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Continuous planting *Eucalyptus* plantations in subtropical China: Soil phenolic acid accumulation and adsorption physiognomies

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As phenolic acids accumulated in monocropping plantations, their allelopathic effects caused the soil's fertility to decline and productivity to decrease. The effects of stand age and generational turnover of phenolic acids in soil remain unclear. Therefore, we conducted an experiment to examine the changes in phenolic acid content and accumulation characteristics in soils from *Eucalyptus* plantations in terms of planting years and generations. Soils were collected from three *Eucalyptus* plantations: a 6-year-old first-generation plantation (6a1g), 9-year-old first-generation plantation (9a1g) and 6-year-old second-generation plantation (6a2g). Seven phenolic acids were identified from different soil samples by high-performance liquid chromatography. In addition, exogenous phenolic acids were added to the soil samples to study the kinetics as well as the adsorption and desorption characteristics of soils. The total of seven phenolic compounds in 6a1g (38.451 $\mu\text{g g}^{-1}$) was higher than that of the 9a1g and 6a2g soils, and the total of 6a2g (27.257 $\mu\text{g g}^{-1}$) was higher than that of the 9a1g (15.536 $\mu\text{g g}^{-1}$). In the tested soil, the *p*-hydroxybenzoic acid accounted for 51.6% (for 6a1g), 51.1% (for 9a1g) and 33.5% (for 6a2g) of the total amount of the seven phenolic acids, the *p*-hydroxybenzoic may have had a higher allelopathic capacity in these *Eucalyptus* plantation soils. Soil bulk density showed a strong positive correlation with vanillic and ferulic acids, and SOM also had an extremely positive correlation with *p*-hydroxybenzoic and coumalic acids. The findings demonstrated that the adsorption and desorption rates and amounts of each phenolic acid in the soil of 6a1g were higher than those in the soil of 9a2g and 6a2g using both a kinetic adsorption experiment and isothermal adsorption and desorption experiments. But among the three soils, 9a1g soil had the lowest adsorption rate, which resulted in the lowest accumulation capacity. It is noticed that the Pseudo-second-order kinetic model can be used to describe the kinetic adsorption better than the Pseudo-first-order kinetic model and the Elovich model, and the Freundlich model could fit the desorption isotherms

better than the adsorption isotherms. However, more study of this phenomenon is essential to better understanding its mechanisms and how they function in order to address issues with soil degradation in future intergenerational *Eucalyptus* plantings.

KEYWORDS

phenolic acids, *Eucalyptus* plantations, adsorption isotherm, desorption isotherm, kinetic of adsorption

1. Introduction

Phenolic acids, organic acids with active carboxyl groups on aromatic rings, are not only secondary plant metabolites in plants, but also major allelopathic compounds, occurring widely in soils, decomposing plant residues and roots (Zhao et al., 2022). Litter leachate, root exudates, and the byproducts of lignin-rich plant debris are the main sources of phenolic acids in soil. The species diversity, vegetation, environmental factors, and soil characteristics all affect the composition of soil phenolics (Zwetsloot et al., 2020), with significant differences between deciduous and conifer forests (Buondonno et al., 2014). Most phenolic acids in soil normally promote the growth of their source plants by allelopathic inhibition of other plants (Lockwood et al., 2013). On the other hand, many studies have suggested that the accumulation of phenolic acids in soil is one of the important factors that hinder continuous mono-cropping (Qu and Wang, 2008; Bao et al., 2022), directly or indirectly alter the soil's microbial population, encourages soil sickness (Wu and Lin, 2020) and excessive concentrations of phenolic acids can inhibit the growth of plants (Kato-Noguchi and Kurniadie, 2021).

This allelopathy also assumes a significant role in different managed forestry systems, and a number of trees have been reported to exhibit this phenomenon (Reigosa and Gonzalez, 2006). It was reported that allelopathy due to phenolics is a cause of natural regeneration deficiency in subalpine spruce (*Picea abies* L. Karst.) forests (Latif et al., 2017), and germination bioassays showed their autotoxicity involving spruce with inhibitory effects on root elongation and seedling growth (Yang et al., 2006). In recent years, with the decline of plantation productivity in monoculturing consecutive forest land, the phenolic acids in soil have attracted the attention of many scholars (Li et al., 2020; Zhao et al., 2022). Increasing evidence from those widely planted tree species in the form of successive monocultures seemed to support this "self-toxicity" caused by phenolic acids (Wu et al., 2016). In the majority of cases, one tree species planted continuously for a long time may suffer from allelochemicals like phenolic acids secreted by the root system and released by litter decomposition, thus resulting in negative allelopathy in replanted forest soil (Abbas et al., 2017; Kato-Noguchi and Kurniadie, 2021).

In China, some fast-growing timber tree species are planted continuously on the same forestlands, such as Chinese fir and *Eucalyptus*, which are representative species of successive mono-cropping for multiple generations. The accumulation of phenolic substances in soil may be one of the important reasons for the yield reduction of Chinese fir under continuous cultivation in

southern China (Tan et al., 2008; Liu et al., 2020). With the consecutively planted generations in poplar plantations, the target phenolic acids and their total amount had an accumulation trend in soils, presenting as rhizosphere soil of generation II > rhizosphere soil of generation I > non-rhizosphere soil of generation II > non-rhizosphere soil of generation I (Tan et al., 2008; Wang Y. et al., 2016). Phenolic acids inhibit photosynthesis, biomass reduction, low organic matter accumulation, leaf cell damage, fine root growth, nutrient uptake efficiency, and nitrogen transformation (Li et al., 2020; Sengupta and Pal, 2021). The evidence found in Chinese fir plantations showed that the water extracts from soils, litters, and fresh tissues inhibited seed germination (Chen and Wang, 2013), organ development, root vigor, and chlorophyll content (Chen et al., 2014; Li et al., 2020) of the fir, linking to vanillin, *p*-coumaric, ferulic, and *p*-hydroxybenzoic acids (Lin et al., 2010; Wei, 2017).

Eucalyptus is a fast-growing, high-yielding, broad-leaved tree species that is widely cultivated in tropical and subtropical regions, contributing economically to the pulp and timber industries. However, the sustainability of *eucalyptus* plantations is a worry in many areas given the current state of global change, which includes an increase in the frequency of drought events, a rise in global temperature, allelopathy, and a rapid spread of pests and diseases (Laclau et al., 2018). In those regions where *Eucalyptus* plantations have expanded to cover more forest land in consecutive monoculture and regenerate by sprouting for multiple generations after clearing, the allelopathic substances secreted by *Eucalyptus* have become a hotspot of research, because of their potential effects on the growth of *Eucalyptus* or other plants (Zhou et al., 2020). In *Eucalyptus* stands, the allelochemicals enter the soil by means of rain washing leaves, decomposition of litter, and root secretion (Becerra et al., 2018), resulted an invasion of exotic plants occurred in the understory as a result of reductions in understory grasses, woody plants, and soil N and P cycling indices (Zhou et al., 2020). Previously, we analyzed the differences in phenolic acid accumulation and the effects on soil enzyme activities between the pure and mixed *Eucalyptus* plantations (He et al., 2017; Liao et al., 2019), but the changes in soil phenolic acid with the planting time of *eucalyptus* on forestland need to be investigated.

