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Biochar

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# Plant performance and soil–plant carbon relationship response to different biochar types

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**Abstract**

Biochar (BC) applications in soil has positive effects on plant performance, particularly for loose soil in agricultural context. However, how biochar types affect plant performance of non-crop species and soil–plant carbon relationships is not clear. We selected five different BC types and three plant species to investigate the responses of plant performance and the soil–plant carbon relationship to BC effects. The result demonstrated that peanut shell BC led to the death of both *R. tomentosa* and *C. edithiae*, due to a reduction in nutrient uptake caused by higher soil electricity conductivity (2001.7 and 976.3 µS $cm^{-1}$). However, the carbon content of *S. arboricola* increased by 57% in peanut shell BC-amended soil, suggesting that *S. arboricola* has a higher tolerance for soil salinity. Wood BC-amended soil led to better stomatal conductance ($g_s$) and leaf area index (LAI) of both *R. tomentosa* and *C. edithiae* due to the higher water retention in the soil (22.68% and 20.79%). This illustrated that a higher amount of water retention brought by wood BC with a great amount of pore volume might be the limited factor for plant growth. The relationship between $g_s$ and LAI suggested that $g_s$ would not increase when LAI reached beyond 3. Moreover, wood and peanut shell BC caused a negative relationship between soil organic carbon and plant carbon content, suggesting that plants consume more carbon from the soil to store it in the plant. Overall, wood BC is recommended for plant growth of *R. tomentosa* and *C. edithiae*, and peanut shell BC is suggested for *S. arboricola* carbon storage.

## Highlights

- Peanut shell biochar enhanced soil salinity which causes the death of *R. tomentosa* and *C. edithiae*, while wood biochar is suitable for these plant species.
- Wood and peanut shell biochar caused a negative relationship between soil organic carbon and plant carbon content.
- The stomatal conductance will not increase when the leaf area reaches the limiting value 3.
- An empirical function is developed to correlate plant carbon content and leaf area index under different biochar applications.

**Keywords** Biochar, Carbon content, Feedstocks, Stomatal conductance, Leaf area index

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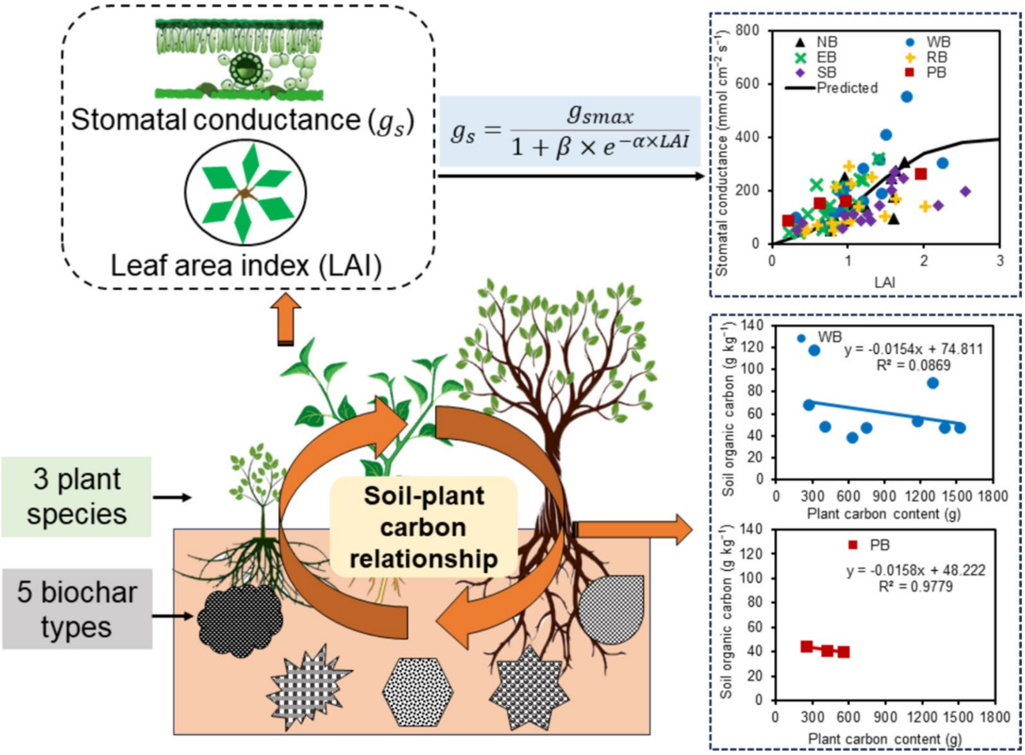
## Graphical Abstract



## 1 Introduction

Biochar (BC) is a carbon-rich porous material that is produced through a process called "pyrolysis" in which organic biomass is heated in a limited oxygen environment (Pardo et al. 2019). BC has been applied to vegetated soil for three major beneficial reasons, i.e. improving plant performance, changing soil nutrient properties, and sequestering stable carbon in the soil (Renforth et al. 2011). On the other hand, soil types and pedoclimatic conditions also influence the BC performance (Schmidt et al. 2021). The positive effects of BC properties on plant growth depend on the feedstock type and have been well-documented in agricultural applications. For instance, Ochiai et al. (2021) reported that manure BC application increased the average biomass production of oat plants by 26% compared with the wood BC application. They have also pointed out that the feedstock type of BC was a more dominant factor than carbonization temperature. The selection of feedstock determines the characteristics of BC, such as ash content, carbon and nitrogen ratio, pH, surface area, and cation exchange capacity (CEC) (Singh et al. 2010). Many feedstocks have been utilized for BC production, such as wood, straw (Kloss et al. 2012), orange peels, bagasse, bamboo (Sun et al. 2014), and manure which are all sourced from locally available biomass. Even though it has been suggested that other factors, including soil type, temperature, and water content (Phillips et al. 2020), may have an impact on BC application effects, the effects of BC types synergistic with soil properties on plant performance are not well understood.

Plant performance, including growth and hydraulic properties, has received considerable attention after BC application, especially for those crop species (Borges et al. 2020). Non-crop species, such as vegetation for ecological restoration and green infrastructure (Garg et al. 2015), have been seldom studied for plant survival parameters such as stomatal conductance ($g_s$), which are required to interpret plant survival in the early transplantation stage (Bordoloi and Ng 2020). Plant hydraulic properties, such as $g_s$, can be influenced by vegetation species, which are also significantly influenced by soil properties. Thus, understanding the hydrological performance of plants is important for engineers to design vegetated geotechnical infrastructure (Ng et al. 2019a). Besides, the major pathway for $CO_2$ to enter plants is also through the stomatal pore of the leaves and $g_s$ acts as an indirect indicator of carbon assimilation (Jezek et al.

2021). Therefore, $g_s$ is a major parameter that will influence both the plant photosynthesis process and carbon assimilation. The measurement of plant $g_s$ is an essential tool for determining the degree of stomatal opening and for analysing the equilibrium and exchange of water and carbon dioxide between plants and the atmosphere (Jasechko et al. 2013). Keenan et al. (2013) have reported that there is a linear relationship between $g_s$ and the photosynthetic uptake of $CO_2$, which can be used to estimate plant carbon assimilation. It is well known from the literature that BC application changed poor soil properties and generally provided more nutrients for plant growth. For crop species, Ahmed et al. (2018) reported that straw wheat BC improved maize $g_s$, because BC addition delayed the time of plant reaching the critical soil water content. However, it has been argued that plants respond to BC differently according to its types (Joseph et al. 2021). In particular, plant morphology governs the $g_s$ response in BC-amended soil (Ng et al. 2021). For non-crop species, different BC types will likely induce varied responses in $g_s$. According to niche differentiation theory, diverse plant species can live by dividing gradients in resource availability and diverging in features that are highly effective in certain niches (Grubb 1977). Thus, the hydraulic properties of different plant species determine water availability, which might be one of the major drivers for plant performance (Comita and Engelbrecht 2009). Markesteijn et al. (2011) pointed out that hydraulic properties varied across species, which might be related to the classical trade-off between secure photosynthesis and stomatal closure. Plant assimilation is the process that transforms carbon dioxide into sugar for plant growth, which contributes to global carbon neutrality (Jansson et al. 2010). BC application improved plant growth and photosynthesis thereby increasing below-ground carbon inputs. For example, a periodic $^{13}CO_2$ pulse-labeling approach has been used to find that adding BC to ferrallisol soil boosted the below-ground recovery of fresh ryegrass root-derived C by 20% (Weng et al. 2017). The uptake and dynamics of photosynthetic C in plant-soil systems will be further modulated by the favourable or negative effects of BC on plant photosynthesis. Even though there is numerous research on the impact of BC on C dynamics, most of them have concentrated on how native soil organic carbon and exogenous organic compounds (i.e., sucrose and humic acid) will change upon BC application (Zimmerman and Ouyang 2019; Kuzyakov et al. 2014). It remains critically important to understand the consequences of photosynthesis assimilated carbon content in plants, both above- and below-ground after BC application.

Unfortunately, due to the commercial value of the non-crop species, the correlations between indicators of early plant establishment such as leaf area index (LAI) with the transpiration parameters and available plant-to-soil carbon ratios were seldom studied. It has been reported that LAI is the major factor in determining canopy photosynthesis by many studies (Schieving and Poorter 1999; Parker 2020). The use of LAI is to reflect the quantitative changes in plant foliage surface to describe the changes in foliage growth (Medina and Klinge 1983). With the help of remote sensing technology, the LAI has been used in modeling forest ecosystem growth and water exchange in forest ecosystems (Running and Nemani 1988). Besides, the tree morphology (i.e., LAI) will directly influence plant hydraulic properties, such as $g_s$ (Bordoloi et al. 2024). Thus, BC effects on plant LAI were investigated in the current study to reflect the relationship between LAI and plant carbon content.

