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Lignite-derived organic fertiliser enhanced the carbon sequestration capacity of woody plant by improving soil quality and promoting plant growth

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Abstract: As essential natural carbon sinks, woody plants play a key role in urban ecological restoration. The lignite-derived organic fertiliser (LOF) may promote plant growth and carbon sequestration by improving soil properties. This study investigated LOF effects on three typical woody plants – *Styphnolobium japonicum* (L.) Schott. with taproots, *Malus* × *micromalus* Makino with fibrous roots, and *Malus domestica* Borkh. with both taproots and fibrous roots – focused on soil properties improvement during a three-year planting experiment (2021–2023). The results indicated that LOF application significantly increased soil organic matter (SOM) content, with and without woody plants, by 82.3% and 54.9%, respectively. Concurrently, LOF influenced soil microbial characteristics, especially enhancing the 16S rRNA gene copy number by 0.99 times. For plant growth, LOF application increased root length, volume, and tip number in *Malus domestica* Borkh. by 37.4, 27.4, and 26.0%, respectively, and in *Styphnolobium japonicum* (L.) Schott by 43.8, 76.7, and 26.6%, respectively. However, in *Malus* × *micromalus* Makino, while root volume increased by 3.8%, root length and tip number decreased by 10.0% and 26.9%, respectively. Additionally, the LOF application increased the soil plant analysis development (SPAD) values of woody plant leaves by 5.3%, indicating improved chlorophyll content and plant health. These findings demonstrate that LOF applications may significantly enhance soil quality and promote plant growth, contributing to improved terrestrial carbon sequestration.

Keywords: terrestrial ecosystem; lignite-based organic fertiliser; soil amendment; soil organic matter; plant roots

Global carbon dioxide (CO_2) emissions reached a record high of 35.8 ± 0.3 Gt CO_2 in 2023 (Liu et al. 2024), prompting many countries to prioritise the development of low-carbon societies. Terrestrial ecosystems serve as a critical carbon sink, having sequestered approximately 225 ± 55 Gt CO_2 since 1850, accounting for 32% of total anthropogenic emissions (Friedlingstein et al. 2023). Among these ecosystems, forests represent the largest organic carbon pool, despite covering only one-third of the Earth's land surface (Tang et al. 2018, Fu et al. 2022, Leng et al. 2024). As a core element of forests,

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woody plants play a crucial role in forest carbon sequestration due to their extensive root systems and high concentrations of recalcitrant compounds such as lignin and cork, which contribute to long-term carbon storage (Moingt et al. 2016, Han et al. 2022, Huang et al. 2023). Meanwhile, many woody plants are strategically planted in urban environments to sequester CO_2, mitigate pollution, and improve environmental quality (Zhang et al. 2023, Fini et al. 2024); however, plant growth, including that of woody species, is influenced by various factors such as water availability, soil quality, and climatic conditions (Cai 2022, Kong and Liu 2022).

Soil organic matter (SOM) is a crucial soil quality indicator that plays a vital role in supporting the growth of woody plants by enhancing soil structure, nutrient retention, and overall rhizosphere conditions (Hoffland et al. 2020). Organic fertilisers, which are rich in organic matter and essential nutrients, are an effective means to enhance SOM levels, improve soil fertility, and promote plant development (Wei et al. 2024). For example, Ji et al. (2017) reported that liquid organic fertilisers significantly enhanced *Chrysanthemum* root and aboveground growth by 10.2–77.8% and 10.7–33.3%, respectively, compared to mineral fertilisers. Similarly, the application of organic fertilisers has been shown to increase SOM levels, which in turn improve leaf chlorophyll content – a key factor in photosynthesis and plant vitality (Kuerban et al. 2021). Ye et al. (2022) found that organic fertiliser application increased total chlorophyll content in Pear jujube by 26.73–43.01%, while Li et al. (2023) demonstrated that different organic fertiliser dosages improved relative chlorophyll content and net photosynthetic rate in *Ziziphus jujuba* cv. Lingwuchangzao by 3.80–8.79% and 4.82–27.31%, respectively. Beyond its impact on plant physiology, SOM plays a vital role in enhancing soil physicochemical properties, sustaining soil fertility, and increasing nutrient availability (Tiessen et al. 1994, Lin et al. 2019, Li et al. 2023). Organic fertilisers contribute to these benefits by improving soil microbial activity, nutrient cycling, and overall soil productivity (Fierer et al. 2021, Chen et al. 2023), ultimately promoting root development, aboveground biomass accumulation, and carbon sequestration capacity (Bradford et al. 2016, Chen et al. 2021).

However, many urban greening projects rely on soils with inherently low SOM levels (Xie et al. 2019), which may limit the carbon sequestration potential of woody plants. Despite the critical role of SOM in plant growth and carbon storage, research on its effects, including woody plant root development, remains limited. Lignite-derived organic fertiliser (LOF) is a novel organic soil amendment originating from lignite, a partially mineralised biomass rich in plant-derived constituents, and a once misused natural resource (as a fossil fuel). Lignite-derived organic fertiliser has a high organic matter content (90.5% dry basis) and provides essential nutrients along with a broad spectrum of micronutrients and trace elements. Studies have shown that LOF can enhance soil physicochemical properties, improve soil fertility, and stimulate plant growth (Akimbekov et al. 2020, Chen et al. 2023, Guan et al. 2023). Therefore, our study evaluates the effects of LOF on soil properties and the growth of three representative woody plant species including *Malus domestica* Borkh. (*M. domestica*), *Malus × micromalus* Makino (*M. baccata*), and *Styphnolobium japonicum* (L.) Schott (*S. japonica*). They are widely planted in the green systems of northern Chinese cities. The *S. japonica* (with taproots), *M. baccata* (with fibrous roots), and *M. domestica* (with both taproots and fibrous roots) have different root structures, which allow for a comprehensive assessment of the LOF impact on woody plants with varying root characteristics (Li et al. 2025). The findings aim to provide insights into the role of LOF in enhancing carbon sequestration through improved soil quality and plant growth, contributing to more effective strategies for urban and ecological restoration initiatives.

MATERIAL AND METHODS

Lignite-derived organic fertiliser characteristics. The LOF product was acquired from Apaxfon Biosciences Technologies Co., Ltd. (Baotou, Inner Mongolia, China), produced from lignite coal fines through a proprietary train of biochemical reactions. The LOF contained a high content of organic compounds of varied molecular sizes, essential nutrients, and abundant medium and trace elements. The organic matter content of LOF was 90.5% (dry basis), humic acid content was 10.2%, total nitrogen content was 2.2%, effective phosphorus content was 2.0% and potassium content was 2.57%. The contents of the remaining elements met the "Organic Fertiliser Standard (NY/T 525-2021)" from China.