Furthermore, phenolic acid accumulation in *Eucalyptus* plantation soils should be dependent on phenolic acid adsorption and degradation abilities as a result of root exudates littering in different forest types (Wang, 2016). However, the relationship between the adsorption dynamics of phenolic acids and their environmental behavior in *Eucalyptus* plantations is still unclear. Therefore, adsorption and desorption isotherms provide valuable information related to the quantification of phenolic acids in

soil, and these data can be used to assess their possible effects in environmental systems. The introduction of a mathematical model provides us with a useful tool to reveal the environmental behavior of phenolic acids (Sun et al., 2012; Mahmoudi et al., 2016). However, some basic research questions need to be addressed, e.g., what factors might be relevant to the accumulation of phenolic acids process? How do the different phenolic acids change during the processes of plantation and regeneration? To solve these acknowledged problems, it might be helpful to understand the productivity decline of *Eucalyptus* plantations from the perspective of phenolic acid allelopathy.

To identify the stand age and generation effects of *Eucalyptus* plantations on soil phenolic acid, we made a comparison at our study location in China's subtropical region. In this case, we hypothesize that there are obvious differences in the soil phenolic acid, and the differences may be different in various stand ages. In order to clarify the performance of phenolic acids in the soils of *Eucalyptus* plantations with planting time, we collected the soils from three intergenerational *Eucalyptus* plantations with research objectives: (1) to investigate the changes of phenolic acids in forestland soils; (2) to study the adsorption or desorption characteristics; and (3) to assess the changes in phenolic acids by using the kinetics and isotherm curves.

2. Materials and methods

2.1. Study sites and soil sampling

This study was performed in mid-2016 at the Experimental Center of Tropical Forestry (21°57'47"–22°19'27"N, 106°39'50"–106°59'30"E; 1045.9 m), Chinese Academy of Forestry, Pingxiang, Guangxi, China. Three forest types of pure *Eucalyptus* plantations were studied: 6-year-old first-generation forest (6a1g), 9-year-old first-generation forest (9a1g) and 6-year-old second-generation forest (6a2g; Table 1). Although the content of phenolic acids in rhizosphere soil is generally higher than that in bulk soil, because the rhizosphere is limited to a few millimeters of space away from the root surface, the non-rhizosphere soil, which accounts for the majority of forest soil, is the key to controlling the category and content of phenolic acids and determining the allelopathic effect in forest land. Thus, in this study, non-rhizosphere soil was collected to describe the content distribution of different soil phenolic acids, and the adsorption dynamic mechanism was further discussed. 12 sample plots of equal size (20 m x 20 m = 400 m²) were randomly selected in each forest type, the number of stems in each plot was calculated, and divided by the plot size. Finally, the average stem number of selected plots was multiplied by 10,000, and the densities ha⁻¹ were recorded. In each plot, 12 non-rhizospheric soil samples were collected randomly, but each sampling point was at least 1 m away from the base of the surrounding tree trunks to ensure that no roots were collected. The depth of the soil collection was 0–30 cm. If there were roots in the soil samples collected, the roots and the surrounding soil particles within at least 1 cm were removed. The soils from the same plot were mixed together for later analysis; each mixed sample is not

less than 500 g. All samples were preserved in separate zip-lock bags, then divided into two parts; one was frozen in an ultralow temperature freezer until use, and the remainder was used to determine the soil density, pH, and soil organic matter (Table 1).

2.2. Determination of soil bulk density, pH and SOM

Soil bulk density (ρ_B) values were determined using the core method (Mualem and Assouline, 1986). Undisturbed soil core samples were collected using a stainless steel coring ring from each plot at 0–30 cm depths. Soil cores were oven-dried at 105°C for 24 h. Bulk density was calculated by dividing the mass of oven dried soil by the core volume accordingly.

$$\text{Bulk density (g/cm}^3\text{)} = \text{Dry soil weight (g)} / \text{Soil volume (cm}^3\text{)}$$

The pH of soil samples was measured using a digital pH meter (Starter 2100 pH Bench, OHAUS) after the soil was shaken with distilled water at a solid-to-water ratio of 1:2.5 (w/v) for an hour. SOC was computed using the oxidation method (Bao, 2000). Soil samples (0.5 g) were digested with 5 ml of 1 mol K₂Cr₂O₇ and 5 ml of concentrated H₂SO₄ and titrated with FeSO₄ after boiling at 175°C for 5 minutes. Soil organic matter calculated by multiplying SOC with 1.72.

2.3. Extraction of phenolic compounds

The soil samples (25 g) needed to stand in 25 mL of 1 M NaOH overnight. NaOH was chosen as the extractant mainly because it is commonly used in soil particle dispersant and organic material solubilization (Siqueira et al., 1991), which is conducive to improving the extraction of phenolic acid. The samples were shaken for 30 min, then the supernatants were filtered through 0.22 μm filter membranes after centrifugation (4,000 rpm, 10 min, 25°C). The filtrates were soured with 12 M HCl until the pH was reduced to 2.5. After standing for 2 hours, the precipitates of humic acid appeared, and then the mixtures were centrifuged for 10 min again. We used 1 mL of the supernatants for high-performance liquid chromatography.

2.4. High-performance liquid chromatography

Seven phenolic standard compounds were purchased from Sigma-Aldrich, including hydroxybenzoic, benzoic, vanilloid, ferulic, coumarate, salicylic, and cinnamic acids. Standard mixtures of these compounds were prepared. For each phenolic standard compound, 1, 5, 10, 50, and 100 mg were weighted, and dissolved in 2 mL of chromatographic grade methanol, then diluted with ultra-pure water (Millipore, Bedford, MA, USA). After methanol volatilization, the soil pH was adjusted by 0.5 M HCl or NaOH and then supplemented by ultra-pure water, resulting in concentrations

TABLE 1 Environmental factors of the selected *Eucalyptus* plantations.

Forest type	Origin	Density (trees/ha)	Tree height (m)	DBH (cm)	Elevation (m)	Slope aspect	Slope position	Slope degree	Bulk density (g·cm ⁻³)	pH	SOM (g kg ⁻¹)
6a1g	Planting	1,200	20.5	13.6	210	NWst	mesoslope	25	1.32	5.37	21.24
9a1g	Planting	1,200	22.8	15.5	225	W	mesoslope	15	1.26	5.17	17.07
6a2g	Sprouting	1,300	20.8	14.0	220	NWst	mesoslope	25	1.37	6.08	18.18

DBH, diameter at breast height; NW, northwest; W, west; SOM, soil organic matter.

of 1, 5, 10, 50, and 100 $\mu\text{g}\cdot\text{mL}^{-1}$. The standard curve was obtained by linear regression of the peak area and concentration of these seven phenolic acids.

Standards and sample analysis were carried out using a Waters-e2695 high-performance liquid chromatograph system (Milford, MA, USA). The phenolic acids were separated on a Luna 5u C18(2) column (250 × 4.6 mm; 100Å; particle size, 5 microns; Phenomenex Inc., Torrance, CA, USA), with elution using 0.1% phosphoric acid (solvent A) and acetonitrile (solvent B) at a flow rate of 1.5 mL min⁻¹. The gradient profile was as follows: 0-1 min, isocratic, 5% B in A; 1-20 min, 5-46 % B in A; 20-21 min, 46-5 % B in A. The eluent was monitored at 230 nm with a Waters 996PAD detector.