The overarching objective of this study is to investigate the effects of different BC types on plant performance and soil–plant carbon relationship, thereby providing a guideline for the selection of BC types used in soil. Five BC types and three non-crop species suitable for ecological rehabilitation in Hong Kong, China were chosen to conduct a pot trail test. The physicochemical properties of BC and BC-amended soil were measured and plant parameters were regularly monitored during the growth stage. At the end of cultivation, plant carbon content and soil organic carbon were measured to reflect the soil and plant relationship. The correlations between the BC and plant properties were discussed in this study to provide new insights that can be useful for ecological rehabilitation.

## 2 Materials and methods

### 2.1 BC production and measurements

Based on the chemical components of feedstocks, the BC types can be classified into four types. They are starch-rich BC (rice), calcium-rich BC (eggshell), protein-rich BC (soybean), and cellulose-rich BC (wood). A commercially available peanut shell BC was also chosen for comparison. These different types indicate different nutrient types for BC application. For instance, the cellulose in woody residue can decay into smaller molecules during the pyrolysis process (Gholizadeh et al. 2019), while the proportions of C, H, and O content will be different for starch-rich BC. The feedstocks of BC, including rice, eggshell, soybean residues, and waste wood were collected from the kitchen and garbage station. The feedstock of peanut shell BC was sourced from agricultural waste, mainly from peanut shells. The collected feedstocks were placed in the oven (100 °C) and dried for 24 h until they reached constant weight. Before pyrolysis, the feedstock was all grounded and sieved at 4.75 mm. The pyrolysis reactions were carried out by using a tube furnace

(CARBOLITE GERO, TS1/3 1200 mm Ø 200 mm), and the pyrolysis temperature was controlled at 500 °C for 1 h (José et al. 2019). The pH and EC were measured by pH meter and EC meter (ASTM 2013). The moisture content, mobile matter, ash content, and resident matter were measured based on the measurements in ASTM (2020). Mobile matter (analogous to volatile matter), reflecting the non-carbonized portion in BC, was determined as the weight loss after heating in a covered crucible at 450 °C for 30 min. Ash content was also measured as the residue remaining after heating at 700 °C in an open-top crucible in a muffle furnace (Ahmad et al. 2012). The specific surface areas were analysed by using the Brunauer–Emmett–Teller equation (BET), and the total pore volume was estimated from $N_2$ adsorption at P/Po around 0.5 (Zeng et al. 2021). The surface physical morphology was examined by using a Scanning Electron Microscope (SEM) (Gabhi et al. 2020). The surface functional groups of BC were explored by Fourier transform infrared spectroscopy (FTIR) (Cai et al. 2021). The elemental composition of BCs including carbon (C), hydrogen (H), nitrogen (N), sulphur (S), and oxygen (O) was determined by using an elemental analyser (Ippolito et al. 2020). The CEC, extractable N, phosphorous (P), and potassium (K) in different BC were measured based on the method by Li et al. (2020).

### 2.2 BC-amended soil, vegetation, and test setup

Sandy loam soil was chosen for the current study, which is commonly used for ecological restoration in Hong Kong, China (Ng et al. 2019a). The soil type in the current study was classified by the unified soil classification system. The original soil pH was 6.56 and electricity conductivity (EC) was 61.2 $\mu S\ cm^{-1}$. The total organic carbon (TOC) and total nitrogen (TN) of the original soil were 1676 $mg\ kg^{-1}$ and 6.0 $mg\ kg^{-1}$, respectively. The phosphorus (P) and potassium (K) of the original soil were 26.5 $mg\ kg^{-1}$ and 260.5 $mg\ kg^{-1}$, respectively. The CEC of the original soil was 2.86 $cmol\ kg^{-1}$. Six test conditions, including soil without BC (control), and soil amended with wood, eggshell, rice, soybean, and peanut shell BC were conducted and labelled as NB, WB, EB, RB, SB, and PB, respectively. Before compaction, the compaction curves of each soil mixed with different types of BC (refer to Fig S1) were plotted to get the maximum dry density (MDD) and optimum water content (OMC) by using the standard test method from ASTM (2015). The MDD of NB, WB, EB, RB, SB, and PB were 1.88, 1.68, 1.71, 1.73, 1.61, and 1.58 $g\ cm^{-3}$, respectively. The OMC of NB, WB, EB, RB, SB, and PB were 12.1%, 16.87%, 17.45%, 18.15%, 20.12%, and 19.53%, respectively. Three vegetation species, including *Rhodomyrtus tomentosa, Schefflera arboricola,* and *Cyclobalanopsis edithiae* were selected for this test to investigate different BC effects on those plant performance. The native non-crop vegetative species *S. arboricola*, is commonly utilized in urban areas for geotechnical infrastructure in Hong Kong, China. It would be the first type of plant to experience the influences of elevated $CO_2$ on the performance and response to carbon assimilation (Wang et al. 2019a). *R. tomentosa* and *C. edithiae* are commonly used for ecological restoration in the early stages (Corlett 1999), and estimating their carbon assimilation helps ecologists calculate the carbon budget. In general, there were 18 groups of tests, and there were three replicates each. The detailed test plan is provided in Table S1. The shoot heights of *S. arboricola*, *R. tomentosa*, and *C. edithiae* seedlings were around 150–200 mm, 400–450 mm, and 800–880 mm, respectively. The root ball sizes of *S. arboricola* seedlings was 50 mm in diameter and 70 mm in height, while the root ball size of *R. tomentosa* and *C. edithiae* seedlings were 70 mm in diameter and 100 mm in height. Thus, it is most likely that root impedance would not be a problem based on the growth period considered in the study. The shoot and root performance of three species after 5 m is shown in Fig. S2. The soil mixed with different BC at 5% ratio by wt. was set up in plastic pots with 260 mm in height and 240 mm in diameter at the top and 200 mm at the base. Chen et al. (2018) have reported that 5% BC application is beneficial for plant performance in compacted soil. It has also been reported by Gao et al. (2021) that the more recommended BC application rate was 10.1–20 $t{\cdot}ha^{-1}$ or 2.01–4% for crop species. Therefore, 5% of the BC application ratio for non-crop species in the current study was selected. The pots were filled with sandy loam soil in OMC, and the soil was compacted at 80% in three equal layers to reach a homogeneous state in each pot (Ng et al. 2014). The soil compaction at 80% degree ensures resistance toward erosion and provides adequate strength under field applications.

### 2.3 Test procedures

In total, 54 pots (18 groups of tests with three replicates) were established to investigate the effects of BC types on the performance of different vegetation species. These pots were placed in a greenhouse with controlled temperature (25 ± 2 °C) and humidity (60 ± 3%) for five months. LED growth light was uniformly provided to all pots throughout the testing period during the day for 12 h. After transplantation, all pots were irrigated every 48–72 h to ensure that field capacity was maintained in the root zone (Ng et al. 2022a). During the growth period analogous to the early plant establishment period of five months, vegetation morphological parameters were regularly measured. Plant shoot height was measured by a flexible rule. Plant $g_s$ was measured by a SC-1 Leaf

Porometer (Meter Devices Inc., USA). LAI was calculated by leaf area and projected area of leaf canopy (Ng et al. 2022a). After five months, plants were meticulously removed from the pots and washed carefully, and then the fresh and dry weight of plants was obtained. The plant carbon content (PCC) in different parts was obtained using an elemental analyser (Lamlom and Savidge 2003) to evaluate the amount of carbon content by plants. The soil carbon content was transformed from soil organic matter by using van bemmelen factor (Heaton et al. 2016). The soil organic matter was determined by Standard method (ASTM 2020). The soil was collected in plastic bags after removing all the plants and then their basic properties, such as pH, EC (ASTM 2013), water-soluble total organic carbon (TOC), and water-soluble total nitrogen (TN) of soil were measured by TOC analyzer (Ng et al. 2022b). 1 g dry soil was used for digestion (Nelson and Sommers 1972), and then it was diluted with deionized water for measuring in TOC analyzer. $BaCl_2$ was used for digesting 1 g dry soil and then analysed by ICP for determining soil CEC (Ross and Ketterings 1995). $NaHCO_3$ was used to extract soil P and then measured with spectrophotometric (Song et al. 2019). The soil was digested with $HNO_3$ and then measured by flame photometer to determine available K (Pratt 1965).

### 2.4 Statistical analysis

The significance between different test groups was performed by using one-way ANOVA (analysis of variance) for analyzing soil and plant parameters by using IBM SPSS Statistics 27. The post-hoc test is LSD, and the significance level is set to 0.05. If the *p*-value of the mean difference is less than 0.05, it can be considered significant. As for some non-normal data, data processing such as logarithmic can be performed to make the data obey normal distribution (Lee 2020). Principal components analysis (PCA) was done using the software Origin (OriginLab Corporation, Norhampton, MA, USA) for BC effects on soil properties.