The LOF has been demonstrated to promote beneficial soil bacteria growth, improve soil structure and increase fertiliser efficiency (Fallgren et al. 2021,

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Chen et al. 2023, Guan et al. 2023). Chen et al. (2022b) reported that the LOF could increase sunflower plant height and dry biomass by 8–76.7 cm and 309–402 g/plant, respectively. Chu et al. (2022) concluded that applying LOF could increase the weight of single ginger by 0.8–15.0%, and the yield of ginger by 5.9–11.6%. Schillem et al. (2019) reported that N-modified lignite granulates could increase the biomass of spring wheat on poor sandy substrates.

Experimental device. A three-year outdoor planting experiment was conducted at the Peiyang Campus of Tianjin University, P.R. China, to evaluate the effects of lignite-derived organic fertiliser on the growth of three typical woody plants: *Malus domestica* Borkh. (*M. domestica*), *Malus* × *micromalus* Makino (*M. baccata*), and *Styphnolobium japonicum* (L.) Schott (*S. japonica*). The woody plants were acquired from Suqian Manshi Xue Gardening Co., Ltd. (Suqian, Jiangsu, China). The experimental setup and layout are illustrated in Figure 1. The experiment utilised columnar planting devices constructed from DN500 PVC pipes with a wall thickness of 10 mm and a height of 90 cm. Each column comprised four distinct layers: (1) aquifer layer (8 cm) between the soil surface and the overflow outlet; (2) gravel layer (2 cm) of 5–10 mm gravel particles to ensure uniform infiltration; (3) soil medium layer (75 cm) as the primary planting substrate; and (4) gravel drainage layer (5 cm) of 5–10 mm gravel particles to facilitate drainage.

The experimental soil was a mixture of Tianjin Jixian soil and natural river sand in the ratio of 7:3 by volume, classified as Technic Cambisol (Humic, Calcaric, Arenic). The mass of soil used in each experimental setup was approximately 200 kg. The pH of the experimental soil was 8.25, the total nitrogen content was 658 mg/kg, the ammonium nitrogen content was 7.05 mg/kg, and the nitrate nitrogen content was 0.42 mg/kg.

A total of 36 experimental treatments were implemented, divided into 12 groups based on plant type and fertiliser application. The groups included no-plant control, *M. domestica*, *M. baccata*, and *S. japonica*. Each plant type was assigned three groups, with each group containing three replicate devices (a total of nine devices per plant species). Within each group, two devices received 3% LOF (applied only in the first year and mixed into the soil), denoted as WF. One device served as a control without LOF application, denoted as NF.

This experimental design allowed for a systematic evaluation of LOF impact on soil properties and plant growth over three consecutive years.

Experimental scheme. The *M. domestica*, *M. baccata*, and *S. japonica* were planted during April–May 2021 according to the layout plan in Figure 1B. The plants were regularly irrigated and dewormed during the experimental period to ensure normal growth. In October and December of each year (2021–2023), destructive experiments were performed on 12 devices (one group each of the no-plant control, *M. domestica*, *M. baccata*, and *S. japonica*; shown in Figure 1B) to test the plant and soil indicators.

Experimental indicators. The soil indicators included SOM, soil aggregates, and soil microorganisms. The plant indicators included root indicators (root length, average root diameter, root projected

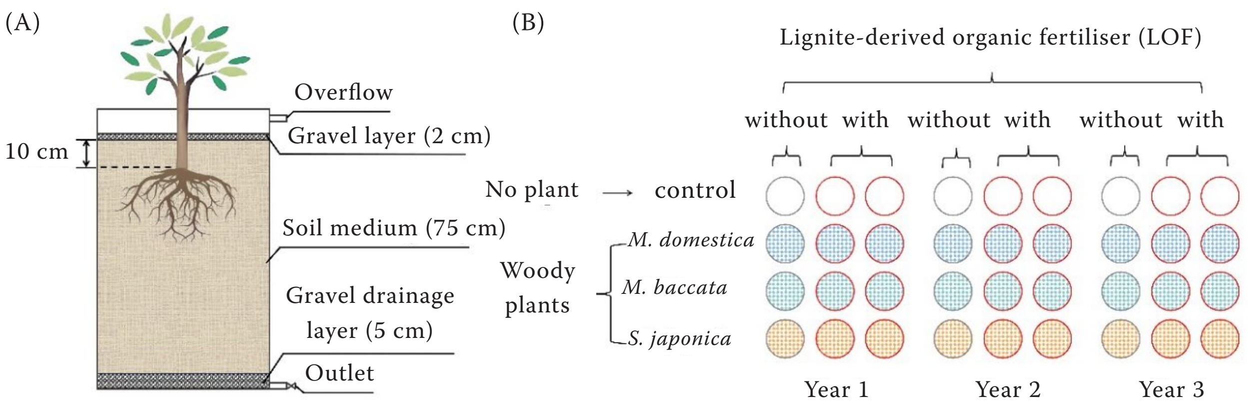

Figure 1. Experimental setup: (A) is the vertical profile view of the experimental columnar setup; (B) is the layout plan of the experiment, where the circles represent the columnar setup. Year 1, 2, and 3 represent the year when the destructive experiments were conducted. The white, blue, green, and orange fills in the circles represent the no woody plant, planted *M. domestica*, *M. baccata*, and *S. japonica* treatments, respectively; grey and red borders in circles represent non-LOF applied and LOF applied treatments

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area, root surface area, root volume, and root tips), as well as leaf relative chlorophyll content, as determined by the soil plant analysis development value (Zhang et al. 2022).

Soil indicators. Soil samples were collected through destructive sampling using the five-point method within the column devices. The physical and chemical indexes of soil samples were measured according to the Chinese national standard. Soil pH was determined according to Soil-Determination of pH-Potentiometry (HJ 962-2018). Soil aggregates were evaluated according to the standards outlined in the Soil Testing-Part 19: Method for Determination of Soil Water Stable Macro-aggregates Distribution (NY/T1121.19-2008). Soil ammonia nitrogen and nitrate nitrogen were determined according to Soil-Determination of ammonium, nitrite and nitrate by extraction with potassium chloride solution – spectrophotometric methods (HJ 634-2012). Soil total nitrogen was assessed following the Soil Quality – Determination of total nitrogen – Modified Kjeldahl method (HJ 717-2014). SOM was assessed following the Soil Testing Part 6: Method for determination of soil organic matter (NY/T1121.6-2006). The soil aggregates and SOM were sent to Apaxfon Biosciences Technologies Co., Ltd. for analysis.