2.5. Accumulation mechanism experiment

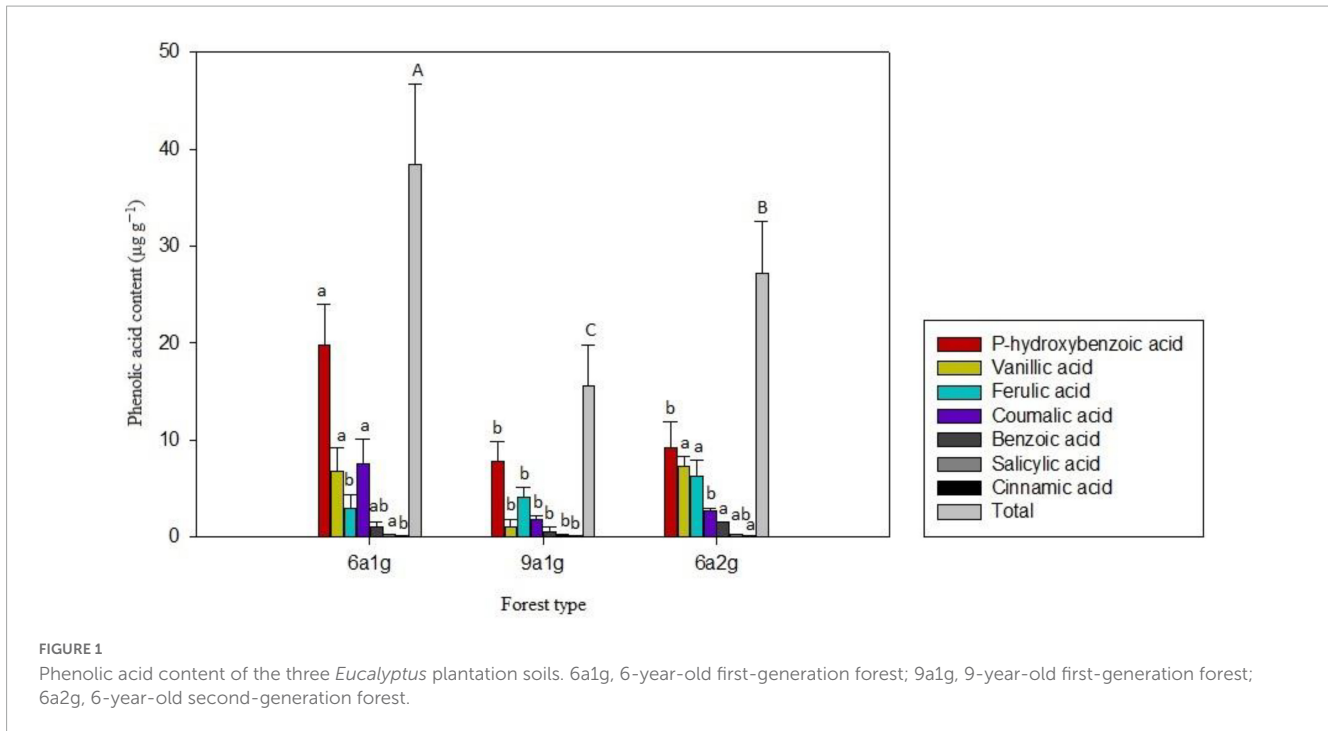
The accumulation mechanism experiment was mainly divided into two parts: a kinetic adsorption experiment as well as isothermal adsorption and desorption experiments. The kinetic adsorption experiments were carried out on a constant shaking incubator at 180 rpm after 50 mL of phenolic acid mixed standard solutions with a specific concentration (100 $\mu\text{g}/\text{mL}$) were added to 5 g of ultraviolet-sterilized soil. After the start of the experiment, under sterile conditions, and at 1 h, 4 h, 8 h, 14 h, 24 h, 36 h, 48 h, 60 h, and 72 h, respectively, mixtures were centrifuged (4,000 × g, 10 min, 25°C), and then 2 mL of each supernatant was analyzed by high-performance liquid chromatography. To further evaluate the kinetics of the adsorption process (Qin et al., 2015), three of the most widely applied kinetic models were used to fit the experimental data, a kinetic model suitable for describing the phenolic acid adsorption process was explored. A pseudo-first-order model is expressed as (Tseng et al., 2003; Tan and Hameed, 2017):

$$q_t = q_e \left(1 - e^{-k_1 t}\right) \quad (1)$$

where q_e and q_t ($\mu\text{g}\cdot\text{g}^{-1}$) are the amounts of phenolic acids adsorbed at equilibrium and at time t (h), respectively. k_1 is the pseudo-first-order rate constant. Pseudo-first-order kinetic model is based on Lagergren's first-order rate equation of solid adsorption capacity. It is mainly applied to the adsorption kinetic equation of the liquid phase and is suitable for the initial stage of adsorption. Based on the hypotheses, one is that adsorption is controlled by the pore diffusion step, and the other is that the adsorption rate is proportional to the difference between equilibrium adsorption capacity and t -moment adsorption capacity. If the adsorption process conforms to a pseudo-first-order kinetic model, the curve drawn by $\lg(q_e - q_t)$ and t should be a straight line. The model considers that the mass transfer resistance of particles is the limiting factor of adsorption.

A pseudo-second-order equation (Wang and Guo, 2020) has been widely used to predict kinetic adsorption. It was expressed as:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \quad (2)$$



where k_2 is the rate constant of second-order adsorption. The pseudo second-order kinetic model is based on an assumption that adsorption rate is controlled by chemical adsorption mechanisms, which involve electron sharing or electron transfer between adsorbent and adsorbate. It is consistent across the entire adsorption equilibrium time range and can generally explain the adsorption mechanism. The main idea of the pseudo-second order kinetic model is chemical adsorption, whole rate depends on chemical adsorption, and the adsorption capacity of the adsorbent mainly depends on active sites on the surface of the adsorbent. If the model is satisfied, a straight line should be obtained for mapping to t/q_t and t . It is considered that the limiting factor of adsorption is the adsorption mechanism, not mass transfer.

The Elovich equation has the effect of characterizing this type of chemisorption and can be given in the following equation (Kajjumba et al., 2018).

$$q_t = (1/b) \ln(ab) + (1/b) \ln t \tag{3}$$

where a is the initial adsorption rate and b is an indication of the number of sites available for adsorption. The phenolic acid uptake at any time, q_t , was calculated using Eq. (4):

$$q_t = \frac{(C_0 - C_e) \cdot V}{W} \tag{4}$$

Whether, W (g) represents the mass of the soil samples and V (mL) is the volume of the drawing solution. C_0 and C_e ($\mu\text{g}\cdot\text{mL}^{-1}$) are the initial and final (after adsorption) concentration of phenolic acid solution, respectively.

The isothermal adsorption experiments were conducted at 25°C. Five g of soil samples was added into 50 mL of phenolic acid mixed as standard solutions of different initial concentrations ($100\text{--}400 \mu\text{g}\cdot\text{mL}^{-1}$) in 100 mL Erlenmeyer flasks until the system reached adsorption equilibrium (shaking 24 h, then standing

for 30 min). The concentrations of phenolic acid left in the supernatants (2 mL removed) were determined to fit the Freundlich (Eq. (5)) isotherm equation:

$$q_t = Kd \cdot C_e^{1/n} \text{ or } \lg q_t = \lg Kd + 1/n \lg C_e \tag{5}$$

where Kd is the constant of adsorption equilibrium and n shows the intensity of sorption. The equation tends to the axiomatic linear form only for $n = 1$ (Hartley, 1976).

