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### **Abstract**

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Bamboo (Phyllostachys sp.) is considered a sustainable resource that can replace fossil fuelbased products. Its additional ability to sequester organic carbon in the soil (SOC) makes it a promising nature-based solution for combating climate change. However, bamboo's soil C storage potential may vary considerably between species or growing conditions and needs to be better quantified, especially in temperate climates where data is lacking. In the present research, the SOC dynamics of plots converted from grassland to plantations of three bamboo species (i.e. Phyllostachys nigra, Phyllostachys aurea and Phyllostachys aureosulcata), planted 12 years ago on podzol (World Reference Base classification) in the Belgian Campine region, have been studied. Soil and root samples were taken until a depth of 40 cm using a 10 cm interval. Besides, the total belowground C stability (mgCO<sub>2</sub>-C g<sup>-1</sup>C h<sup>-1</sup>) was assessed by measuring during three months the carbon dioxide (CO<sub>2</sub>) efflux relative to the belowground C stock. Based on an equivalent soil mass, only P. aureosulcata, the species with the highest culm basal area, had a significant (p < 0.001) SOC increase of 5.0 kgC m<sup>-2</sup> (relative increase of + 94 %) as compared to grassland. Considering the sum of C stocks in the soil, roots and leaf litter, all bamboo species showed significant (p < 0.001) C storage, i.e. +3.6 kgC m<sup>-2</sup> (+64 %), +5.3 kgC m<sup>-2</sup> (+94 %) and +8.6 kgC m<sup>-2</sup> (+151 %) for P. nigra, P. aurea and P. aureosulcata, respectively. In addition, bamboo's relative basal CO<sub>2</sub> efflux (0.007, 0.006 and 0.008 mgCO<sub>2</sub>-C g<sup>-1</sup>C h<sup>-1</sup> respectively) was remarkably lower than in the grassland (0.012 mgCO<sub>2</sub>-C g<sup>-1</sup>C h<sup>-1</sup>), though it was only significant for P. aurea. This study highlights that converting temperate permanent grassland into *Phyllostachys* bamboo plantation can result in net and rapid organic C storage by increasing the total belowground C stability and C input. Further research regarding the net CO<sub>2</sub> balance of bamboo-derived products is still required to fully assess its climate change mitigation potential.

- 61 Keywords: Bamboo, Phyllostachys, nature-based solution, Soil organic carbon, Soil
- 62 respiration, CO<sub>2</sub> efflux

#### 1. Introduction

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The soil may play a key role in regulating the global climate through C storage following land use or agro-management changes, as illustrated by the 4 per 1000 initiative (Chenu et al., 2019). The initiative aims to increase the C stock in the top 30 to 40 cm of soil by 0.4% per year in order to mitigate the CO<sub>2</sub> increase in the atmosphere considerably. Between 1500 and 2400 Pg of organic C is stored in the first two meters of soil, where C sequestration is most active in the first 30 centimetres (Balesdent et al., 2018). In comparison, about three to four times less C is present in vegetation (450 to 650 PgC) and three times less in the atmosphere (~829 PgC) (Paustian et al., 2016; Bispo et al., 2017). In addition to the traditional de-carbonation approaches, soil-based greenhouse gas mitigation strategies should be pursued (Paustian et al., 2016; Bispo et al., 2017). With this respect, bamboo has already been identified as one species that efficiently sequesters CO<sub>2</sub> in subtropical regions (Zhou et al., 2011). Bamboo belongs to the subfamily Bambusoideae under the family Gramineae. Nearly 1500 species have been recorded in Asia, America and Africa (Li & Kobayashi, 2004), where they can be found between c. 47°S to 50°N latitude and between 0 and 4300 meters above sea level (Liese & Köhl, 2015). Although the world's forest area has decreased since 1990, especially in Africa and Latin America, the bamboo area, estimated at 35 million ha in 2020, is increasing, mainly in China and India (FAO, 2020). In addition to its cultural importance (Dlamini et al., 2021), bamboo has multiple advantages compared to other forest types. For example, it grows remarkably faster (30-100 cm day<sup>-1</sup>) than tree species (Zhou et al., 2011; Desalegn & Tadesse, 2014) and, due to a highly developed root system, does not need to be replanted (Rocky & Thompson, 2018). As a result, bamboo can reduce soil erosion, conserve water efficiently, and be used for land rehabilitation (Ben-zhi et al., 2005; Dlamini et al., 2021). Bamboo also has antibacterial properties and, therefore, does not require pesticides (Rocky & Thompson, 2018; Lombardo, 2022). Due to its carbon-fixing capabilities, the culms can also be utilized for