For soil microorganisms, the 16S rRNA gene sequencing method was used for quantitative measurement of bacteria. Sequencing was performed following a basic high-throughput sequencing process using deoxyribonucleic acid (DNA) extraction, polymerase chain reaction amplification, and MiSeq high-throughput sequencing. First, the DNA of the soil samples was extracted, and then the 16s rDNA V3 + V4 region (primer sequence 341F/806R) was amplified. Operational taxonomic unit (OTU) cluster analysis, in-depth species taxonomical analysis, and statistical data analysis (Zhang et al. 2023). Additionally, the diversity of microbial communities in the samples was evaluated using α-diversity indexes, including the Shannon index and Chao1 index (Jeanne et al. 2019, Li et al. 2022).

Plant indicators. Plant roots were destructively sampled yearly from October to December (2021–2023). Root images were captured using a scanner (Epson Expression 1680, Seiko Epson Corporation, Nagano, Japan). The root indicators including root length, average diameter, projected area, surface area, volume, and tips were statistically analysed with the WinRHIZO root analysis system (Shanghai Zequan Technology Co., Ltd., Shanghai, China). The SPAD values, indicating relative chlorophyll content, were measured using a SPAD chlorophyll detector, model TYS-A (Beijing Zhongke Weiho Technology Development Co., Ltd., Beijing, China).

Data analysis. For statistical analyses, the normal distribution of the data was tested using the Shapiro-Wilk test (A'Aqoulah et al. 2024). The Student's *t*-test was used to investigate the significant ($P < 0.05$) differences in indictors under different treatments (Zhao et al. 2021). All statistical analyses were done by IBM SPSS Statistics for Windows (version 26.0; IBM Corp., Armonk, USA) (Lin et al. 2021).

The degree of correlation between different indicators was analysed using the Pearson correlation coefficient (Liu et al. 2020): the calculation formula is shown in Eq. (1). The calculation results are displayed by OriginPRO (version 2021; Origin Lab, Northampton, USA) (Anwar et al. 2024, Lee et al. 2024).

$$r = \frac{\sum_{i=1}^{n}(x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum_{i=1}^{n}(x_i - \bar{x})^2 \sum_{i=1}^{n}(y_i - \bar{y})^2}} \quad (1)$$

Where: x_i and y_i – values of different indicators, the correlation coefficient r takes the value range of [–1,1], $r > 0$ is a positive correlation, and $r < 0$ is a negative correlation.

Reproducibility assessment. To evaluate the reproducibility of the results of this study, we analysed the errors of the column experimental data under the treatment of LOF application. For soil indicators, the error ranges for the no-plant control, *M. domestica*, *M. baccata*, and *S. japonica* were 0.19–15.62%, 0.57–19.90%, 0.01–16.64%, and 1.25–40.16%, respectively. For *M. domestica*, the average error of root indicators and SPAD were 18.32%, and 2.67%, respectively. For *M. baccata*, the average error of root indicators and SPAD were 27.01%, and 6.95%, respectively. For *S. japonica*, the average error of root indicators and SPAD were17.89%, and 13.56%, respectively. In larger-scale outdoor simulation experiments, only 3% LOF was applied during the first year, and considering the growth differences among woody plants, the above data shows the results of this study are reproducible.

RESULTS

SOM characteristics. The impact of LOF application on SOM content that is presented in Figure 2 demonstrates a significant increase in SOM levels following LOF treatment. Three years after LOF application, SOM content increased by an aver-

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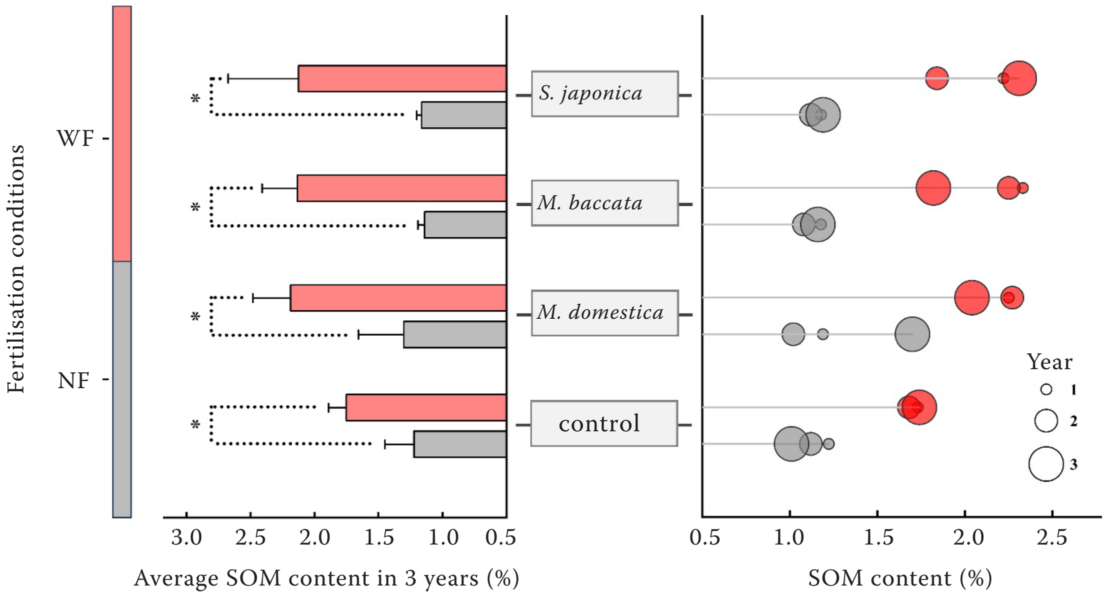

Figure 2. The soil organic matter (SOM) content under different fertilisation conditions. WF – lignite-derived organic fertiliser (LOF)-applied treatment; NF – non-LOF applied treatment. For no plants control, planted *M. domestica*, *M. baccata*, and *S. japonica* treatments, the left figure shows the three-year average of SOM content in WF and NF treatments, and *represent there is a statistically significant difference ($P < 0.05$); the right figure shows the SOM content with WF and NF treatments in the different years

age of 0.6% in soil without woody plants and by 0.9% in soil planted with *M. domestica*, *M. baccata*, and *S. japonica*. The enhancement of SOM content was more pronounced in treatments where woody plants were present, suggesting a synergistic effect between LOF application and plant-root interactions. Statistical analysis confirmed that SOM content in LOF-treated soils with woody plants was significantly higher ($P < 0.05$) compared to the no-plant control. Specifically, LOF application increased SOM content by 0.9% in *M. domestica*, and 1.0% in both *M. baccata* and *S. japonica*. This increase may be attributed to factors such as root exudates and root mortality, which contribute to soil organic matter accumulation (Adamczyk et al. 2019, Chen et al. 2023).