After removing 8 mL of supernatants from the excess solutions of isothermal adsorption experiment, the above amount of solution that remained was 50 mL by adding 10 mL Milli-Q water. The desorption solutions were determined until the system reached equilibrium (like the adsorption experiment). The amount (C_s') and rate (R_d) of desorption were calculated according to Eqs. (6) and (7), respectively:

$$q_t = \frac{(C_e - C_e') \cdot V}{M} \tag{6}$$

$$R_d = \frac{(q_t - q_t')}{q_t} \tag{7}$$

where R_d is the desorption rate and C_e' ($\mu\text{g}\cdot\text{mL}^{-1}$) is the concentration of phenolic acid solution after desorption equilibrium.

2.6. Statistical analysis

The obtained data were collated in MS Excel and analyzed in SPSS 19.0. The content of phenolic acid is expressed by Mean \pm SD. The differences of phenolic acid content among different forest types were detected with one-way ANOVA and LSD multiple comparisons. Because soil clay, organic matter, and pH are related

to the amount of phenolic acid adsorbed by soil, R (3.2) software was used to conduct the correlation analysis between soil bulk density (instead of clay content, because of a significant positive correlation between them ($R^2 > 0.9$), organic matter, pH, and phenolic acid accumulation in different soils.

3. Results

3.1. Quantitative analysis of soil phenolic acids

Seven phenolic acids, including *p*-hydroxybenzoic, benzoic, vanillic, ferulic, coumarate, salicylic, and cinnamic acids, were identified and quantified in the three types of *Eucalyptus* soils (Figure 1). The total of seven phenolic compounds in 6a1g ($38.451 \mu\text{g}\cdot\text{g}^{-1}$) was higher than that of the 9a1g and 6a2g soils, whereas in 6a2g ($27.257 \mu\text{g}\cdot\text{g}^{-1}$) was found higher than the 9a1g ($15.536 \mu\text{g}\cdot\text{g}^{-1}$). The percentages of *p*-hydroxybenzoic, vanillic, ferulic and coumarate acid varied among the different types of forest: 6a1g (51.6, 17.7, 7.8, and 19.7%), 9a1g (51.1, 7.1, 26.4, and 11.8%) and 6a2g (33.5, 26.6, 22.8, and 9.6%), although the other phenolic compounds made up less than 5% of the total. Benzoic, and cinnamic acids in 6a2g were significantly higher than in 9a1g, while coumalic acid in 6a2g and 9a1g was significantly lower than in 6a1g. In addition, no significant differences were observed for the vanillic, benzoic and salicylic acids between 6a1g and 6a2g.

3.2. Relationship between phenolic acid content and soil characteristics

Soil bulk density, pH and soil organic matter were chosen to explore the relationship between them and phenolic acids in non-rhizosphere soil. Clay is usually considered to be positively correlated with soil phenolic acid content, mainly because of its strong adsorption capacity. We used the bulk density to replace the clay content because there is a positive correlation between them in general. Except for benzoic acid, the correlation analysis showed that pH had little effect on other phenolic acids. Soil bulk density showed a strong or an extremely strong positive correlation with vanillic, and ferulic acid, and SOM also had a strong or extremely strong positive correlation with *p*-hydroxybenzoic and coumalic acid (Figure 2).

3.3. Kinetic adsorption study

Figures 3A, B shows the results of kinetic adsorption experiments conducted to determine the equilibrium time and adsorption quantity required for the uptake of different phenolic acid substances on the various soils of *Eucalyptus*. The equilibrium times in different types of *Eucalyptus* plantations were basically identical but varied for the different phenolic acids. The equilibrium times of *p*-hydroxybenzoic, vanilloid, ferulic, and benzoic acids were all 8 h in 6a1g, 9a1g, and 6a2g, while salicylic and cinnamic acids lasted nearly 36 h. Coumarate acid had the longest

equilibrium time, lasting 60 hours. In terms of the adsorption quantity, in addition to coumarate acid, the other acids showed that the content in 6a1g was higher than in 9a1g or 6a2g. No obvious difference was observed for coumarate acid among the three plantation types. In the three different types of *Eucalyptus* plantations, cinnamic acid had the highest equilibrium adsorption quantity (about $700\text{--}1,000 \mu\text{g}\cdot\text{g}^{-1}$) and *p*-hydroxybenzoic and benzoic acids were the lowest ($100\text{--}200 \mu\text{g}\cdot\text{g}^{-1}$) among the seven phenolic acids. Table 2 shows the kinetic adsorption parameters of three models. Based on R^2 , $q_{e,\text{exp}}$ and $q_{e,\text{cal}}$ values, the pseudo-second-order model ($R^2 > 0.95$, $q_{e,\text{exp}}$ and $q_{e,\text{cal}}$ are similar) can be used to describe this kinetic adsorption better than the pseudo-first-order model ($R^2 < 0.9$, $q_{e,\text{exp}}$ and $q_{e,\text{cal}}$ show differences). In addition to *p*-hydroxybenzoic in 6a2g, the Elovich model may also fit these data well ($R^2 > 0.8$).

3.4. Adsorption and desorption experiments of three *Eucalyptus* plantation soils

The characteristics of the adsorption of seven phenolic acids were studied in three different soils and characterized through sorption isotherms. Sorption isotherms were described by the Freundlich equation with regression coefficients $R^2 > 0.9$, except for the sorption isotherms of ferulic, coumarate, and benzoic acids in 9a1g and *p*-hydroxybenzoic, vanilloid, and salicylic acids in three soils (Table 3). The values of K_d were higher in 6a1g (0.645–4.364) than in 6a2g (0.046–1.493) for all of the phenolic acids, while the values of $1/n$ were the opposite. In addition to cinnamic acid, the Freundlich equation was unsatisfactory for the other phenolic acids in 9a1g. The K_d for cinnamic acid in the soils could be ranked in decreasing order: 6a1g > 6a2g > 9a1g. However, 6a1g soil had the highest average adsorption rate (12.313–22.807%) among the three soils for all seven phenolic acids.

Through the Freundlich model (Table 4) the desorption isotherms fit very well. The regression coefficients were all greater than 0.9, except for the desorption isotherms of hydroxybenzoic, vanilloid, and salicylic acids in 9a1g soil and benzoic acid in 6a2g soil. The Freundlich coefficient (K_d) showed the order was 9a1g > 6a2g > 6a1g for *p*-hydroxybenzoic, vanillic, ferulic, coumalic, and salicylic acids, while for benzoic and cinnamic acids the highest ones were 6a2g and 6a1g, respectively. However, $1/n$ was the opposite of K_d .