88 sustainable construction, and form an important income source for local producers (Zhou et al., 89 2011; Li et al., 2015; Liese & Köhl, 2015). 90 Globally, the potential of bamboo as a sustainable resource has been studied mainly in Asia 91 (Gupta & Kumar, 2008; Dlamini et al., 2021) and Africa (Desalegn & Tadesse, 2014). The 92 subfamily Bambusoideae is not endemic in Europe (Liese & Köhl, 2015). However, some 93 species are suitable for temperate climates and have been introduced into the European 94 horticulture industry for ornamental use (Potters et al., 2013; Liese & Köhl, 2015; Akinlabi et 95 al., 2017). Recently, its planting in a professional context has also been gaining interest in 96 Europe (Lombardo, 2022). This is because of its great potential to be used as a sustainable 97 resource in multiple products (e.g. flooring or furniture) and the fact that it is easy and 98 economical to cultivate (Potters et al., 2013; Desalegn & Tadesse, 2014; Lombardo, 2022). 99 Although the growth rate of bamboo in Europe may be lower than that in tropical climates, 100 Potters et al. (2013) argued that bamboo could help the European economy transition from a 101 fossil fuel-based economy to a more sustainable bio-based one. Bamboo is a very popular 102 resource in Europe and is important to its economy. In 2019, the continent was the world's 103 largest importer of bamboo products and the second largest exporter (after China), accounting 104 for 10% of exports on the international market (INBAR, 2021). 105 Phyllostachys sp. is a widely known genus of running bamboo with over 100 species 106 predominantly native to China and Japan (Liese & Köhl, 2015; Akinlabi et al., 2017). The root 107 system of these monopodial species forms a very dense mat that can spread laterally thanks to 108 long cylindrical rhizomes (Ben-zhi et al., 2005; Liese & Köhl, 2015). One of the most well-109 known species is P. pubescens (Moso bamboo), which occupies between 56 and 59% of the bamboo area in China (Fu et al., 2014; Dlamini et al., 2021). The species of interest in the 110 111 present study are P. nigra, P. aurea and P. aureosulcata, which are black, green and yellow, 112 respectively. Stands of P. nigra, P. aurea and P. aureosulcata are reported throughout Europe (Lombardo, 2022). These take the form of professional plantations, experimental plots or ornamental gardens. They have been widely transplanted across the globe, for example, to make furniture (Liese & Köhl, 2015; Akinlabi et al., 2017). Together with P. pubescens, P. nigra is one of the major bamboo species found in Japan (Inoue et al., 2013). In addition, P. aurea is also cited in South Africa, where it is used as a source of biomass or for revegetation and rehabilitation of mined areas (Bada et al., 2016). Through photosynthesis, bamboo stands sequester atmospheric CO<sub>2</sub> in the aboveground biomass, i.e. culms, branches and leaves, and in the belowground parts, i.e. the root system and the soil, in the form of organic C (Chen et al., 2009). The bamboo species, growth characteristics and soil properties impact the C sequestration rate in soil (Zhou et al., 2011). The type of management also affects soil C storage (Fu et al., 2014). For example, in the Moso bamboo ecosystem in China, about four to five times more C is present in the soil (i.e. 10.8 versus 12.0 kgC m<sup>-2</sup> under intensive and extensive management, respectively) than in the aboveground biomass (i.e. 1.9 versus 2.5 kgC m<sup>-2</sup> under extensive and intensive management, respectively) (Fu et al., 2014). These proportions of C (in vegetation and soil) align with Li et al., (2015) and Yuen et al., (2017), who report that SOC accounts for 76% of total ecosystem C in bamboo stands. Moreover, Lin et al., (2018) measured a 12% decrease in SOC (from 7.2 to 6.3 kgC m<sup>-</sup> <sup>2</sup> in the top 40 cm) after converting a natural evergreen broadleaf forest into intensively managed Moso bamboo, indicating that bamboo SOC storage is also impacted by previous land use. Scientific research on the SOC storage of bamboo has gained importance in recent years. Bamboo is an appealing climate-smart commercial crop, yet most research on its potential has focused on Asia and Africa. There is still a lack of knowledge about its growth and soil C storage potential in temperate climates, such as in Europe. Furthermore, C dynamics exhibit a relatively slow rate of change, and it often takes many years to observe or detect changes in

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SOC stocks. Taking this into consideration, the present study benefits from having two comparison sites: the standard treatment (a temperate permanent grassland) and its equivalent converted into a bamboo plantation 12 years ago. Moreover, the sandy region where the study site is located is generally less attractive for conventional crops (i.e. grassland is an important agricultural land use type), further justifying the potential for planting bamboo in this ecosystem. Therefore, this study's primary objectives were to (i) quantify SOC storage 12 years after converting a temperate permanent grassland into a bamboo plantation and (ii) compare root and leaf litter C stocks, as well as soil CO<sub>2</sub> efflux between the permanent grassland and the bamboo plantation.