According to Granier et al. (2000), $g_s$ and LAI have a positive relationship until they reach threshold values. Based on this, we have summarized the relationship between $g_s$ and LAI from the results of this paper. The empirical Eq. 1 was proposed below:

$$g_s = \frac{g_{smax}}{1 + \beta \times e^{-\alpha \times LAI}} \tag{1}$$

where $g_{smax}$ is the maximum $g_s$ of plant. For each soil treatment, the $g_{smax}$ was selected as the maximum $g_s$ among all the plant species in the same soil treatments. For instance, the $g_{smax}$ of NB, WB, EB, RB, SB, and PB were 307.6, 551.5, 320.4, 293.6, 273.4, and 318.5 mmol $cm^{-2}$ $s^{-1}$, respectively (Table 1). Where α and β are the coefficient parameters to describe the relationship between LAI and $g_s$, and the specific value of these two parameters for each plant species are presented in Table 1. The value of LAI is between 0 to 3. Therefore, $g_s$ corresponding to different LAI can be calculated based on the Eq. 1 subsequently. For each plant species, the different growth period was recorded to investigate the relationship between LAI and $g_s$. The fitting process was produced by Microsoft Excel 2023. R-squared can be used to explain the goodness of fit for some common nonlinear regression models (Cameron & Windmeijer 1997). Therefore, the $R^2$ value for different soil treatments is presented in Table 1.

**Table 1** Summary of fitting coefficient for the correlation of LAI with carbon content

| Parameter | $g_{smax}$ | α | β | $R^2$ |
|---|---|---|---|---|
| NB | 308 | 3 | 25 | 0.9577 |
| WB | 552 | 2 | 30 | 0.9443 |
| EB | 320 | 3.5 | 35 | 0.9117 |
| RB | 294 | 3 | 20 | 0.8803 |
| SB | 273 | 2 | 20 | 0.9152 |
| PB | 319 | 1 | 3 | 0.8682 |

*α* and *β* are the coefficient parameters. Soil without biochar (NB), soil with wood biochar (WB), soil with eggshell biochar (EB), soil with rice biochar (RB), soil with soybean biochar (SB), soil with peanut shell biochar (PB)

According to Keenan et al. (2013), the photosynthetic uptake of $CO_2$ ($A_{net}$) can be calculated by following equation (2).

$$A_{net} = g_s(c_a - c_i) \tag{2}$$

where $g_s$ can be replaced by Eq. 1. $C_a$ is the external $CO_2$ concentration in the atmosphere, and $C_i$ is the internal $CO_2$ concentration in a plant leaf. Therefore, the fitted parameters for the correlation between carbon content and LAI based on the empirical function proposed above can be deduced in the below equation (3).

$$A_{net} = \frac{g_{smax}}{1 + \beta \times e^{-\alpha \times LAI}} \times (c_a - c_i) \tag{3}$$

where the $g_{smax}$ is the maximum $g_s$ of plant. The $g_{smax}$ of *R. tomentosa*, *C. edithiae*, and *S. arboricola* were 551, 284, and 319, respectively (Table 2). $C_a$–$C_i$ represents the difference in $CO_2$ concentration inside and outside of the plant leaves. The external $CO_2$ concentration in the atmosphere is a consistent value (i.e., 400 ppm) (Ng et al. 2019b). Thus, $C_i$ is different according to different plant

**Table 2** Summary of fitting coefficient for the correlation of LAI with carbon content

| Parameter | *R. tomentosa* | *C. edithiae* | *S. arboricola* |
|---|---|---|---|
| $g_{smax}$ | 551 | 284 | 319 |
| α | 2 | 5 | 3 |
| β | 15 | 5 | 20 |
| $C_a$-$C_i$ | 2 | 5 | 1 |
| $R^2$ | 0.7025 | 0.9726 | 0.7955 |

$g_{smax}$ is the maximum stomatal conductance of plant. α and β are the coefficient parameters. $C_a$-$C_i$ is the difference in $CO_2$ concentration inside and outside of the plant leaves

species. Where α and β are still the coefficient parameters to describe the relationship between LAI and $g_s$, which will change based on different LAI values. The $R^2$ has also been used to describe the fitting performance of this equation. Detailed information on the parameters can be found in Table 2. The fitting process was also explored by Microsoft Excel 2023.

## 3 Results and discussion

### 3.1 Plant biomass and soil properties response to different BC types

Total biomass growth for the non-crop species grown in soil amended with different BC types is shown in Fig. 1. Except for the case of PB amended soil, BC application in soil increased overall plant biomass with respect to control. This increase for all three species could be attributed to plant available soil nutrients (N, P, and K) from BC application (Table 3) as well as expected improvement in plant available water content (Razzaghi et al. 2020). BC overall has been reported to improve soil nutrient retention capacity by reducing leaching as it accommodates soluble nutrients in the intrapore and retains ionized nutrients through surface charge effects (Hossain et al. 2020). In particular, the dramatic increase ($p < 0.05$) in *C. edithiae* biomass was found for the wood and eggshell BC that had a relatively higher surface area facilitating the aforementioned positive effects (Table 3). High surface area and pore volume also provide a habitat for beneficial bacteria and fungi, thereby stimulating plant growth (Ng et al. 2023). In the excellent lab test of Guo et al. (2023),

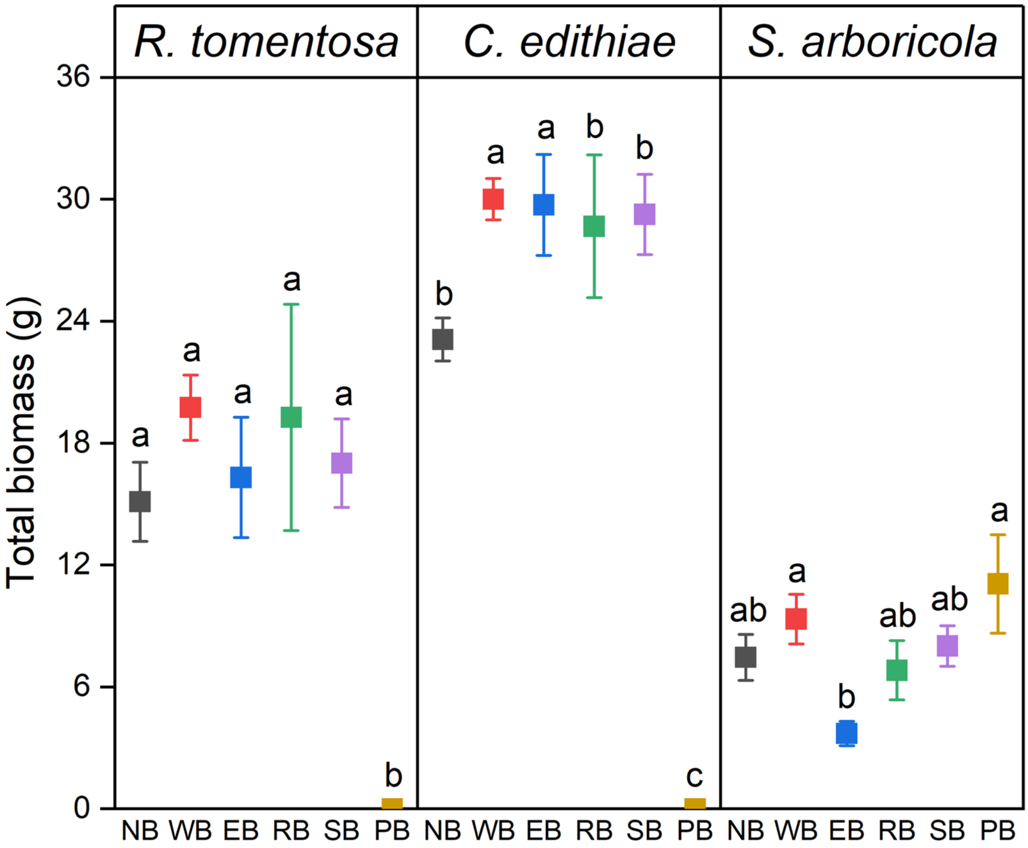


**Fig. 1** The effects of biochar types on vegetation biomass of *R. tomentosa*, *C. edithiae*, and *S. arboricola*. (NB: no biochar effects; WB: wood biochar effects; EB: eggshell biochar effects; RB: rice biochar effects; SB: soybean biochar effects; PB: peanut shell biochar; Value is reported as mean ± standard error, n = 3; and different letters indicate significant difference ($p < 0.05$; ANOVA))

**Table 3** The basic properties of different biochar types

| Feedstock | Unit | WB | EB | RB | SB | PB |
|---|---|---|---|---|---|---|
| pH | – | 8.4 | 9.7 | 7.0 | 10.0 | 8.0 |
| EC | $\mu$S cm$^{-1}$ | 8800 | 200 | 4900 | 6600 | 2300 |
| CEC | cmol kg$^{-1}$ | 2.33 | 12.26 | 0.93 | 14.99 | 24.74 |
| Water content | % | 3 | 0 | 4 | 8 | 41 |
| Mobile matter | | 46 | 0 | 8 | 18 | 23 |
| Ash content | | 46 | 3 | 90 | 41 | 35 |
| Residue content | | 8 | 97 | 2 | 41 | 42 |
| Surface area | m$^2$ g$^{-1}$ | 3.23 | 0.68 | 0.66 | 0.95 | 2.70 |
| Total pore volume | 10$^{-3}$ cm$^3$ g$^{-1}$ | 1.48 | 0.37 | 0.34 | 0.35 | 1.37 |
| Extractable N | mg kg$^{-1}$ | 1.7 | 0.3 | 2.3 | 188.7 | 7.4 |
| Extractable P | | 3.0 | 5.2 | 4.2 | 17.2 | 9.0 |
| Extractable K | | 53.0 | 3.7 | 4.9 | 174.0 | 71.6 |
| Carbon [C] | % | 78.50 | 13.04 | 82.73 | 77.69 | 32.44 |
| Nitrogen [N] | | 0.92 | 0.09 | 2.89 | 2.96 | 1.38 |
| Hydrogen [H] | | 1.85 | 0.15 | 2.24 | 1.74 | 2.70 |
| Sulfur [S] | | 0.46 | 0.28 | 0.59 | 0.43 | 0.42 |
| Oxygen [O] | | 14.64 | 28.12 | 10.14 | 16.07 | 26.36 |
| H/C | – | 0.02 | 0.01 | 0.03 | 0.02 | 0.08 |
| O/C | | 0.19 | 2.16 | 0.12 | 0.21 | 0.81 |
| (O + N)/C | | 0.20 | 2.16 | 0.16 | 0.24 | 0.86 |
| (O + N + S)/C | | 0.20 | 2.18 | 0.16 | 0.25 | 0.87 |