4. Discussion

4.1. Quantitative analysis of soil phenolic acids

We can usually separate and identify a variety of allelopathic phenolic acids in soil. For example, Chou and Muller (1972) identified *p*-hydroxybenzoic, *p*-coumaric, ferulic, syringic, and *o*-coumaric acids during research related to the allelopathic mechanisms of *Arctostaphylos glandulosa* var. *zacaensis*. Li et al. (1992) found that *p*-coumaric, ferulic, vanillic, and

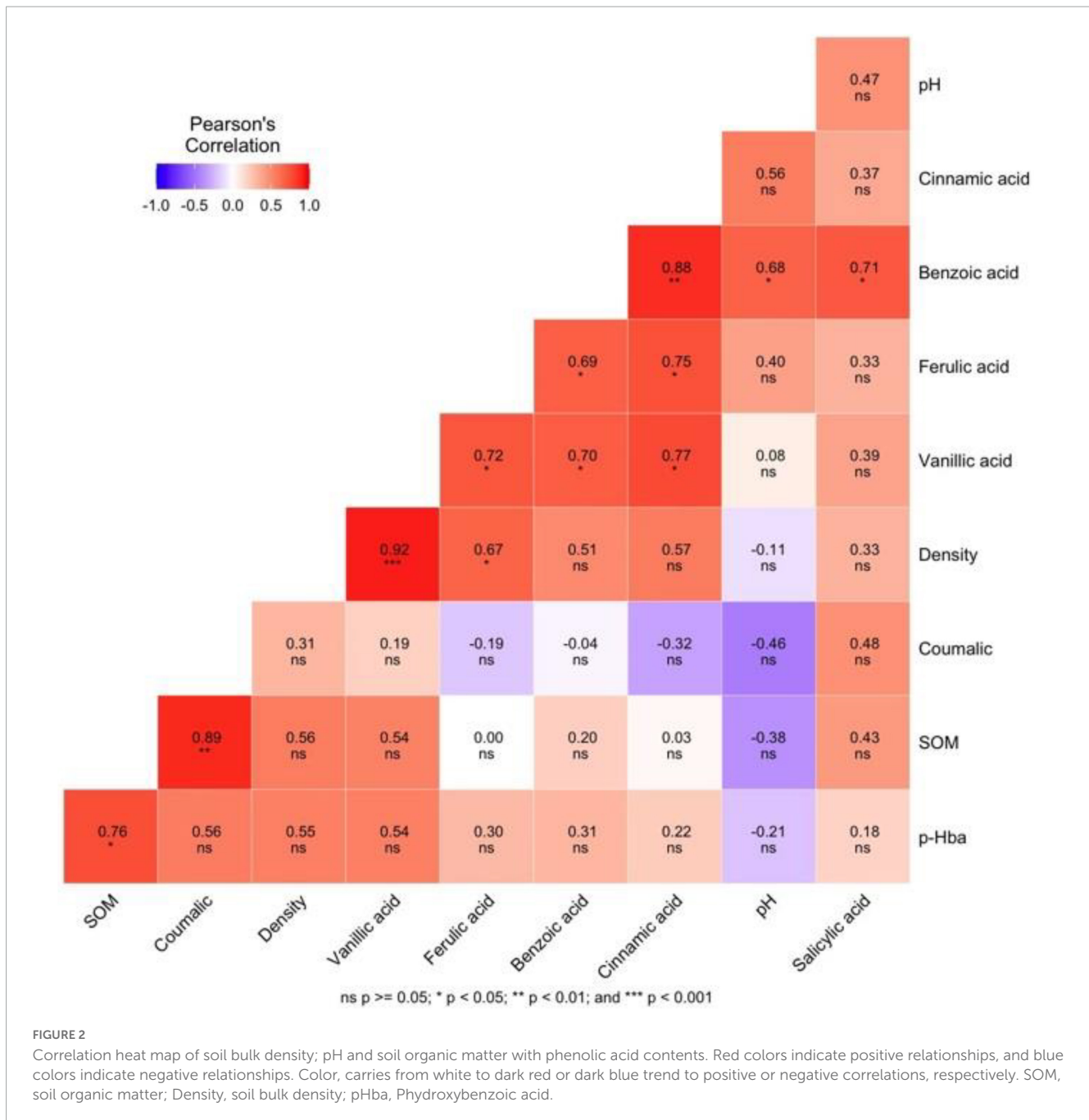


FIGURE 2

Correlation heat map of soil bulk density; pH and soil organic matter with phenolic acid contents. Red colors indicate positive relationships, and blue colors indicate negative relationships. Color, carries from white to dark red or dark blue trend to positive or negative correlations, respectively. SOM, soil organic matter; Density, soil bulk density; p-Hba, Phydroxybenzoic acid.

p-hydroxybenzoic acids, as well as *p*-hydroxybenzaldehyde, were the main allelochemicals in Sasa soil. Huang et al. (2013) also identified caffeic, ferulic, *p*-coumaric, chlorogenic, and *p*-hydroxybenzoic acids as the main phytotoxins. These phenolic acids can be divided into volatile, water-soluble, and compound phenolic acids. Volatile phenolic acids are difficult to accumulate in the soil. Several common phenolic acids are water-soluble phenolic acids, including the tested ones here. However, due to the adsorption, the content in the soil is very low, generally $0.1\text{--}30 \mu\text{g g}^{-1}$. When the content of water-soluble phenolic acids in soil is high, they can be adsorbed by soil humus substances and mineral colloids to form compound phenolic acids (Muscolo et al., 2020). As a result of being adsorbed by soil colloids, compound phenolic acids are insoluble in water and cannot be absorbed by plants, it is generally harmless to plants.

When the content of water-soluble phenolic acids in soil decreases, the compound phenol acids are released from soil colloids and transformed into water-soluble phenolic acids, which is a dynamic balance. Li et al. (2002) reported that the content of water-soluble phenolic acids in the soils of Chinese fir forest and broad-leaved forest was between 6.58 and $35.88 \mu\text{g}\cdot\text{g}^{-1}$, and the content of compound phenolic acids was between 420.10 and $1,907.47 \mu\text{g g}^{-1}$.

All of the seven target phenolic acids in this study were extracted and identified from soils of different types of *Eucalyptus* plantations, their mean total amounts were $15.28\text{--}38.37 \mu\text{g g}^{-1}$. The amplitude range was greater than that reported by Wang Y. et al. (2016), who reported that phenolic acid concentrations in the bulk soil of Poplar plantation forest soils ranged from 14.22 to 15.03 g g^{-1} . However, Djurdjevic et al. (2004) reported that the