Soil aggregate characteristics. The application of LOF in this study was limited to 3%, which may not have been sufficient to induce significant soil aggregate formation (Shao et al. 2019). Following LOF treatment, notable fluctuations were observed only in soil aggregates smaller than 0.25 mm (Figure 3). After LOF application, aggregates < 0.25 mm increased consistently in *M. baccata* across all three years and in the second year for *M. domestica* and *S. japonica*, with increases ranging from 1.0% to 21.2%. In contrast, these aggregates decreased in the first and third years for *M. domestica* and *S. japonica*, with reductions ranging from 0.6% to 18.3%. Aggregate size > 1.0 mm was observed only during the second and third years, irrespective of plant presence. These findings suggest that soil aggregate composition is influenced not only by SOM but also by multiple factors, including soil microorganisms, plant root activity, nutrient dynamics, and cementing substances (Chaplot and Cooper 2015, Li et al. 2017, Tang et al. 2018, Yao et al. 2024).

Soil microbial characteristics. The bacterial 16S rRNA gene copy number (GCN) in soils with or without three woody plants and LOF are presented in Figure 4. Overall, the application of LOF could enhance the GCN in soil. In soils without woody plants, the GCN increased by 1.8 times in the first year and by 1.3 times in the second year after LOF application; however, the GCN decreased slightly by 17.6% in the third year after the LOF application. This may be due to the large number of exogenous microorganisms in LOF entering the soil, which could have a beneficial effect for a short period. The soil microorganisms were selective to exogenous microorganisms, which disturbed the microbial environment of the soil after a period of time and could affect the bacterial GCN (Allison and Martiny 2008, Semenov et al. 2021).

The planting of woody plants could also lead to an increase of GCN in soil. Compared with non-woody

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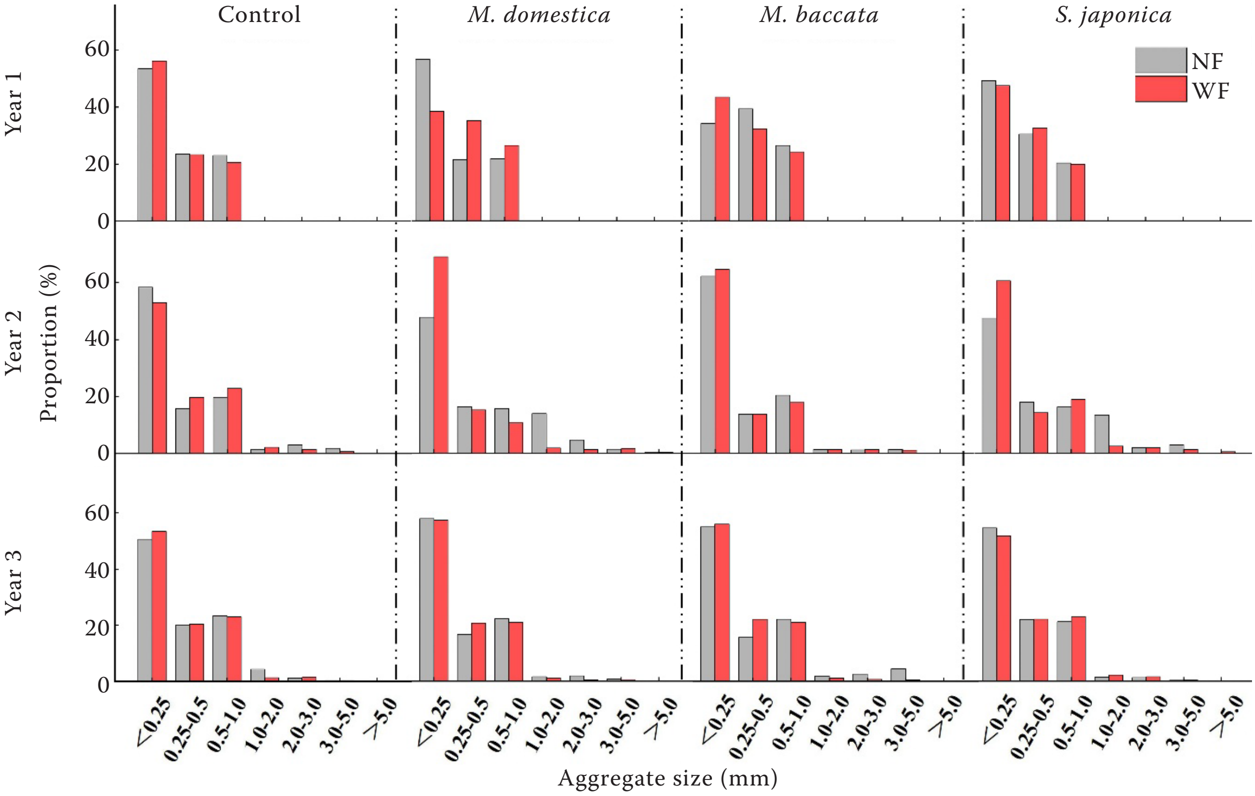

Figure 3. The characteristics of soil aggregates under different fertilisation conditions. WF – lignite-derived organic fertiliser (LOF)-applied treatment; NF – non-LOF applied treatment

plants, in soils without LOF, the GCN increased by 0.5–7.9 times with three woody plants; while in soils with LOF applied, it increased by 0.3–3.0 times with three woody plants. In addition, LOF further promoted the increase of GCN in soils with woody plants. In soils without LOF, the GCN gradually increased from 3.7–5.0 × 10^7/g to 3.6–4.6 × 10^8/g with three years of woody plants planting. In contrast, in soils with LOF, the GCN rapidly increased from 1.3–7.2 × 10^7/g in the first year to 4.2–6.1 × 10^8/g in the second year with woody plants; however, in the third year, the GCN (2.5–3.9 × 10^8/g) in soils with LOF and woody plants decreased to similar levels in soils without LOF and with woody plants (3.6–4.6 ×