Wood biochar (WB), eggshell biochar (EB), rice biochar (RB), soybean biochar (SB), peanut shell biochar (PB)

it was reported that BC application increased a non-crop species (*S. arboricola*) LAI by 51% due to the enriched nutrients in BC. Xiang et al. (2022) reported that BC affords positive attributes that make it a suitable bacterial carrier and soil health enhancer. Additionally, calcium-enriched eggshell BC might be able to stabilize cations in the soil because of its calcium-enriched properties and stable carbonate adsorption effects. Islam et al. (2021) explored the effects of eggshell BC on soil cationic pollutants and found that eggshell BC application reduced the effects of cationic pollutants on rice plants. The effects of BC type on plant specific growth were quite evident as both *R. tomentosa* and *C. edithiae* died in peanut shell BC-amended soil. This may be due to the fact that peanut shell BC exhibited the highest soil EC (Table 4), which means soil salinity stress was higher which is a stressor to plant growth. This may be because soils in which *R. tomentosa* and *C. edithiae* were grown might be overproducing reactive oxygen species (ROS) that likely cause oxidative stress resulting in oxidative damage to cell organelles and membrane components (Hasanuzzaman et al. 2021). However, the biomass of *S. arboricola* was significantly higher ($p < 0.05$) in peanut shell and wood BC than in eggshell BC application. This might be because eggshells are a common biomass waste with plenty of $CaCO_3$ (94%) and marginal organic matter (6%) (Li et al. 2023), which contain fewer nutrients for plant growth compared with wood and peanut shell BC (Table 3). On the other hand, peanut shell BC improved soil nitrogen content (Table 4), which might also be the reason for the better performance of *S. arboricola*. In total, the biomass of *R. tomentosa* and *C. edithiae* was highest in wood BC-amended soil, while *S. arboricola* biomass was highest in peanut shell BC-amended soil. The aforementioned discussion on the interrelationship between the effects of BC type on plant species does not have a simple linear trend, rather a complex relationship exists.

To further understand the reason why different vegetation species performed differently in different types of BC, principal component analysis (PCA) was conducted to decipher the complex interrelationship. Figure 2 shows that measured soil properties and BC types explained with 81.6% of the variations (PC1 = 42.7%, PC2 = 38.9%), revealing three major different groups of BC types applied in the soil. Sample scores were plotted for the first two PCs, and differences in soil properties emerged on BC type applications. Three well-differentiated groups, including peanut shell BC-amended soil, soybean BC-amended soil, and other soil treatments (i.e., NB, WB, EB, and RB) were clustered. It indicated that soil properties had been changed by peanut shell BC and soybean BC applications. The first PC was associated with

**Table 4** Variation of soil properties in different biochar type application

| Test ID<br>Unit | pH<br>– | EC<br>μS cm$^{-1}$ | TOC<br>mg kg$^{-1}$ | TN<br>mg kg$^{-1}$ | P<br>mg kg$^{-1}$ | K<br>mg kg$^{-1}$ | CEC<br>cmol kg$^{-1}$ | Water content<br>% |
|---|---|---|---|---|---|---|---|---|
| *R. tomentosa* | | | | | | | | |
| NB | 6.88 ± 0.27c | 63.3 ± 12.5b | 1736 ± 57c | 7.8 ± 1.0b | 26.2 ± 19.6c | 276.7 ± 188.2c | 3.12 ± 0.06b | 17.82 ± 0.02ab |
| WB | 6.88 ± 0.03c | 114.9 ± 8.43b | 1949 ± 266c | 8.2 ± 1.9b | 60.5 ± 13.9bc | 456.7 ± 23.2c | 3.16 ± 0.06b | 22.68 ± 0.03a |
| EB | 7.60 ± 0.13b | 89.3 ± 48.1b | 1947 ± 146c | 7.8 ± 1.5b | 58.7 ± 21.7bc | 459.2 ± 59.7c | 2.65 ± 0.10bc | 18.98 ± 0.01ab |
| RB | 7.22 ± 0.12bc | 106.1 ± 12.4b | 1813 ± 250c | 10.7 ± 2.9b | 91.5 ± 22.2b | 443.2 ± 6.1c | 2.36 ± 0.07c | 15.53 ± 0.02b |
| SB | 8.86 ± 0.06a | 185.8 ± 1.3b | 3464 ± 483a | 33.5 ± 6.6b | 203.3 ± 9.0a | 1311.8 ± 43.5a | 1.04 ± 0.03d | 17.01 ± 0.02b |
| PB | 6.29 ± 0.12d | 2001.7 ± 321.2a | 2750 ± 237b | 2327.8 ± 327.4a | 138.7 ± 45.1b | 652.7 ± 21.9b | 9.47 ± 0.71a | 13.97 ± 0.03b |
| *C. edithiae* | | | | | | | | |
| NB | 7.03 ± 0.06b | 94.0 ± 15.9b | 2485 ± 515b | 26.8 ± 14.0b | 25.3 ± 0.2c | 413.0 ± 11.6d | 2.77 ± 0.05b | 20.30 ± 0.05a |
| WB | 7.03 ± 0.25b | 102.5 ± 3.3b | 1849 ± 160ab | 21.0 ± 9.5b | 29.0 ± 0.4c | 440.2 ± 15.7 cd | 2.73 ± 0.07b | 20.79 ± 0.03a |
| EB | 7.41 ± 0.26b | 125.3 ± 8.9b | 2418 ± 89ab | 14.2 ± 2.1b | 57.8 ± 4.6b | 508.0 ± 8.2bc | 2.67 ± 0.24b | 17.97 ± 0.05a |
| RB | 7.16 ± 0.11b | 100.3 ± 16.9b | 2367 ± 760ab | 13.7 ± 4.9b | 44.8 ± 3.1bc | 463.3 ± 5.6 cd | 2.05 ± 0.05bc | 15.08 ± 0.05a |
| SB | 8.81 ± 0.08a | 172.9 ± 6.87b | 3359 ± 719a | 32.3 ± 7.8b | 142.8 ± 27.2a | 1148.3 ± 82.7a | 0.99 ± 0.04c | 12.83 ± 0.03a |
| PB | 6.49 ± 0.08c | 976.3 ± 320.8a | 2422 ± 182ab | 856.8 ± 468.9a | 66.3 ± 6.5b | 565.7 ± 30.3b | 6.25 ± 1.27a | 16.75 ± 0.06a |
| *S. arboricola* | | | | | | | | |
| NB | 7.17 ± 0.04c | 68.9 ± 9.4c | 1783 ± 32b | 6.5 ± 0.0b | 33.3 ± 9.4bc | 414.7 ± 16.6c | 2.98 ± 0.04b | 20.57 ± 0.01ab |
| WB | 7.21 ± 0.03c | 83.0 ± 10.1c | 1498 ± 105b | 7.3 ± 0.2b | 28.2 ± 1.0c | 445.3 ± 30.5bc | 2.78 ± 0.04bc | 24.45 ± 0.08a |
| EB | 7.83 ± 0.08b | 128.6 ± 11.9bc | 2013 ± 417b | 9.0 ± 2.4b | 53.3 ± 7.7b | 480.7 ± 28.6bc | 2.78 ± 0.16bc | 18.67 ± 0.05ab |
| RB | 7.04 ± 0.09c | 113.7 ± 5.3bc | 1938 ± 185b | 10.8 ± 0.2b | 50.3 ± 7.6bc | 477.0 ± 10.0bc | 2.38 ± 0.10c | 21.30 ± 0.04ab |
| SB | 8.87 ± 0.10a | 202.0 ± 11.1b | 2860 ± 216a | 19.2 ± 1.2b | 199.3 ± 20.6a | 1349.5 ± 91.4a | 0.98 ± 0.05d | 14.03 ± 0.02b |
| PB | 6.76 ± 0.07d | 677.0 ± 84.8a | 2821 ± 554a | 523.2 ± 142.7a | 56.3 ± 3.7b | 530.8 ± 28.4b | 5.63 ± 0.44a | 23.88 ± 0.01a |

EC represents electricity conductance, TOC represents total organic carbon, TN represents total nitrogen, P represents available phosphorous, K represents available potassium, CEC represents cation exchange capacity, and WC represents water content. (NB: no biochar effects; WB: wood biochar effects; EB: eggshell biochar effects; RB: rice biochar effects; SB: soybean biochar effects; PB: peanut shell biochar; Value is reported as mean ± standard error, n = 3; and different letters indicate significant difference ($p < 0.05$; ANOVA))