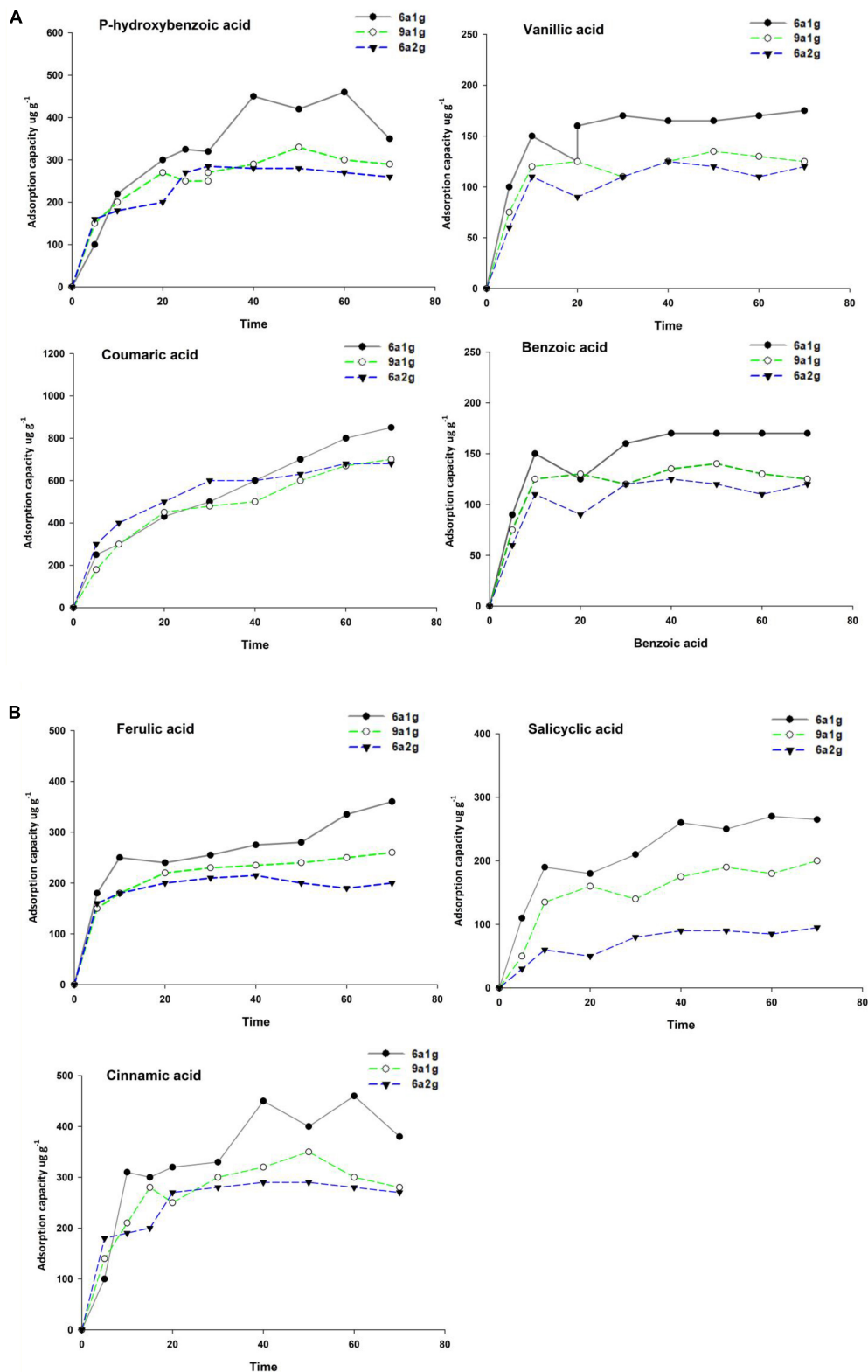


FIGURE 3 (A,B) Kinetic adsorption of seven phenolic acids in non-rhizospheric soils of different *Eucalyptus* plantations. 6a1g, 6-year-old first-generation forest; 9a1g, 9-year-old first-generation forest; 6a2g, 6-year-old second-generation forest.

soil of *Allium ursinum* L. contained 160 $\mu\text{g g}^{-1}$ free and 1,610 $\mu\text{g g}^{-1}$ total bound phenolic acids. In our study, *p*-hydroxybenzoic acid from the soil of 6a1g was highest (19.81 $\mu\text{g g}^{-1}$). In the soils,

the total of seven phenolic acids in 6a1g soil was obviously higher than in 9a1g and 6a2g soils. Therefore, it was anticipated that the *p*-hydroxybenzoic acid could be a key source for phenolic acids to

TABLE 2 Kinetic adsorption rate model equation constants and correlation coefficients for the adsorption of phenolic acids of three *Eucalyptus* plantation soils.

Phenolic acids	Forest type	qe,exp ($\mu\text{g}\cdot\text{g}^{-1}$)	Pseudo-first-order kinetic model			Pseudo-second-order kinetic model			Elovich model		
			qe,cal ($\mu\text{g}\cdot\text{g}^{-1}$)	K ₁	R ²	qe,cal ($\mu\text{g}\cdot\text{g}^{-1}$)	K ₂	R ²	A	B	R ²
p-hydroxybenzoic acid	6a1g	188	–	–	–	182	0.0020	0.988	6.6×10^4	0.0735	0.866
	6a2g	94	–	–	–	107	0.0015	0.869	–	–	–
	9a1g	115	–	–	–	129	0.0022	0.968	6671	0.0867	0.733
Vanillic acid	6a1g	258	225	1.349	0.512	247	0.0022	0.991	7.5×10^5	0.0620	0.844
	6a2g	182	168	1.457	0.431	182	0.0041	0.998	5.3×10^5	0.0824	0.929
	9a1g	219	182	0.812	0.450	213	0.0015	0.986	3992	0.0465	0.920
Ferulic acid	6a1g	380	298	0.810	0.324	376	0.0005	0.976	3200	0.0254	0.864
	6a2g	248	210	1.041	0.528	236	0.0019	0.987	4.1×10^4	0.0519	0.841
	9a1g	280	231	0.339	0.456	272	0.0009	0.982	1269	0.0311	0.902
Coumalic acid	6a1g	870	816	0.066	0.741	970	0.00009	0.959	406	0.0066	0.878
	6a2g	734	703	0.129	0.839	804	0.0002	0.995	576	0.0076	0.983
	9a1g	741	691	0.080	0.850	826	0.0001	0.977	312	0.0072	0.936
Benzoic acid	6a1g	180	164	0.733	0.589	184	0.0025	0.998	3998	0.0524	0.898
	6a2g	115	114	0.644	0.642	120	0.0081	0.993	3314	0.0782	0.767
	9a1g	135	132	0.641	0.610	140	0.0063	0.992	4352	0.0688	0.807
Salicylic acid	6a1g	276	259	0.196	0.681	301	0.0007	0.994	417	0.0230	0.972
	6a2g	117	108	0.103	0.725	123	0.0011	0.971	78	0.0502	0.883
	9a1g	204	198	0.117	0.897	230	0.0006	0.989	108	0.0246	0.957
Cinnamic acid	6a1g	447	426	0.146	0.850	467	0.0005	0.977	296	0.0119	0.920
	6a2g	307	304	0.142	0.895	325	0.0007	0.978	175	0.0159	0.902
	9a1g	306	318	0.131	0.918	404	0.0002	0.850	123	0.0135	0.904

6a1g, 6-year-old first-generation forest; 9a1g, 9-year-old first-generation forest; 6a2g, 6-year-old second-generation forest.

access soils and cause allelopathy. It is generally believed that the continuous tree planting will increase the phenolic acids in the soil year by year, and the reduction in forest production is caused by this accumulation of phenolic acids (Bai et al., 2019). But the result of our study shows that the phenolic acids were not accumulating with replanting and inter-generation in *Eucalyptus* plantations. The results of this study supported the hypothesis that the content of phenolic acids in soil usually varies with soil pH and nutrient status. Tan et al. (2008) identified five soil phenolic acids from the first, second, and third generations of *P. nigra* plantations and found that the total content of phenolic acids in the soils of the second and third generation forests was 137.87 and 64.18%, respectively. Therefore, continuous planting does not necessarily lead to the accumulation of soil phenolic acids. Phenolic acid accumulation is dynamically controlled by biotic and abiotic factors in the soil. Despite the lack of microbial evidence here, previous studies have confirmed that fungal growth and reproduction can be stimulated by phenolic acids as available C sources. According to He et al. (2001), some fungi, such as *Endomycopsis* sp. F2, *Aspergillus oryzae* F3, *Penicillium* sp. F4, *Oznyun* sp. F7, and *Papulaspora* sp. F15, were good at degrading ferulic and p-hydroxybenzoic acids. Decarboxylase, hydroxylase, and oxygenase degrading phenolic acids and their coding genes have been isolated and identified

(Wang W. B. et al., 2016). These gains partly explain why soil phenolic acids from *Eucalyptus* plantations failed to accumulate with continuous planting.