### 2. Material and methods

### 2.1 Site description

The study site was located at the botanical garden De Kleine Boerderij (51°20'53"N, 4°49'38"E, 29 m above sea level), located in Merksplas (northern Belgium) (Fig. 1). The climate is temperate and the annual average temperature and rainfall are 10.8°C and 894 mm, respectively. The soil is a freely drained, moderately dry podzol (92.4% sand, 4.2% silt, 3.4% clay). Four types of vegetation have been considered, i.e. a mowed permanent grassland mainly composed of perennial ryegrass (*Lolium perenne L.*) and clover (genus *Trifolium*), and three distinct bamboo plantations of different monopodial species, i.e. *Phyllostachys nigra*, *Phyllostachys aurea* and *Phyllostachys aureosulcata* (Supplementary Fig. 1), all of which were planted on the same grassland 12 years prior to sampling, i.e. in 2010.

The root system of the grassland is mainly composed of thin roots with a diameter < 1 mm but also contains some thicker roots (up to 3 mm). The root system of the three bamboo species has a broader range of diameters. Both very fine (< 2 mm in diameter) and fine roots (2 to 5 mm) are present. The rhizomes are the most considerable coarse roots, with a diameter in the range

of 15–30 mm. Kleinhenz & Midmore (2001) presented the annual growth rate of a typical monopodial bamboo in a northern hemisphere temperate climate. The new rhizomes grow around the beginning of June, followed by the finer roots, which develop from September onwards. Maximum culm growth occurs in spring, after a winter period during which growth of the belowground and aboveground parts are at a standstill. As groundwater table depth strongly influences SOC stock (Meersmans et al., 2008), the study plots were selected by analysing the digital soil map of Flanders, ensuring that they all belonged to the same soil type, especially regarding drainage conditions, and that no land use change occurred between 1979 and 2010. Although the bamboo plots are small, located in a botanical garden, and have no economic purposes, they are managed as a plantation. Approximately 30% of the culms are cut down annually and removed from each plot to allow for regrowth of the culms. A square of 9 m<sup>2</sup> was delineated to count the bamboo culms. The square included the soil respiration measurement area and the soil samples. A digital caliper measured the culms' diameter at breast height (DBH), i.e. 1.3 m high, as described by Liese & Köhl (2015)These measurements were then used to calculate the culm basal area, i.e. the percentage of the soil occupied by bamboo culms, for each bamboo plantation.

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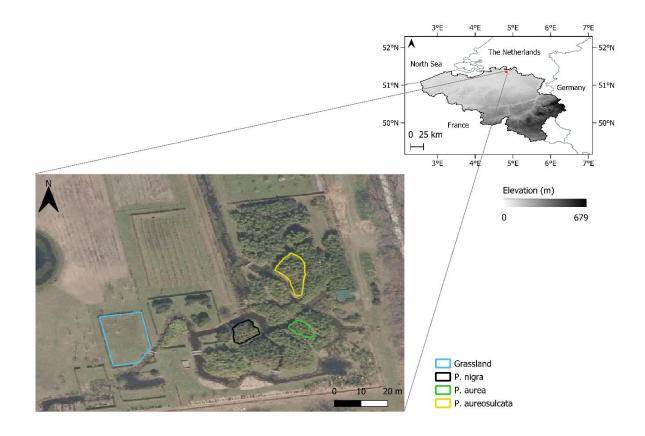


Fig. 1. Description of the four vegetation types: a permanent grassland and three bamboo species of *Phyllostachys sp*. The study site (29 meters above sea level) is highlighted in red on the Digital Elevation Model (DEM) of Belgium.