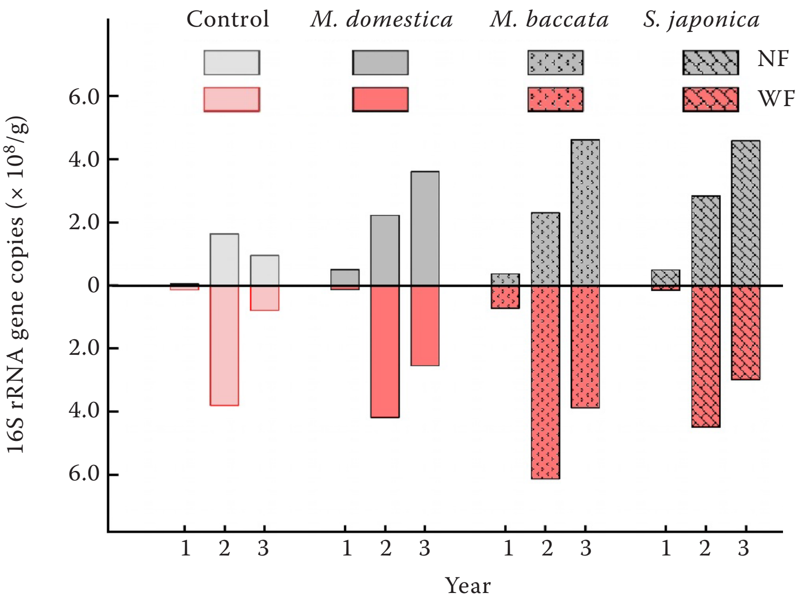

Figure 4. The 16S rRNA gene copy number (GCN) of soil under different fertilisation conditions. WF – lignite-derived organic fertiliser (LOF)-applied treatment; NF – non-LOF applied treatment

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10^8/g). This may be due to a combination of soil microbial selectivity for exogenous microbes in the LOF and root secretions (Podmirseg et al. 2019, Yang et al. 2024).

The application of LOF could enhance microbial diversity and abundance in soil without woody plants (Figure 5). After lots of beneficial bacteria in LOF entered the soil (Fallgren et al. 2021, Chen et al. 2023), the original flora in the soil was significantly affected in the first year, and the microbial Shannon index and Chao1 index decreased. After that, the soil gradually adapted to the beneficial flora brought by LOF, and the soil microbial Shannon index and Chao1 index increased by 7.9% and 6.1% in the third year with LOF applied. However, the positive effects of LOF on soil microbial diversity and abundance were diminished under the combined influence of woody plants and LOF. In soils planted with different woody plants, the soil microbial Shannon index (except for *M. domestica* in the first year) and Chao1 index (except for *M. baccata* in the second year and *S. japonica* in the third year) decreased by 1.1–54.7% and 0.04–51.1% respectively, in the three years with LOF applied. This reduction may be attributed to the interactions between soil microorganisms and the roots of woody plants, including root secretions, which warrant further investigation (Aydogan et al. 2018, Sasse et al. 2018, Zhao et al. 2021).

Woody plants' root characteristics. The root characteristics of the three woody plants with or without LOF application are illustrated in Figure 6. Overall, the application of LOF significantly improved the root characteristics of the woody plants. In the first and second years after the LOF application, the root indicators (all positive indicators) for the three woody plants increased by 52% and 23%, respectively. Among these indicators, root volume shows the most notable improvement, with an average increase of 61%. Specifically, the root volume of *S. japonica* increased by 151% and 84% in the first and second years with LOF applied, respectively.

The average root diameter of the woody plants, in contrast, was less influenced by LOF, with values ranging from 0.89 to 1.33 times that of the non-LOF treatment in the first and second years. This was primarily due to the accelerated growth of the root system after LOF application, which could lead to an increase in the number of lateral and fine roots, thus impacting the average root diameter. The characteristics of root tips of different woody plants varied during the first and second years after LOF application, compared with non-LOF treatment, the root tips of *M. domestica* and *S. japonica* increased by 36% and 52%, respectively, and those of *M. baccata* decreased by 38%.

The root indicators of the woody plants decreased in the third year after LOF application. Compared to the non-LOF treatment, the root indicators of *M. domestica*, *M. baccata*, and *S. japonica* in the third year were 0.98–1.06, 0.63–0.95, and 0.71–1.15 times of those under non-LOF treatment, respectively. This reduction may be attributed to the constraints on root system growth imposed by the limited size of the experimental device in the third year.

Woody plants' SPAD characteristics. The SPAD values of the three woody plants with or without LOF

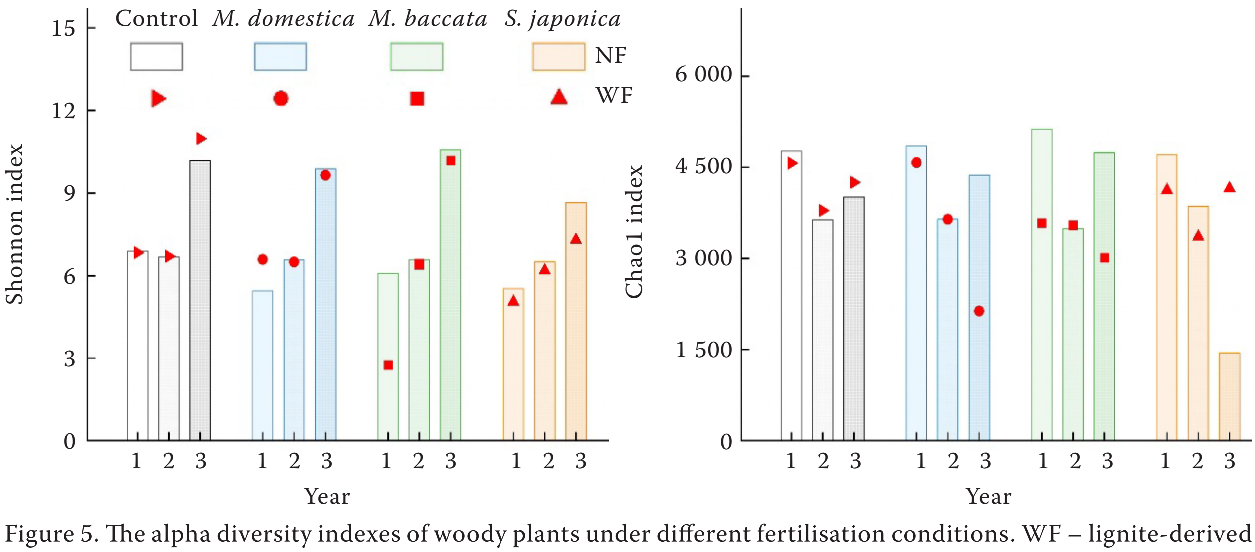

Figure 5. The alpha diversity indexes of woody plants under different fertilisation conditions. WF – lignite-derived organic fertiliser (LOF)-applied treatment; NF – non-LOF applied treatment