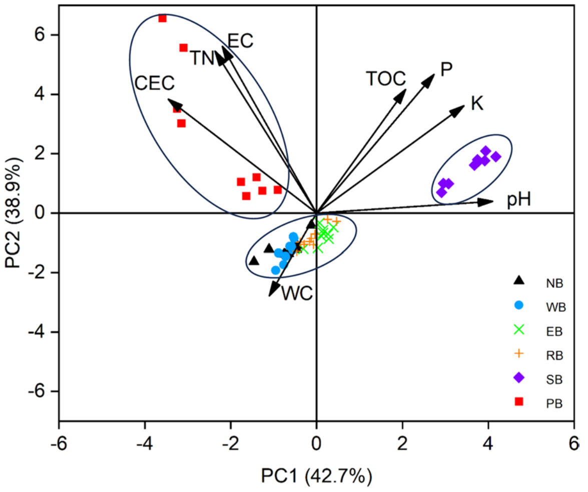


**Fig. 2** Principal component analysis (PCA) of soil properties under different biochar types application. (NB, no biochar effects; WB, wood biochar effects; EB, eggshell biochar effects; RB, rice biochar effects; SB, soybean biochar effects; PB, peanut shell biochar. EC, electricity conductance; TOC, total organic carbon; TN, total nitrogen; P, available phosphorous; K, available potassium; CEC, cation exchange capacity; WC, water content)

**Table 5** Loadings of the soil properties for six soil treatments of Principal Component Analysis on the first two Principal Components (PC1 and PC2)

| Soil property | PC1 | PC2 |
|---|---|---|
| pH | 0.51302 | 0.03344 |
| EC | −0.27445 | 0.48167 |
| TOC | 0.25927 | 0.35686 |
| TN | −0.29597 | 0.46716 |
| P | 0.34225 | 0.40021 |
| K | 0.42868 | 0.31031 |
| CEC | −0.43209 | 0.32882 |
| WC | −0.13805 | −0.23812 |

EC represents electricity conductance, TOC represents total organic carbon, TN represents total nitrogen, P represents available phosphorous, K represents available potassium, CEC represents cation exchange capacity, and WC represents water content

soil pH and K of soil, while the other soil properties had a strong association with PC2 (Table 5). Overall, the pH of BC-amended soil positively correlated with soybean BC application. Thus, the higher pH of soybean BC (Table 3) translates into the highest soil pH. It is known that BC can form carbonates, such as $CaCO_3$ and $MgCO_3$, during BC pyrolysis. These carbonates react slowly with $H^+$ in the soil, thereby enhancing soil pH (Oladele 2019). Generally, soil pH governs many plant-soil chemical relations due to its influence on solubility, especially the availability of micronutrients and toxic ions. For instance, high-pH soils are often rich in Cr, Co, Ni, Fe, and Mg, and deficient in N, P, K, and Ca, which might inhibit plant growth (Offord et al. 2014). However, three vegetation species biomasses did not decrease in soybean BC applications (Fig. 1) in the current study, which can be attributed to overall pH being not excessively alkaline ($pH < 9$). When the pH is more than 9, the soils are considered highly alkaline and often have toxic amounts of bicarbonate, carbonate, aluminium, and iron (Smith 2022). Nutrient deficiency is also likely to be a major problem and the high amount of exchangeable sodium in these soils further reduces soil physical fertility (Hall et al. 2009). Peanut shell BC-amended soil had the lowest pH among the six soil treatments, which might be related to the functional group on the peanut shell BC. For instance, peanut shell BC has a higher functional group of $OH^-$ on the surface based on the FTIR results (Fig S3). Those base cations in soil solutions will be reacted with $OH^-$, which breaks the adsorption–desorption equilibrium, resulting in the production of exchangeable $H^+$ and then acidified soil (Li & Li 2000). Peanut shell BC-amended soil overall had a positive relationship with CEC, TN, and EC. This suggested that peanut shell BC improved soluble soil nutrient cations, such as Ca, Mg, and K, which can be easily absorbed by plants. It has been noted that peanut shell BC application led to higher soil TN, while the highest extractable N was found in soybean BC. This suggested that the extractable N of BC does not have a huge influence on the soil-soluble TN. The EC can be used as an index for estimating the number of dissolved salts in soil solution (Hossain et al. 2011). Therefore, the positive relationship between peanut shell BC and soil EC suggested that soil salinity increased with peanut shell BC application (Fig. 2), which can be used to explain the death of *R. tomentosa* and *C. edithiae* in peanut shell BC-amended soil. Some cations on the surface of the peanut shell BC may not strongly adhere to or bond by electrostatic forces and thus dissolve as soluble salts (Chintala et al. 2014), which might be the reason for the highest soil EC after peanut shell BC application. Soil water retention has a significant impact on plant physiology and yield by influencing plant adsorption and transportation (Razzaghi et al. 2020). In the current study, soil water content was positively related to wood, rice, and eggshell BC-amended soils, and it was also noted that the water content in wood BC-amended soil was always higher than in other soil conditions (Table 4). This can be explained by the highest pore volume and surface area of wood BC (Table 3). It has been reported that BC with a large surface area and pore volume could store more water in soil through the capillary phenomenon (Mao et al. 2019), which might be the reason for higher soil water retention in wood BC-amended soil. However, water content in the soil is also influenced by plant species. For example, water content in the same soil treatments was different when different species were planted with (Table 4). This might be because plant root structure and growth might lead to a change in soil pore volume and structure (Ng et al. 2016), which might lead to variation in soil water content.

### 3.2 The effects of BC types on LAI and $g_s$

The effects of BC types on temporal change in LAI for different plant species are shown in Fig. 3. The LAI is a quantitative measure of plant growth and indirectly indicates carbon assimilation. It should be noted that LAI is an important parameter in many carbon models for estimating carbon assimilation (Liu et al. 2008). The relationship between LAI and carbon assimilation has been studied to predict the response of ecosystem energy, water, and carbon budgets (Gan et al. 2018). As for *R. tomentosa* species, the highest LAI (2.01) was observed in soil amended with rice BC with LAI increased by 114% during the early plant establishment period (Fig. 3A). The fact that rice BC has relatively high carbon and nitrogen content might be the reason for this result (Table 3). According to Lawlor (2002), the interaction between carbon dioxide and nitrate assimilation is of key importance for plant production. An adequate supply of nitrogen is essential for protein synthesis, leaf growth, and photosynthesis. This might be the reason why LAI was highest in rice BC-amended soil. Nevertheless, the lowest LAI of *R. tomentosa* was found in eggshell BC-amended soil at around 1.41. The LAI increase rate (58%) was also lower in eggshell BC-amended soil than that in rice BC-amended soil. This may be related to the nutrient content of these two types of BC. The LAI only increased by 37% after five months growing period when soybean BC was applied. This slow growth of *R. tomentosa* might be related to higher soil pH after soybean BC application. However, the LAI growth of *C. edithiae* showed the highest in soybean BC application, around 1.73. The difference between the effects of wood, eggshell, and rice BC application on *C. edithiae* LAI was not significant. The increase rate was higher than 60% during the early plant transplantation stage (Fig. 3B). For the control soil, the LAI of *C. edithiae* showed the lowest value (0.96). This indicates that BC stimulates *C. edithiae* leaf area growth regardless of their type. Feng et al. (2021) found that BC application stimulates crop LAI due to improvement in

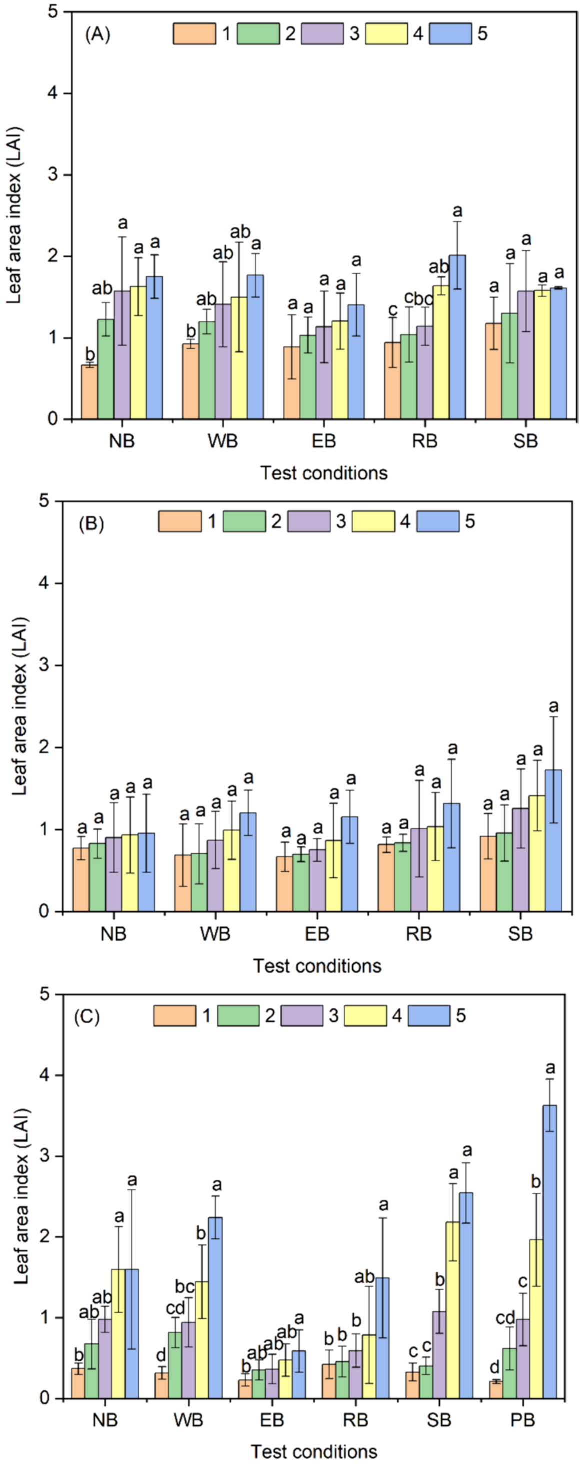


**Fig. 3** Leaf area index of (**A**) *R. tomentosa*, (**B**) *C. edithiae*, and (**C**) *S. arboricola* response to different biochar types (n = 3) with different growth periods. The legend in the figure indicates the number of months that plants spend for growth (i.e., 1 represents plant growth for one month, etc.). The different letters on the column indicate a significant difference ($p < 0.05$)