### 2.2 Soil and biomass sampling

To estimate the C storage after 12 years of bamboo plantation, we adopted a space-for-time substitution approach, considering the current grassland as the reference (t = 0) and the adjacent bamboo stand as the conversion (t = 12 years). An initial survey to a depth of 1 m was conducted to inspect the soil profile in each vegetation type. Between the four plots, the soil profiles differed below a depth of 40 cm, especially in the position of the horizons. Moreover, the roots of the three bamboo species stopped at 40 cm in depth. Soil samples from 10 randomly selected points (n = 10) considering four depth increments (i.e. 0-10 cm, 10-20 cm, 20-30 cm and 30-40cm) were collected from each vegetation type, of which 7 with an Edelman auger and 3 with a root auger. The latter is characterised by a fixed volume of 503 cm<sup>3</sup> in order to obtain a reliable

measure for soil bulk density and volumetric root mass. Finally, leaves from the soil surface were collected from a 50x50 cm square (n = 3 for each bamboo plot).

# 2.3 Sample processing and SOC stocks estimation

All soil samples were oven-dried at 40°C, homogenised, ground and passed through a 2 mm sieve. The samples collected with the root auger were weighed fresh and dry to calculate their bulk density. The C contents of the dried subsamples were measured with an elemental analyser (Primacs, Skalar Analytical, The Netherlands) by the Provincial Centre for Agriculture and Rural Affairs laboratory in La Hulpe, Belgium. The SOC stock was then calculated using the following formula:

$$SOC_{stock} = \rho_s * \frac{SOC}{1000} * Th$$
 (1)

Where  $SOC_{stock}$  is the SOC stock per unit area (kgC m<sup>-2</sup>), SOC the SOC concentration (gC kg<sup>-1</sup>),  $\rho_s$  the soil bulk density (kg m<sup>-3</sup>) and Th the soil layer thickness (0.1 m). Subsequently, also the top 40 cm SOC stock was calculated by summing each layer's SOC stock. The roots of the root auger samples were manually picked, washed with water to remove small soil particles, and dried. They were then weighed to obtain a root mass per unit volume. C stocks per unit area in bamboo roots (of each plot) and leaves (of bamboo only) were calculated with the same formula, assuming a 44 % (Fukushima et al., 2015) and 42 % (Amoah et al., 2020) C concentration, respectively. For grassland, a root C concentration of 40 % was considered (McNally et al., 2015). We assumed that these percentages remained constant throughout the growth period.

However, our fixed depth approach may bias SOC stock calculations because soil bulk density varies substantially between the grassland and the bamboo plantations (Mikha et al., 2013; Rovira et al., 2022). The equivalent soil mass (ESM) approach has been considered more appropriate for comparing SOC stocks across sites that differ in soil bulk density. Therefore, it

is a complementary method to the traditional fixed depth (Rovira et al., 2022). Initially described by Ellert & Bettany (1995) and recently applied in bamboo by Yang et al. (2019), the comparison relies on using the same soil masses M across the vegetation types. The lowest soil mass measured in the top 40 cm ( $M_{\rm min}$ ) is used as a reference to correct the other vegetation types. The  $M_{\rm min}$  in our study was 398.29 kg m<sup>-2</sup> (i.e. 2.0 kg of dry soil), observed in P. aureosulcata. This value was obtained by multiplying the average (n = 3)  $\rho_{\rm s}$  (kg m<sup>-3</sup>) with Th (m) for each layer and summing each layer's soil mass. The equivalent soil mass SOC stocks (kgC m<sup>-2</sup>) of grassland, P. nigra and P. aurea were then calculated using the equation:

$$SOC_{stock,ESM} = \sum_{i=1}^{n} M_i * SOC_i * 0.001 - \Delta M_i * SOC_n * 0.001$$
 (2)

Where  $M_i$  and  $SOC_i$  are the soil mass (kg m<sup>-2</sup>) and SOC content (gC kg<sup>-1</sup>) in the i layer, respectively,  $\Delta M_i$  (kg m<sup>-2</sup>) is the difference between the original soil mass and the  $M_{min}$ , and  $SOC_n$  is the SOC content of the deepest depth increment. The latter considers the non-uniform variation of bulk density and carbon concentration throughout the soil profile (Poeplau et al., 2011).