Adsorption behavior of phenolic acids can be regulated by soil physical and chemical characteristics, such as soil organic matter, clay, and pH, thereby acting on their accumulation in soil. Soil organic matter is colloidal with absorbability, mainly covered by negative charges, but if amino groups ($-\text{NH}_2$) absorb H^+ , they become $-\text{NH}_3^+$ to adsorb phenolic acids. In addition, Zwetsloot et al. (2020) believed that compared with water extracts, partially solubilizing soil organic material by NaOH solutions should contribute to higher phenolic acid concentrations in NaOH extracts, suggesting that phenolic acids in soils exist predominantly in chemically bound rather than free forms. Cecchi et al. (2004) and Wang et al. (2010) studied the adsorption characteristics of phenolic acids and suggested that the adsorption capacity of benzoic acid strongly and positively depended on the content of soil organic matter and clay particles, thus affecting its retention in soil. Therefore, it is not surprising that there is a positive correlation between phenolic acids and organic matter and bulk density, while non-significant relationship of SOM and bulk density in the present study. According to research (Chaudhari et al., 2013), bulk density is a critical feature that may be used to estimate

TABLE 3 Phenolic acid adsorption isotherm parameters for the Freundlich model of three *Eucalyptus* plantation soils.

Phenolic acids	Forest type	1/n	Freundlich coefficient (Kd)	R ²	% adsorbed
<i>p</i> -hydroxybenzoic acid	6a1g	0.513	3.456	0.517	12.313
	6a2g	1.354	0.405	0.739	8.116
	9a1g	–	–	–	9.260
Vanillic acid	6a1g	0.730	2.310	0.720	14.619
	6a2g	1.419	0.404	0.870	10.625
	9a1g	–	–	–	11.877
Ferulic acid	6a1g	0.653	2.677	0.979	13.655
	6a2g	1.631	0.233	0.982	9.426
	9a1g	–	–	–	8.753
Coumalic acid	6a1g	0.547	4.364	0.930	22.807
	6a2g	0.994	1.493	0.985	19.631
	9a1g	0.418	5.399	0.695	19.972
Benzoic acid	6a1g	1.281	0.645	0.925	13.960
	6a2g	2.360	0.046	0.999	11.314
	9a1g	1.156	0.773	0.630	11.966
Salicylic acid	6a1g	0.596	3.361	0.883	16.871
	6a2g	1.012	1.095	0.899	11.710
	9a1g	–	–	–	14.009
Cinnamic acid	6a1g	1.292	0.703	0.953	17.116
	6a2g	2.200	0.085	0.938	16.656
	9a1g	1.558	0.329	0.959	13.103

6a1g, 6-year-old first-generation forest; 9a1g, 9-year-old first-generation forest; 6a2g, 6-year-old second-generation forest.

the concentration and transport of nutrients in the soil. Bulk density decreases inversely with soil organic matter concentration, meaning that the more organic matter there is, the lower the bulk density will be (Leifeld and Kögel-Knabner, 2005). Interestingly, with continuous planting time and generations, the variation in phenolic acid content may be related to the gradual accumulation of organic matter derived from first to second generation forests over time. This might be due to humic acid, fulvic acid, and humin are three components of soil organic matter that each have unique functional groups that are crucial to the cation exchange properties of soil. It affects the maintenance of the soil's structure, moisture retention, nutrient turnover, cation exchange capability, and pollutant degradation (Min et al., 2015). Cecchi et al. (2004) showed that phenolic acid content was also regulated by soil pH; the higher the soil pH, the faster the decomposition rate of phenolic acids (Li et al., 2010). There is evidence that the adsorption capacity of phenolic acids is negatively correlated with soil pH (Diao et al., 2005). The adsorption of variable charges on organic acids, including phenolic compounds, is proportional to the soil acidity (Mitra et al., 2020). With the increase in soil pH, anionic organic acids preferentially adsorb on the surface of Fe and Al oxide minerals. As a result, the ability of soil to adsorb phenolic acids decreases. Therefore, more phenolic acids will enter the soil

TABLE 4 Phenolic acid desorption isotherm parameters for the Freundlich model of three *Eucalyptus* plantation soils.

Phenolic acids	Forest type	Freundlich coefficient(Kd)	1/n	R ²
<i>p</i> -hydroxybenzoic acid	6a1g	2.443	1.068	0.994
	6a2g	6.653	0.886	0.941
	9a1g	12.078	0.770	0.815
Vanillic acid	6a1g	2.388	1.091	0.987
	6a2g	5.998	0.927	0.941
	9a1g	12.823	0.777	0.806
Ferulic acid	6a1g	5.321	0.956	0.978
	6a2g	4.519	0.957	0.976
	9a1g	9.078	0.832	0.981
Coumalic acid	6a1g	22.856	0.750	0.962
	6a2g	26.242	0.704	0.947
	9a1g	41.591	0.627	0.918
Benzoic acid	6a1g	1.119	1.231	0.986
	6a2g	17.022	0.720	0.808
	9a1g	5.834	0.920	0.956
Salicylic acid	6a1g	4.699	0.981	0.979
	6a2g	9.616	0.838	0.917
	9a1g	17.100	0.738	0.869
Cinnamic acid	6a1g	10.162	0.854	0.973
	6a2g	2.917	1.055	0.998
	9a1g	5.176	0.982	0.973

6a1g, 6-year-old first-generation forest; 9a1g, 9-year-old first-generation forest; 6a2g, 6-year-old second-generation forest.

solution and be degraded. However, the correlation between pH and phenolic acids was not observed in Pearson's analysis in the current study (Figure 2). In addition, metal ion content, molecular structure, and properties of phenolic acids are closely related to the content dynamics of different phenolic acids in soil. For example, the adsorption of cinnamic acid was stronger than that of benzoic acid in soil, and their adsorption could be enhanced by a methyl oxygen group or an acrylic acid side chain on the cyclic structure.

It should be noted that although *p*-hydroxybenzoic, vanillic, ferulic, and coumaric acids were rich in these soils, we are not sure that they were able to lead to self-poisoning. Just listed as the allelopathic candidates at present, there are three reasons as follows: (1) Did the content of these soil phenolic acids reach or even exceed the threshold of toxicity to *Eucalyptus*? We cannot give a positive response. (2) There is no effective evidence to distinguish allelopathy from other pathological or environmental stresses on phenotypic, physiological, and biochemical levels. (3) If different phenolic acids are similar in their action sites and modes, antagonism or synergism probably occurs between them, showing their separate effects. In soils, phenolic chemicals can exist in three different forms: dissolved, sorbed, and polymerized. Despite the numerous studies on phenol, there is still considerable disagreement, for instance regarding phenol transformations in soil and the impact on the rate at which soil organic matter decomposes. It's interesting to note that some authors claim phenols' abundance

in soils might vary depending on their forms, rather than their chemical makeup (Conant et al., 2011). By altering soil pH, nutrient availability, and enzyme activity, physically and chemically protected phenolics can survive longer than dissolved forms and provide feedback to SOM degrading microbes (Dungait et al., 2012). But the action site and mode of various phenolic acids still need to be further revealed. In an instant, we need to point out that the soil phenolic acid content has an intimate association with soil adsorption mechanisms and microbial decomposition. Adsorption is a complex process involving multiple factors, to be clear, the dynamic mechanism for adsorption of soil phenolic acids is conducive to how they accumulate in the soil.