soil fertility. As for non-crop species, the LAI of *S. arboricola* showed the highest increase in peanut shell BC application during the early plant transplantation stage, increasing from 0.21 to 3.63 (Fig. 3C). Not all BC types are suitable for the LAI growth of *S. arboricola*, eggshell, and rice BC-amended soil did not show a great stimulus compared to soil without BC. The lower surface area of eggshell (0.68 $m^2$ $g^{-1}$) and rice (0.66 $m^2$ $g^{-1}$) BC might be the reason for this result. As for *R. tomentosa*, rice, and wood BC significantly ($p < 0.05$) improved LAI when compared to the 1 m and 5 m of the incubation time. However, soybean and eggshell BC did not show high ($p > 0.05$) growth on the LAI of *R. tomentosa* (Fig. 3A). It has been reported that rice-husk BC also significantly improved *R. tomentosa* growth, which is plausible as BC increases the capacity of the soil to retain water and nutrients, reducing the need for inorganic fertilizers (Baharudin et al. 2023). The highest value of *C. edithiae* was observed in soybean BC treatment after a 5 m growth period, suggesting that soybean BC is much more suitable for *C. edithiae* in terms of LAI.

The $g_s$ of different plant species subjected to BC application are reported in Fig. 4. The application of wood BC resulted in the greatest increase of $g_s$ in *R. tomentosa* and *C. edithiae* for the early plant establishment period. However, for *S. arboricola*, peanut shell, and wood BC showed similar effects on $g_s$ (around 300 mmol $cm^{-2}$ $s^{-1}$). The $g_s$ of all the plant species increased with cultivation time in all the soil treatments. This might be caused by higher plant growth requiring more water to support a higher amount of LAI (Fig. 3) as reported by Ng et al. (2022a, b). During the first 2 m, the difference in $g_s$ of *R. tomentosa* between soils with five types of BC and without BC was not significant (Fig. 4A). Nevertheless, the $g_s$ increased distinctly in wood BC-amended soil after 3 m of growth, and it reached the highest value at 551 mmol $cm^{-2}$ $s^{-1}$ after 5 m of growth. Except for wood BC, adding other types of BC usually reduced the $g_s$ of *R. tomentosa*, while this reduction by BC application was not statistically significant (Fig. 4A). Most BC applications enhanced *C. edithiae* $g_s$ except for that of soybean BC at the initial 3 m of growth (Fig. 4B). The lower pore volume and surface area of soybean BC may have led to a lower water retention capacity, which may have led to the decrease in $g_s$ of *C. edithiae*. It has been reported that BC application increases plant $g_s$ due to its enhanced soil water retention (Tanure et al. 2019). It has been clarified in the current study that wood BC-amended soil led to better $g_s$ of *C. edithiae* due to its higher water retention ability in the soil (Table 4). As for the $g_s$ of *S. arboricola*, rice, eggshell, and soybean BC did not show positive effects compared with the control test after a 1 m incubation period. However,

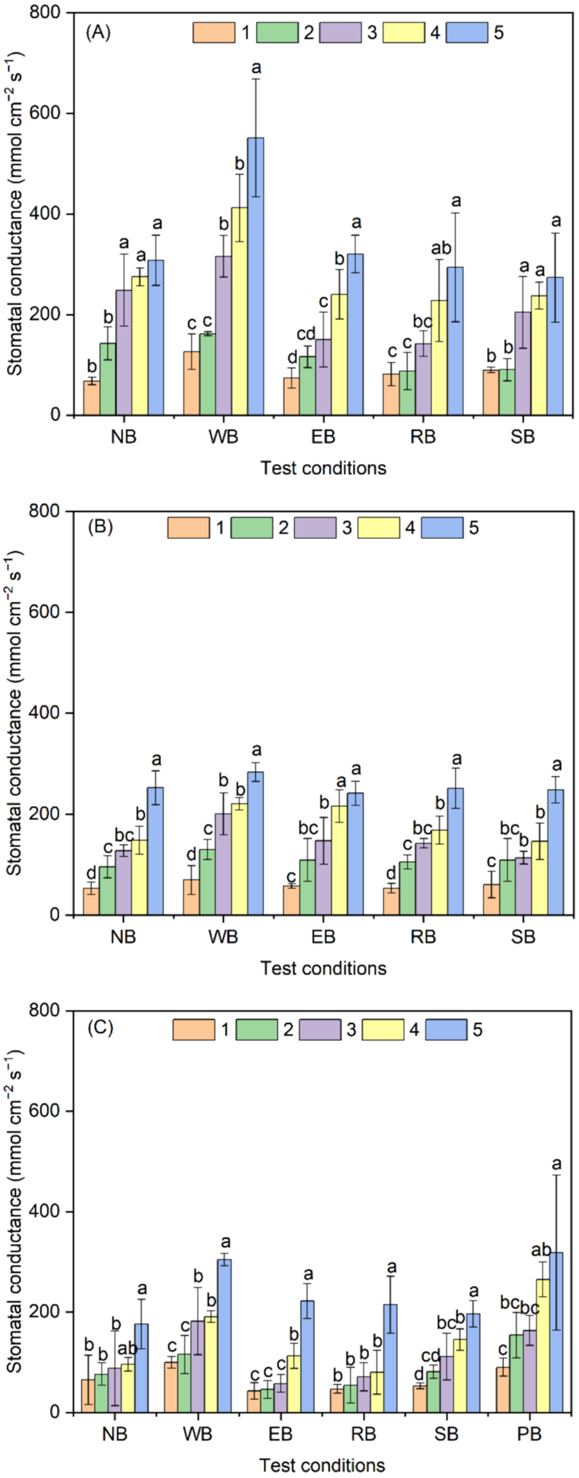


**Fig. 4** The effects of different biochar types on stomatal conductance of (**A**) *R. tomentosa*, (**B**) *C. edithiae*, and (**C**) *S. arboricola* (n = 3) with different growth periods. The legend in the figure indicates the number of months that plants spend for growth (i.e., 1 represents plant growth for one month, etc.). The different letters on the column indicate a significant difference ($p < 0.05$)

soybean BC stimulated the $g_s$ of *S. arboricola* after 2 m growth. When peanut shell BC was used to grow *S. arboricola*, the $g_s$ also increased by 20% in comparison to the control (Fig. 4C). As the plants with higher $g_s$ normally lose more water through evapotranspiration, the plants need to uptake more water from the soil to compensate for the water loss attributed to evapotranspiration (Xue et al. 2021). Therefore, the higher $g_s$ is likely to correlate with the absorption of soil water and thus nutrients by the plants. This may explain why the application of wood BC significantly improved the plant biomass of *R. tomentosa* and *C. edithiae*, while peanut shell BC is most beneficial to the growth of *S. arboricola*, as shown in Fig. 1. $g_s$ regulates plant internal water balance, which requires chlorine to function properly. Chlorine participates in the Hill reaction during photosynthesis and maintains cation transport and equilibrium inside the plant. It also controls sugar translocation in plants and thus regulates plant water use efficiency (Chen et al. 2010). Therefore, wood and peanut shell BC-amended soil might imply an enhancement in soil chlorine content and lead to an increase in plant $g_s$.

### 3.3 Evaluation of carbon content in plant-soil system with BC

The effects of BC types on the carbon content of the soil–plant system are discussed in Fig. 5. BC can stimulate plant growth and thereby increase PCC (Liu et al. 2021). However, Wang et al. (2016) used meta-analysis to investigate BC stability in soil and found that 97% of BC contributes directly to long-term carbon sequestration in soil. This suggests that BC is relatively stable in the soil. As for short-term tests (i.e., 5 m), the BC decomposition rate can be neglected in the current study. The PCC for the three species grown under different BC applications is shown in Fig. 5A. It is noted that peanut shell BC enhanced the PCC of *S. arboricola* by 57% compared with the control test. Wood BC application only improved 18% of PCC by *S. arboricola* compared with the control test. For *C. edithiae* species, all the BC applications increased their carbon content, except for peanut shell BC. Wood BC stimulated *C. edithiae* carbon content by 29%, followed by soybean BC (27%), eggshell BC (28%), and rice BC (25%). Wang et al. (2020) reported that carbon sequestration by plants depends on their species and characteristics. This might be the reason why PCC is higher in *C. edithiae* than that in *R. tomentosa*. Wood BC-amended soil enhanced the PCC of *R. tomentosa* by 21%, while rice and soybean BC increased the PCC of *R. tomentosa* slightly compared to the control. The results suggested that the ability of plant carbon sequestration depends on the application of BC types. Plant growth is