### 2.4 Soil respiration measurement

Soil CO<sub>2</sub> efflux, including heterotrophic respiration of soil organic matter decomposition and autotrophic respiration of living roots, was measured once every two weeks from April to June 2022, resulting in six measurement days. On each plot, three PVC collars (104 mm diameter, 70 mm high), placed about 0.5 m apart, were inserted 30 mm into the soil, leaving 40 mm headspace above the soil surface for CO<sub>2</sub> monitoring. In order to prevent measurement disturbance due to collar installation, they were placed two weeks before recording the first CO<sub>2</sub> efflux. In addition, any vegetation inside the PVC collars was hand-clipped and removed to avoid CO<sub>2</sub> contribution from aboveground plants. This step was repeated just before the soil

respiration measurement if new vegetation had emerged, as well as removing leaves that fell into the collars. The CO<sub>2</sub> efflux from the soil through the PVC collars was measured by an infrared gas analyser (EGM-5 Portable CO<sub>2</sub> Gas Analyzer, PP Systems, USA) connected to an SRC-2 Soil Respiration Chamber (100 mm diameter, 150 mm high) in a closed loop configuration. The chamber has a stainless steel ring that maintains a seal with the collar and also includes an internal fan for flushing and mixing the air. The EGM-5 measurement range was 0-1000 ppm with an accuracy <1% of span concentration. In order to compare soil respiration between the four vegetation types (i.e. grassland and the three bamboo species), the air CO<sub>2</sub> concentration was recorded for seven minutes, and the order of passage on the measurement points was selected randomly. Moreover, the soil efflux variability within each collar was also considered by conducting three successive repetitions on each of the three collars (n = 9 measurements in total per plot, including three dependent measurements per collar). Soil respiration rate was then calculated as the slope value of the linear regression fitted to assess the temporal increase in air CO<sub>2</sub> concentration. Soil CO<sub>2</sub> efflux was initially measured in µmolCO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>. As soil respiration included heterotrophic and autotrophic respiration, especially in this context where an extensive bamboo root biomass was found, we transformed this measurement into a proxy for the stability of total belowground C by expressing it as a relative fraction of the total C mass (i.e. SOC + root C) in the top 40 cm (mgCO<sub>2</sub>-C g<sup>-1</sup>C h<sup>-1</sup>). The latter gives a measure to compare total belowground C stability across the vegetation types regardless of their C amount. More precisely, soil respiration (µmolCO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) was divided by the total belowground C stock of the top 40 cm. We did not consider the leaf litter C stock as part of the current belowground system. As such, leaves were removed from the surface of the collar when measuring the CO<sub>2</sub> efflux. We also

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- reported raw CO<sub>2</sub> efflux values (µmolCO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) in the supplementary material to allow comparison with previous studies.
- The temperature dependence of soil respiration was modelled using a Q<sub>10</sub> function to control
- for differences in soil temperature, as described by multiple authors (Curiel Yuste et al., 2004;
- Davidson et al., 2006; Schindlbacher et al., 2009):

$$R_S = R_{10} * Q_{10}^{(T_S - 10)/10}$$
(3)

- Where  $R_S$  is the measured soil respiration rate (mgCO<sub>2</sub>-C g<sup>-1</sup>C h<sup>-1</sup>),  $R_{I0}$  is the basal respiration rate at 10°C (mgCO<sub>2</sub>-C g<sup>-1</sup>C h<sup>-1</sup>), which corresponds more or less to the average annual temperature in northern Belgium, and  $Q_{I0}$  is the temperature sensitivity of soil respiration that represents the proportional increase in  $R_S$  with a 10°C increase in soil temperature ( $T_S$ ). The function was fitted to each vegetation type's measured  $R_S$  and temperature using the lsqcurvefit command (i.e. a non-linear least-squares solver) in Matlab. Basal respiration was used to compare the total belowground C stability of the different plots, avoiding the potential impact of differences in soil temperature due, for example, to variability in sun radiation through the canopy, which has characteristics depending on vegetation type.
- Two approaches were used to disentangle the confounding effect of temperature and humidity.
- First, univariate linear regressions (Equation 4) were used to test whether soil temperature  $(T_S)$
- or water content  $(S_w)$  significantly predicted respiration. Bivariate regressions containing both
- $T_S$  and  $S_w$  as independent variables (Equation 5) were also tested:

$$R_S = b_0 + b_1 * T_S \text{ or } R_S = b_0 + b_1 * S_w$$
 (4)

$$R_S = b_0 + b_1 * T_S + b_2 * S_w \tag{5}$$

Where  $T_S$  is the soil temperature (°C),  $S_w$  is the volumetric water content (%), and  $b_0$ ,  $b_1$ ,  $b_2$  are fitted parameters. The model performance of both regressions has been evaluated considering the adjusted coefficient of determination ( $R^2_{adj}$ ). Second, the model residuals (i.e. the difference

between the soil respiration measurements and the  $Q_{10}$  function) versus  $S_w$  were analysed (Tan et al., 2013).