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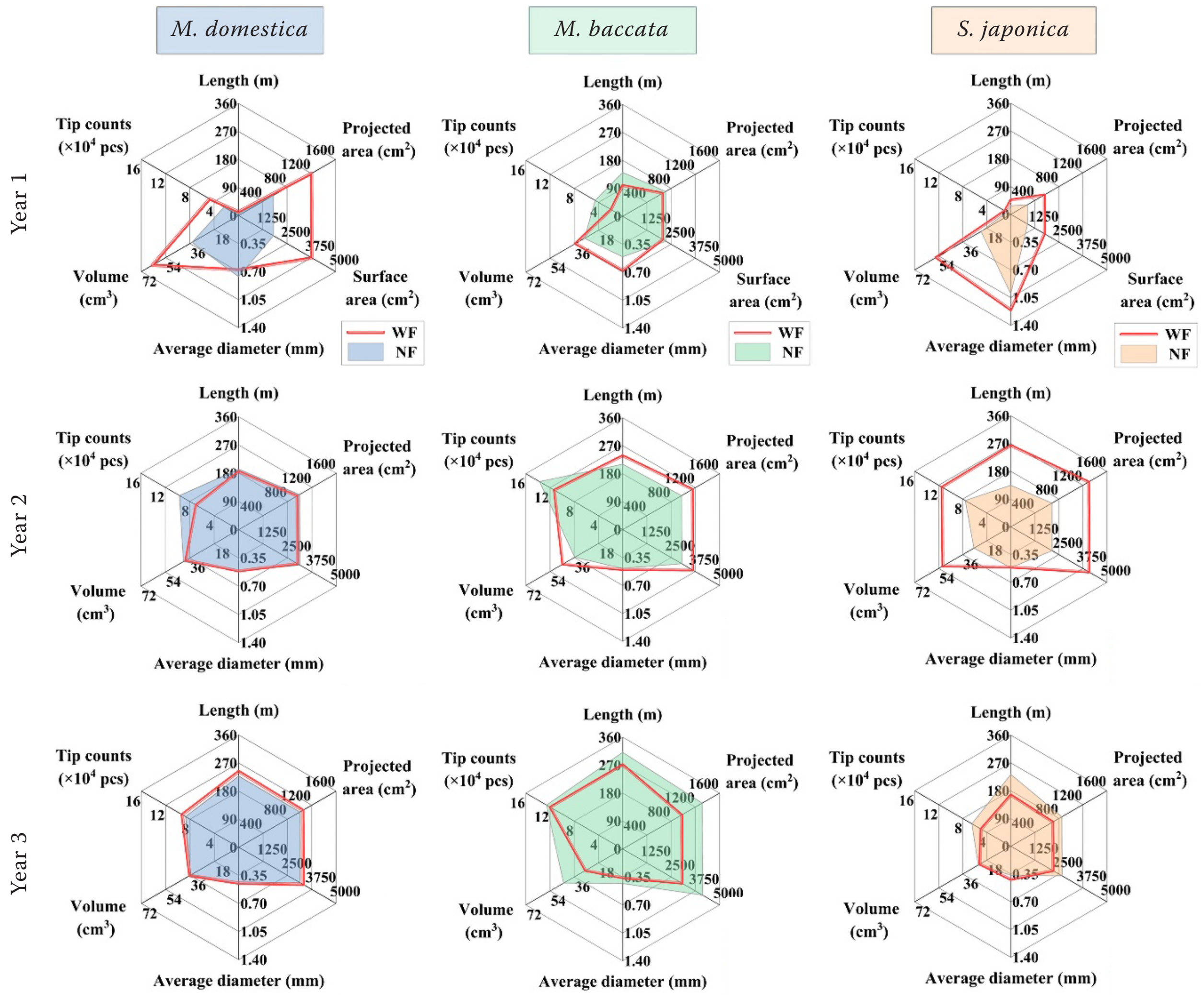

Figure 6. The characteristics of woody plant roots under different fertilisation conditions

are shown in Figure 7. Compared to the non-LOF treatment, the SPAD values of the woody plants increased by 3.1% and 3.7% in the first and second years with LOF application, respectively. Among three different woody plants, *M. baccata* exhibited the most significant response to LOF, with an average SPAD value increase rate of 16.8% in the first and second years compared to the non-LOF treatment.

The SPAD values of *M. domestica* and *M. baccata* in the third year with LOF applied increased by 0.8% and 2.9%, respectively, compared to the non-LOF treatment. However, the SPAD value of *S. japonica* decreased by 7.9%. This decline may be due to the limitations imposed by the experimental space on *S. japonica*'s well-developed root system during the later stages of growth, which adversely affected plant growth and development.

The SPAD values of the different woody plants without LOF application generally followed a trend of initial decline followed by an increase over the three years. The SPAD value of *M. baccata* fluctuated the most (SPAD value ranging from 26.9% to 43.5%), while *S. japonica* exhibited the least fluctuation in SPAD value (ranging from 27.8% to 38.1%). Overall, LOF application could enhance the SPAD values of woody plants to some extent, but its effectiveness gradually diminishes over time.

DISCUSSION

Correlation of root and soil characteristics among different woody plants. The results of the correlation analysis between soil properties and plant growth characteristics are presented in Figure 8.