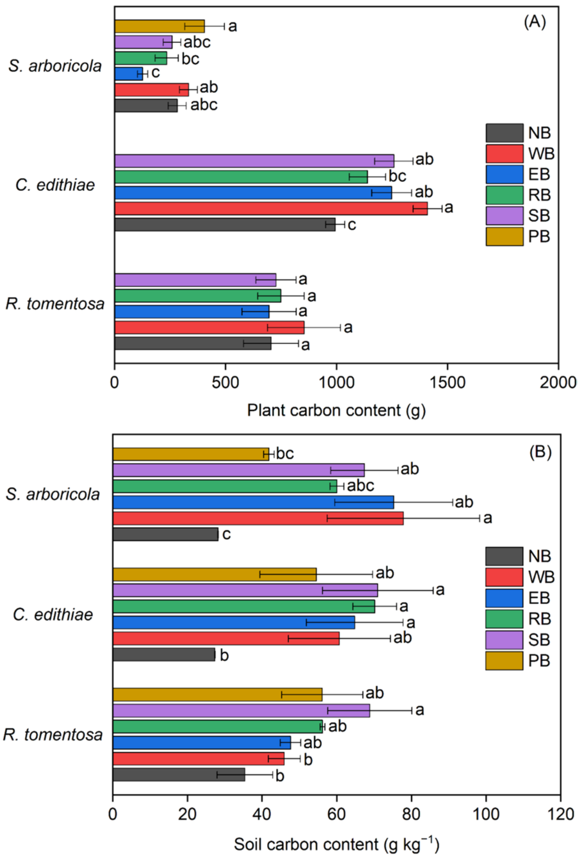


**Fig. 5** The effects of biochar types on carbon content of plant (**A**) and soil (**B**) (n = 3). Any two conditions in the same plant species sharing unlike letters indicate a significant difference ($p < 0.05$)

dependent on the BC based on its feedstocks. For example, wood-based BC enhanced plant productivity by 29.4%, while rice husk BC increased plant productivity by 54% (Wang et al. 2019b). In general, wood BC-amended soil stimulated *R. tomentosa* and *C. edithiae* to store more carbon, while peanut shell BC greatly enhanced the carbon content of *S. arboricola*. Furthermore, the carbon content of *S. arboricola* was much lower compared with *C. edithiae* regardless of BC application types. This might be because woody species of plant have a higher ability to store more stable carbon compared with shrub species (Reda et al. 2020). Soil carbon content was all enhanced by BC application regardless of BC types (Fig. 5B). BC is usually as a strategy for carbon storage due to its lower decomposition rate in the soil (Mašek et al. 2019). Although the performance of *S. arboricola* was great in peanut shell BC application, the soil carbon content in the same condition was lower compared with other BC types of application. As for the soil planted with *C. edithiae* and *R. tomentosa*, soybean BC application showed the highest carbon content in the soil. Overall, BC types had a significant impact on soil carbon content, while vegetation species were also an important factor. The relationship between plant carbon content and soil organic carbon in different BC applications is presented in Fig. 6. It has been shown that the *p*-values of these six treatments were all greater than 0.05, indicating that their relationship was not significant. However, wood and peanut shell BC caused a negative relationship between PCC with soil organic carbon. It has been reported before that these two BC can stimulate plant growth due to their higher water retention ability. It should be noted that the water content of the soil with peanut shell BC was only higher than that of soil without BC when planted with *S. arboricola*. This suggests that plants will consume soil organic carbon for its carbon storage only when wood and peanut shell BC are applied. A positive correlation was found in soil with eggshell, rice, and soybean BC, which indicates that the plants are more likely to release carbon into the soil through exudation and root turnover in such soil conditions (Rees et al. 2005). This might be the reason for the increase in soil organic carbon when PCC is increased.

### 3.4 Relationship of LAI with $g_s$ and PCC

Figure 7 represents the correlation fitted with a proposed empirical function for soil without BC and soil with different BC applications, which explains the relationship between LAI and $g_s$. There was a positive relationship between $g_s$ and LAI in all soil treatments, regardless of BC types. Stomatal apertures serve as pressure regulators to prevent inadequate vapor pressure and the necessary $CO_2$ flux for photosynthesis (Brodribb et al. 2020). Therefore, for larger LAI, higher water movement is necessary to reduce any cavitation and facilitate water for photosynthesis in the leaves (Patanè 2011). It is found that when LAI reaches the limiting value (LAI = 3), plant $g_s$ does not increase with LAI increase. It has also been reported by Granier et al. (2000) that canopy conductance is proportional to LAI up to a limiting value, such as LAI = 6. The difference between this limiting value of LAI might be related to different vegetation species. Compared to forests, lower height plants like crops, grass, and shrubs exhibit a different $g_s$ response to increasing LAI. Normally, the $g_s$ and transpiration are saturated at a much lower LAI threshold (about 3 to 4) for lower height vegetation (Saugier 1996). The saturation of forest transpiration at LAI higher than 6 can be explained by the important shading of low canopy strata by the upper levels when LAI increases (Granier et al. 2000). On the other hand, plant species with different radiation absorbing properties might also influence the $g_s$ (Shuttleworth 1989). LAI is therefore crucial for explaining

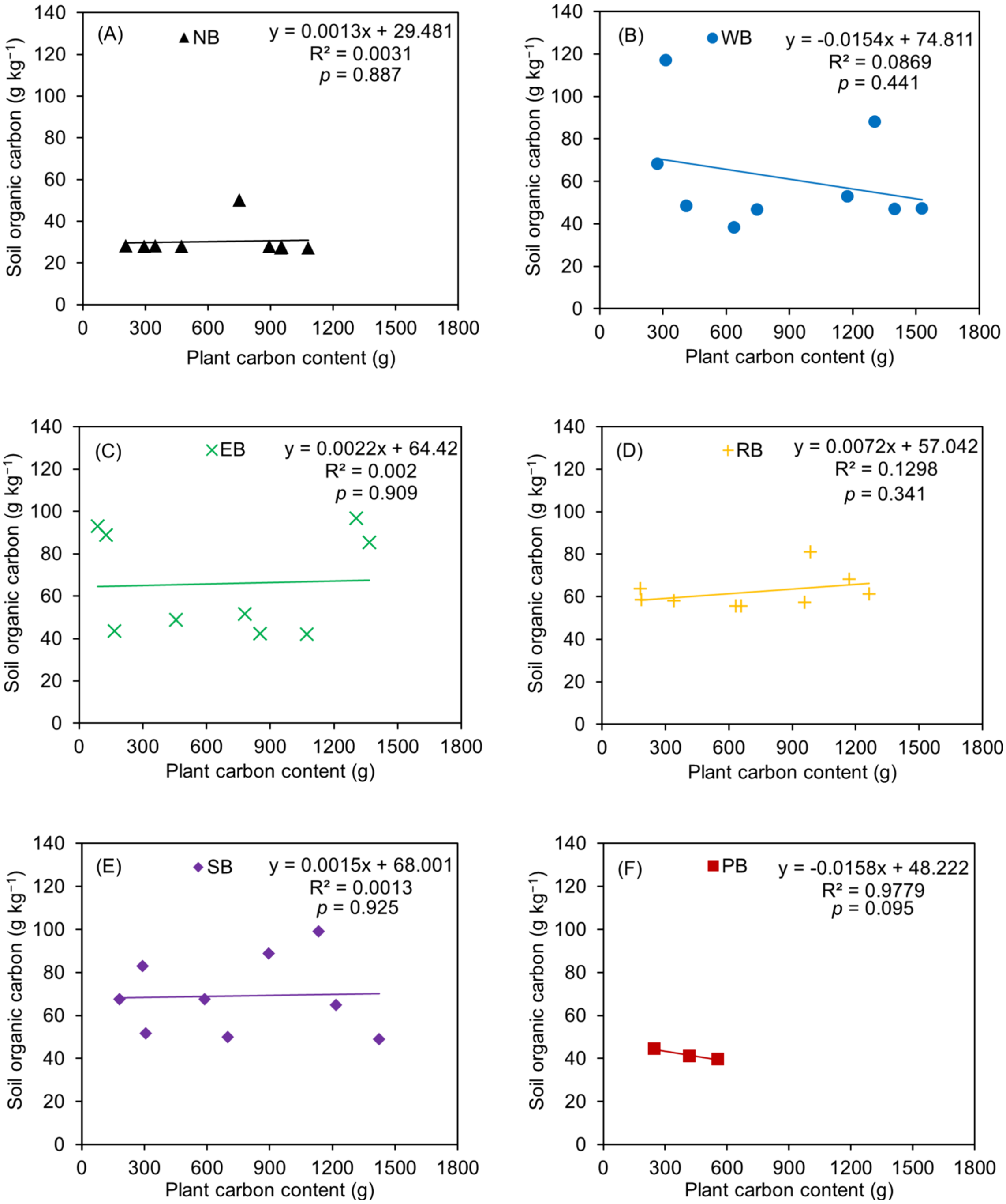


**Fig. 6** The relationship between plant carbon content and soil organic carbon in different soil conditions ((**A**) soil without BC, (**B**) soil with wood BC, (**C**) soil with eggshell BC, (**D**) soil with rice BC, (**E**) soil with soybean BC, (**F**) soil with peanut shell BC)

between-stand variation in transpiration. It should be noted that wood BC provoked more $g_s$ when LAI was lower than 3 (Fig. 7). It is suggested that wood BC should be considered when higher $g_s$ is required.