4.2. Adsorption of different *Eucalyptus* plantation soils

Allelopathy must be based on a certain mass concentration of allelochemicals, which has been confirmed in many controlled experiments. The process of allelopathy is not only related to the intensity of allelochemicals secreted by plant roots but is also strongly influenced by microbial activities in soil and the adsorption capacity of soil colloids as well as allelochemicals. The study of the environmental behavior characteristics of allelochemicals in soils has become an important content to reveal the accumulation mechanism of allelochemicals. Among them, the study on the adsorption and desorption behavior of allelochemicals in soil can provide an important theoretical basis for solving this problem. In the prediction of the adsorption process, a rate-limiting step is an important factor. Kinetic studies help to identify the adsorption process and predict the required design of an accumulation mechanism (Hameed et al., 2008; Subramanyam and Das, 2009). For a solid-liquid adsorption, the solute transfer during the adsorption process can be either an external mass transfer for a nonporous medium or intra-particle diffusion for a porous medium, or both combined (Ncibi et al., 2008). Results of kinetic adsorption measurements in our study showed that the equilibrium timeframes in various *Eucalyptus* plantations were essentially the same; however, they differed for the various phenolic acids. Along with coumarate acid, the other acids demonstrated that the content in 6a1g was larger than that in 9a1g or 6a2g in terms of adsorption quantity (Table 2).

In this study, the second-order model could be used to very well describe the kinetic data for the adsorption of phenolic acids in different types of soil. The $q_{e,cal}$ values of the second-order model were very close to the experimental $q_{e,exp}$ (Fu et al., 2015), and the correlation coefficients (R^2) were all greater than 0.95, with the exception of *p*-hydroxybenzoic in 6a2g soil and cinnamic acid in 9a1g soil (Table 2). Previous studies also found that the adsorption of pesticides from aqueous solutions in soil and highly specific area-activated materials follows the pseudo-second-order kinetics model (Salman and Hameed, 2010; Long et al., 2015). Based on these data, the pseudo-second-order kinetic model was found to be the rate-limiting step (Subramanyam and Das, 2009). In this study, the adsorption of phenolic acids in different forests was also well described by the Elovich equation, indicating that the rate-limiting step involves chemisorption (Li et al., 2015). Phenolic concentrations in alkaline extracts are several-fold higher than in

water extracts for most soils, because alkaline solutions partially solubilize organic material in soil; this indicates that phenolics in soils exist predominantly in chemically bound rather than free forms (Zwetsloot et al., 2020). The amount of phenolic acids adsorbed at equilibrium, q_e , was highest in 6a1g than in the other forest types (Figures 3A, B). This result showed that 6a1g soil had the highest maximum adsorption capacity for the seven phenolic acids among the three types of soil analyzed here.

The adsorption and desorption isotherms were interpreted by the Freundlich model. The high values derived for the Freundlich coefficient, K_d , are an indication of strong adsorption or weak desorption of phenolic acids by soil particles (Cecchi et al., 2004; Mahmoudi et al., 2016). In terms of an adsorption isotherm, K_d , which was greater in 6a1g than in 6a2g, indicated that 6a1g soil had a higher sorptive capacity than 6a2g soil for all seven phenolic acids found here. Nevertheless, the Freundlich equation was not applicable to 9a1g soil. This might occur because it leads to some changes in the characteristics of the soil with the extension of continued planting years in 9a1g, while the stand was felled and regenerated by sprouting in 6a2g. Just like Cecchi et al. (2004) reported, the sorption of the phenolic acids was dependent on a variety of soil characteristics. More follow-up studies are needed to verify this conjecture. In addition, from the adsorption rate of phenolic acids, it could be determined that the adsorption amounts of phenolic acids were very low and 6a1g soil had the highest adsorption rate among the three types of soil. In addition, the desorption capacities of benzoic and cinnamic acids were weakest in 6a2g soil and 6a1g soil, respectively. Meanwhile, 9a1g soil had the lowest desorption potential for the five other phenolic acids. When the $1/n$ value is between 0.1 and 1, the process is favorable (Vazquez et al., 2007). A $1/n$ value of 1.068, 1.091, and 1.231 clearly indicated the desorption processes of *p*-hydroxybenzoic, vanillic, and benzoic acids in 6a1g were worse than in 6a2g (0.886, 0.927, 0.720) and 9a1g (0.815, 0.777, 0.920).

This could be due to its accumulation and tendency to gather in the soils of first-generation plants, though how they accumulate in soils is not fully understood. Drought and nutrient-poor soils are two environmental factors that may encourage the metabolism and release of phenolic acids from live plants (Scavo et al., 2018). The results of the other study revealed that several phenolic acids were rapidly absorbed in the continuous cropping poplar plantation's rhizosphere soils, whose rate of accumulation was higher than that of intergenerational planting soils (Tan et al., 2008). According to Narwal's (2012) investigation, nutrient stress caused an increase in the total phenolic content. Such data suggested that plants under stress released more, which was probably related to the buildup of phenolic acids in soils, particularly in the rhizosphere. On the other hand, the distribution and degradation of soil were strongly affected by the environmental behavior of allelochemicals, namely their adsorption and retention. Therefore, the environment's behavior may determine the fate of phenolic acid (Wang W. B. et al., 2016).

5. Conclusion

The results showed that the 6a1g soil had 38.451 $\mu\text{g g}^{-1}$ of the seven phenolic acids, a high proportion of the total. *p*-hydroxybenzoic accounted for the highest percentage of the seven phenolic acids (benzoic, vanillic, ferulic, coumarate,

salicylic, and cinnamic acids), followed by vanillic, ferulic, and coumalic acids. The allelopathic potential of p-hydroxybenzoic may be greater in these *Eucalyptus* plantation soils. Furthermore, compared to second generation (6a2g) and first generation (9a1g) planting in *Eucalyptus* plantations, it was established that the total phenolic acids were highest in first generation (6a1g) forest types. Vanillic and ferulic acids positively correlated with soil bulk density, but p-hydroxybenzoic and coumalic acids positively correlated with SOM. Using both a kinetic adsorption experiment and isothermal adsorption and desorption experiments to describe this phenomenon, the results showed that the adsorption and desorption rates and amounts of each phenolic acid in 6a1g soil were higher compared with the other forest types. Therefore, the adsorption capacity might be an important factor causing 6a1g soil to have a high accumulation capacity for phenolic acids. In contrast, 9a1g soil had the lowest adsorption rate among the three soils; as a result, its accumulation capacity was also the lowest. Interestingly, the soil phenolic acid accumulation in the second-generation *Eucalyptus* plantation was not the highest. In order to address issues with soil deterioration in successive multi-generational *Eucalyptus* plantings, further research into this phenomenon will help to better understand its causes and how they function.

Data availability statement

The original contributions presented in this study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

SU, YX, CL, and MY conceived the main idea of research and wrote the manuscript. SU, YX, SY, and MY revised the manuscript and provided suggestions. FC, SU, CL, and YX analyzed the data.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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