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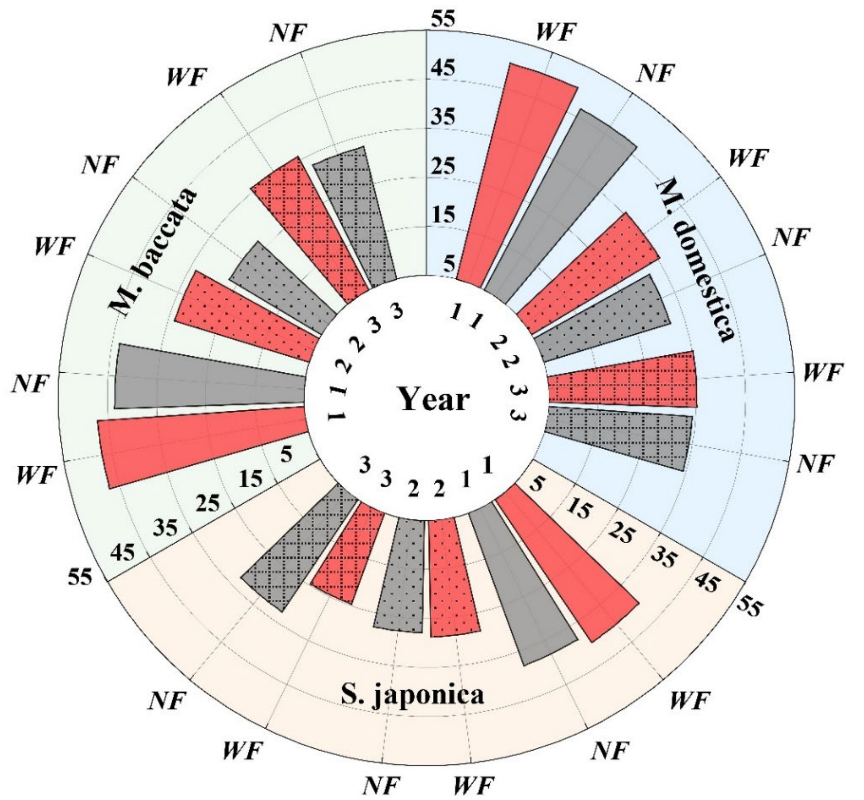

Figure 7. The soil plant analysis development (SPAD) characteristics of woody plants under different fertilisation conditions. WF – lignite-derived organic fertiliser (LOF)-applied treatment; NF – non-LOF applied treatment

The application of LOF significantly enhanced SOM content, which in turn contributed to improvements in both soil quality and plant growth parameters. To account for potential spatial constraints in the experimental setup, correlation analysis was conducted using data from the first and second years. This approach was chosen because, by the third year, plant growth may have been restricted by the limited size of the experimental columns, potentially confounding the observed relationships.

The analysis revealed that SOM was positively correlated with soil aggregates < 0.25 mm, suggesting an influence of SOM on fine aggregate stability. Soil bacterial gene copy number (GCN) increased with higher SOM, indicating a beneficial effect of SOM on microbial abundance. In addition, root development was enhanced by higher SOM, indicated by root diameter (R-Diam), root surface area (R-Area), and root volume (R-Vol). Moreover, SPAD value increased with higher SOM, reflecting improved chlorophyll content and overall plant vitality. These findings highlight the critical role of SOM in fostering both soil microbial activity and plant physiological responses, further reinforcing the importance of organic amendments like LOF in enhancing soil fertility and plant carbon sequestration capacity.

Soil aggregate formation is influenced by multiple factors, including SOM content, chemical composition, plant root systems, and soil biology (Tang et al. 2018). In this study, correlations between SOM and soil aggregate size varied among different woody plant species. In *M. domestica* and *S. japonica*, SOM was positively correlated with < 0.25 mm soil aggregates (r = 0.87 and 0.52, respectively). In *M. baccata*, SOM was negatively correlated with < 0.25 mm soil aggregates (r = –0.14), suggesting species-specific differences in root interactions with soil structure. Previous studies indicate that root diameter, root length density, root hairs, fine root percentage, and root secretions all play critical roles in influencing aggregate size (Poirier et al. 2018). The effects of roots on soil aggregation may also vary depending on SOM (Erktan et al. 2016). Further investigation is required to elucidate the specific mechanisms driving soil aggregate dynamics in this experiment. SOM was positively correlated with soil bacterial 16S rRNA gene copy number (GCN) (r = 0.29), indicating that higher SOM levels support bacterial proliferation and aggregation in the soil. This effect was most pronounced in soils planted with *M. domestica* and *M. baccata*. Similar findings have been reported in previous studies, where organic fertiliser application

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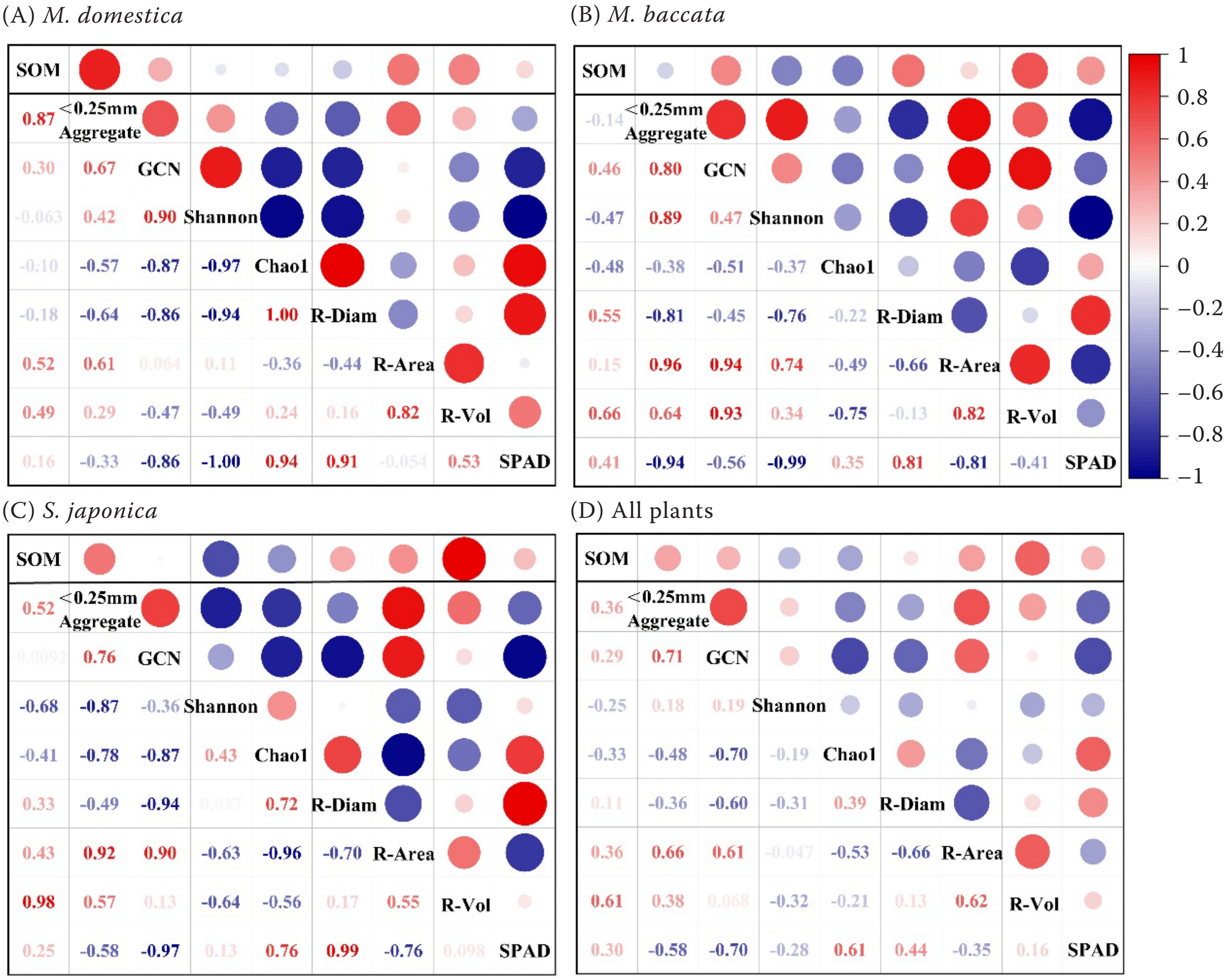