A further deduced correlation function for evaluating the relationship between PCC and LAI is proposed. Since vegetation species is an important factor that influences LAI and $g_s$, the relationship between LAI and carbon content is then divided into three parts based on the plant species (Fig. 8). It should be noted that the influence of plant species on PCC is more significant. As for different plant species, $C_a$–$C_i$ was highest in plant species

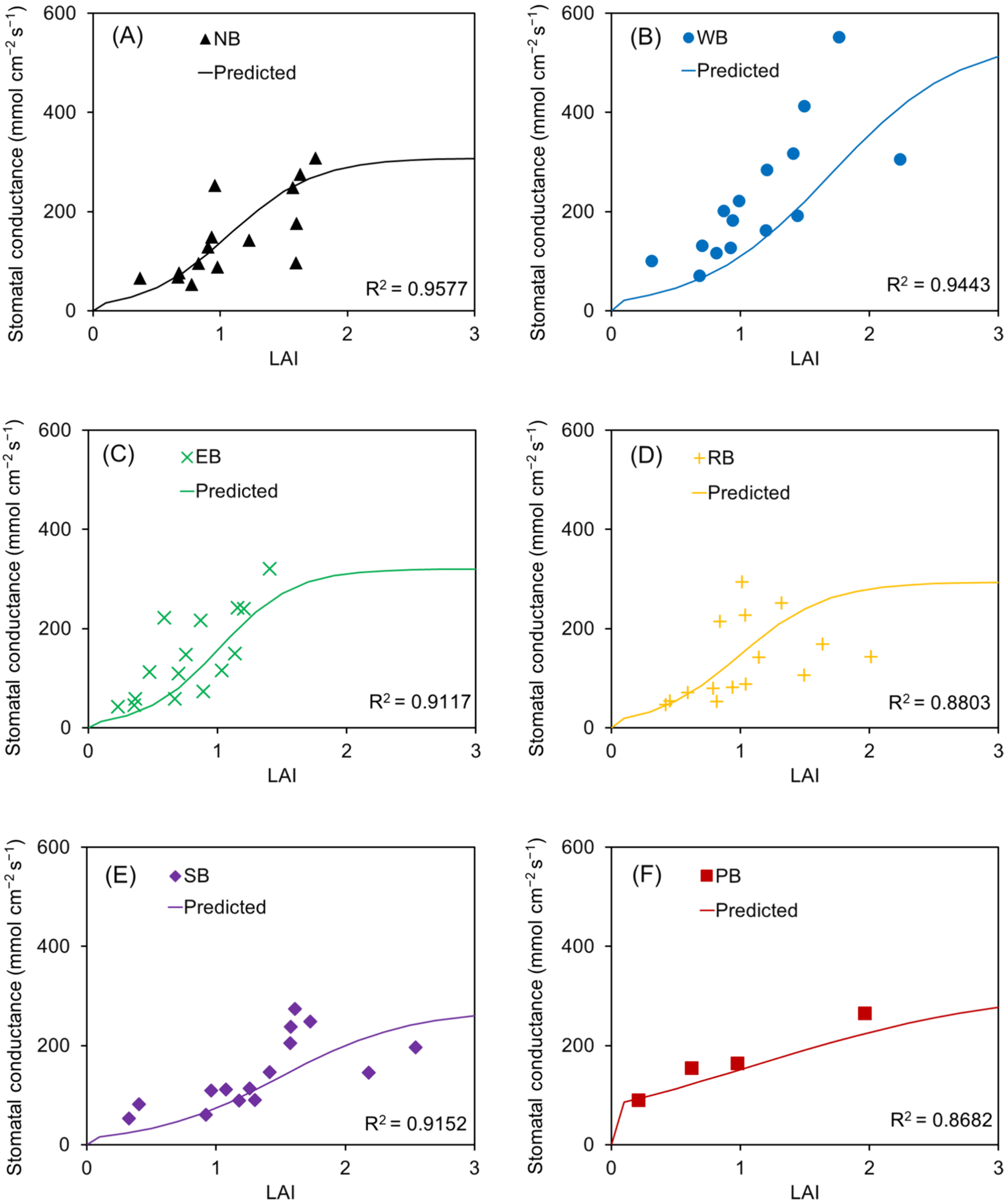


**Fig. 7** The relationship between stomatal conductance and leaf area index under different biochar implementations. ((**A**) soil without BC, (**B**) soil with wood BC, (**C**) soil with eggshell BC, (**D**) soil with rice BC, (**E**) soil with soybean BC, (**F**) soil with peanut shell BC)

of *C. edithiae*, followed by *R. tomentosa* and *S. arboricola*. This indicates that internal $CO_2$ concentrations in a plant leaf are varied in plant species. It has been also reported that the presence of regulation of internal $CO_2$ concentrations by the stomatal resistance depends on the plant species. Normally, $C_4$ plants can maintain a $CO_2$ concentration inside the stomatal cavity that is two times lower than the $CO_2$ concentration outside compared to $C_3$ plants (Poorter et al. 2022). Besides, the internal $CO_2$ concentration varies inside plants when photosynthesis is active (Sage and Khoshravesh 2016). There is also variation between plant species in the rate of increase

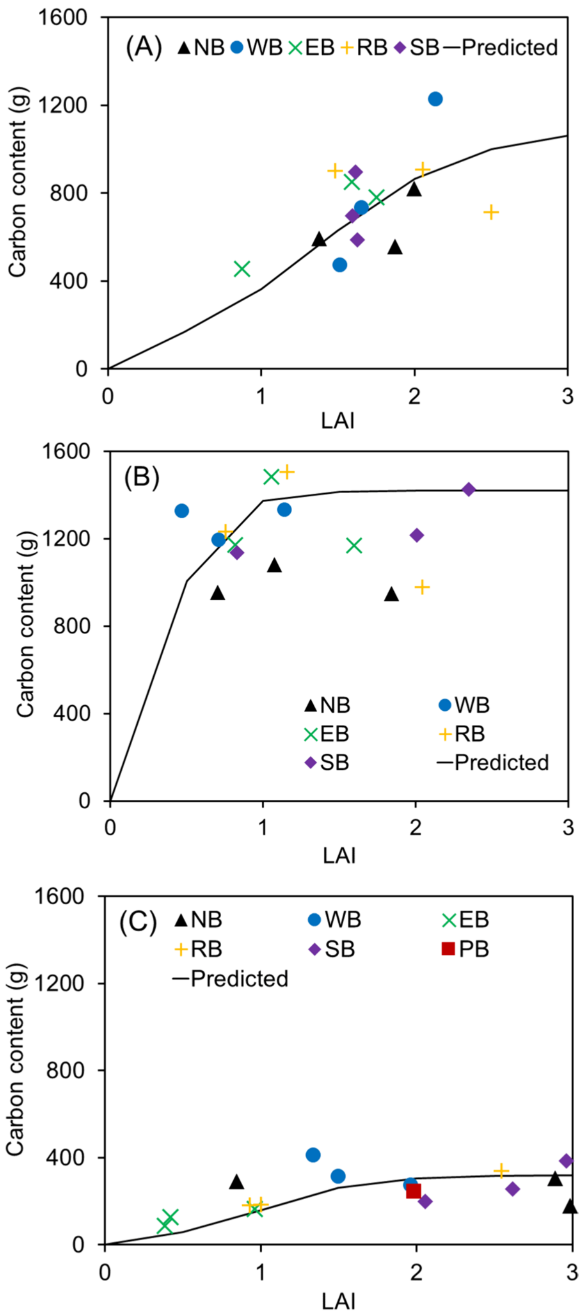


**Fig. 8** The relationship between plant leaf area index of *R. tomentosa* (**A**), *C. edithiae* (**B**), *S. arboricola* (**C**) with carbon content in different biochar effects

in photosynthesis, and thus, the $C_a$–$C_i$ was different for plant species in the current study (Table 2). The correlation function illustrated that the carbon content of plants might not always increase with LAI increase. However, it is noted that BC application can enhance the PCC of *C. edithiae* when LAI was lower than 3, except for rice BC. This correlation function can be used to fit different plant species by regulating the fitting parameters and knowing the internal $CO_2$ concentrations in a plant leaf to predict their carbon content. Thus, it is recommended to estimate the carbon budget and model forest ecosystem growth when facing different plant species.

## 4 Conclusions

1. Wood BC-amended soil led to better $g_s$ of both *R. tomentosa* and *C. edithiae* due to its higher water retention ability in the soil. This illustrated that a higher amount of water retention brought by wood BC with a great amount of pore volume might be the limited factor for *R. tomentosa* and *C. edithiae* growth. Peanut shell BC enhanced the carbon content of *S. arboricola* by 57%, and the performance of *S. arboricola* was well in this soil treatment. It suggests that peanut shell BC is suitable for the carbon assimilation of *S. arboricola*.
2. Wood and peanut shell BC caused a negative relationship between soil organic carbon and plant carbon content, suggesting that plants consume more carbon from the soil organic carbon for its carbon storage.
3. An empirical function for describing the correlation between $g_s$ and LAI is proposed, suggesting that when LAI reaches the limiting value (LAI = 3), $g_s$ does not increase with LAI increase. It should be noted that the BC application can enhance the PCC of *C. edithiae* when LAI is lower than 3. This empirical function can be used for estimating carbon budget and modeling forest ecosystem growth.

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1007/s42773-024-00355-w.

Supplementary Material 1.

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**Author contributions**
J.X. Liao: Data collection, Formal analysis, Methodology, Writing-original draft, Conceptualization. P.S. So: Conceptualization, Formal analysis Investigation, Writing-review & editing. S. Bordoloi: Writing-review & editing, Analysis, and Supervision. D.N. Li: Validation, Methodology, Investigation, Writing-review & editing. H.R. Yuan: Conceptualization, Supervision, Writing-review & editing. Y. Chen: Conceptualization, Writing-review & editing. L. Q. Xin: Writing-review & editing, Analysis.

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**Availability of data and materials**
The corresponding author can provide the datasets upon an adequate request.

## Declarations

**Competing interests**
We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service, and/or company that could be construed as influencing the position presented in, or the review of, the manuscript entitled.

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