no conflict of interest.

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