Figure 8. The correlation of root traits and soil characteristics among different woody plants. SOM – soil organic matter; GCN – gene copy number; SPAD – soil plant analysis development

enhanced bacterial gene abundance and influenced microbial community structure (Lian et al. 2021, Ren et al. 2022, Fang et al. 2024).

A significant positive correlation was observed between SOM and root volume (r = 0.61), with variations among species. *S. japonica*: (r = 0.98) showed the highest correlation, likely due to its robust root system and well-developed primary roots. *M. baccata* (r = 0.66) and *M. domestica* (r = 0.49) also aligned with previous studies demonstrating that organic fertilisers enhance root growth across multiple plant species (Yang et al. 2001, Zhou and Fan 2008, Fan and Yang 2009, Wang et al. 2019, Qiu et al. 2024). For example, Huai et al. (2020) found that increased organic fertiliser application enhanced root length density in maize by an average of 0.18 cm/cm^2 across all soil layers.

Soil organic matter positively correlated with SPAD values (r = 0.30), suggesting that increased SOM enhances chlorophyll content through organic fertiliser application. Previous studies have demonstrated that organic fertilisers significantly increase chlorophyll content, improving plant growth and photosynthetic efficiency. For example, Wei et al. (2024) reported that organic fertiliser application increased SPAD values in *Pinellia ternata* and promoted plant growth. Ji et al. (2024) observed a 25.90% increase in chlorophyll content in crops fertilised with organic fertiliser derived from Chinese medicinal residue.

These findings highlight the multifaceted role of SOM in enhancing soil structure, microbial activity, root development, and chlorophyll content, ultimately improving plant growth and carbon sequestration potential. The species-specific variations observed in soil aggregation and microbial responses underscore the complexity of plant-soil interactions and the need for further research to optimise organic fertiliser applications for different woody plant species.

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Effect of LOF on soil microorganisms. Soils exposed to external disturbances, such as organic fertiliser application, often exhibit varying effects on microbial community composition, activity, and abundance. These disturbances can either reshape the microbial structure by selecting for a new community or allow the original microbial parameters to recover or resist environmental changes (Allison and Martiny 2008, Podmirseg et al. 2019).

No significant difference ($P > 0.05$) in soil microbial characteristics was observed between LOF-treated and untreated soils in this study. While organic fertilisers typically enhance microbial biomass and diversity by activating native soil microbial communities (Semenov et al. 2021), soil microorganisms are inherently selective toward exogenous microorganisms. As a result, most foreign microorganisms introduced through LOF were unable to establish themselves long-term under soil conditions, contributing to the lack of significant microbial changes observed in this study. Similar findings have been reported in related studies, which suggest that organic fertilisers often do not significantly impact soil microbial diversity or activity (Reilly et al. 2013, Shu et al. 2023). However, organic fertilisers can alter soil microbiota structure and increase the abundance of prokaryotic organisms, potentially exerting long-term effects on microbial communities (Hartmann et al. 2015, Semenov et al. 2020). It is also important to note that only 3% LOF was applied in the first year, which may have been insufficient to induce substantial changes in soil microbial composition. The low application rate of LOF could be a key factor in the observed lack of significant microbial effects, as higher doses of organic amendments are often required to drive noticeable shifts in microbial dynamics.

The findings suggest that while LOF did not significantly alter soil microbial characteristics in the short term, its potential long-term influence on soil microbiota structure warrants further investigation. Future studies should explore higher LOF application rates and extended monitoring periods to determine whether larger doses or prolonged exposure could lead to more pronounced microbial shifts.

Effect of LOF on carbon sequestration in woody plants. Overall, the application of organic fertiliser significantly enhances soil organic matter, influences soil aggregate composition and microbial characteristics, improves soil physicochemical properties, promotes root development in woody plants, and ultimately enhances their carbon sequestration capacity. According to the Global Forest Resources Assessment 2020 by the Food and Agriculture Organisation of the United Nations (FAO), total forest carbon stock reached 662 gigatons in 2020, with most carbon stored in living biomass (44%) and SOM (45%). A lot of reports indicate that for every cubic meter of forest growth, an average of 1.83 tons of CO_2 is absorbed while 1.62 tons of oxygen is released. This highlights the crucial role of forests as natural carbon sinks. Organic fertilisers have the potential to further enhance plant growth and carbon storage capacity, thereby strengthening carbon sequestration and accelerating progress toward carbon neutrality. In this study, LOF application increased SOM content by an average of 0.82 times, effectively improving soil health and plant development, which in turn could enhance plant carbon sequestration capacity and improve urban greening (Luo et al. 2023, Wu et al. 2024, Xiong et al. 2024).

These findings emphasise the importance of organic soil amendments, such as LOF, in restoring soil health, enhancing urban greening, and mitigating climate change. But accurate calculation of carbon sink flux from woody plants with LOF should be considered in the future. Further research and large-scale implementation of organic fertilisers could contribute significantly to improving urban greening, enhancing terrestrial carbon sinks and global carbon management strategies.

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