During the six respiration measurement days,  $T_S$  and  $S_w$  at the soil surface (average over the layer 0-10cm) were measured close to each collar (n = 3 per plot) using a 5TM Soil Moisture and Temperature Sensor (Decagon Devices, USA). The 5TM measures the soil's dielectric constant at a frequency of 70 MHz (accuracy  $\pm 2\% S_w$ ). The sensor was previously calibrated with a third-degree polynomial equation (Shaikh et al., 2019).

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### 2.5 Statistical analysis

SOC contents and stocks (n = 10 per vegetation type) were first tested for normality of residuals using Shapiro-Wilk's and homoscedasticity using Levene's tests. Analysis of variance (ANOVA) (Shapiro-Wilk, p > 0.05, Levene, p > 0.05) and Kruskal-Wallis test (Shapiro-Wilk, p < 0.05) were then used to test the effect of the vegetation type on SOC contents and stocks. Data from each 10 cm soil layer, the top 40 cm and the top 2 kg (ESM) were investigated. Depending on the results of the previous tests, Tukey HSD (honestly significant difference) or Dunn (with the Bonferroni correction) post-hoc test was performed to identify significant differences between the four plots. A normal distribution was assumed for soil bulk densities, roots and leaf litter C stocks because the normality could not be tested as the number of replications was low (n = 3). The corresponding standard errors (SE) were then calculated by dividing the standard deviation (SD) by the square root of n. Finally, to test the effect of vegetation type on soil  $CO_2$  efflux (n = 9), a nested ANOVA, followed by a post-hoc comparison of least-square means, was performed on each measurement day. This approach was used to reflect the dependence of the three repeated measurements per collar and, therefore, considers the variation introduced by the collars nested in each vegetation type. The soil CO<sub>2</sub> efflux was the response variable, and the vegetation type was the fixed variable within which

the collar was the nested variable. Statistical analyses were performed using RStudio 2023.03.0+386 software.

### 3. Results

## 3.1 SOC content and bulk density

Table 1. Characterisation of grassland, P. nigra, P. aurea and P. aureosulcata. Except for the standing culm density (STD) and the culm basal area of the bamboo plantations, data are presented as the mean  $\pm$  SD. Different letters within the same depth indicate significant differences between vegetation types at p < 0.05

	Grassland	P. nigra	P. aurea	P. aureosulcata
STD (culms m <sup>-2</sup> )	/	19	15	9
DBH (cm) $(n = 40)$	/	$1.57\pm0.58^{b}$	$1.64 \pm 0.54^b$	$3.69\pm1.06^a$
Culm basal area (%)	/	0.42	0.35	1.07
Bulk density (g cm <sup>-3</sup> ) ( $n = 3$ )				
0-10 cm	$1.30\pm0.03^{\rm a}$	$0.78\pm0.12^{b}$	$0.74\pm0.04^{b}$	$0.32 \pm 0.06^{c}$
10-20 cm	$2.00 \pm 0.11^a$	$1.39\pm0.15^{\text{b}}$	$1.25\pm0.13^{b}$	$0.99 \pm 0.11^{\text{c}}$
20-30 cm	$1.90 \pm 0.01^a$	$1.61\pm0.11^{b}$	$1.76\pm0.28^{ab}$	$1.00\pm0.36^{c}$
30-40 cm	$1.84 \pm 0.07^{\text{b}}$	$1.91\pm0.05^{ab}$	$1.96\pm0.07^a$	$1.66 \pm 0.05^{c}$
SOC (gC kg <sup>-1</sup> ) ( $n = 10$ )				
0-10 cm	$16.22 \pm 3.55^{b}$	$27.86\pm7.66^{ab}$	$28.83 \pm 21.09^{b}$	$87.52 \pm 48.78^{a}$
10-20 cm	$11.85\pm2.74^{b}$	$15.16 \pm 3.52^{b}$	$16.30\pm4.93^{ab}$	$35.32 \pm 18.30^{a}$
20-30 cm	$9.92\pm1.68^{b}$	$12.60\pm1.97^{ab}$	$13.89\pm3.74^\mathrm{a}$	$18.66\pm6.24^{\mathrm{a}}$
30-40 cm	$8.55\pm1.81^{b}$	$10.36\pm2.49^{ab}$	$11.54\pm1.47^a$	$12.58\pm1.93^{\text{a}}$

The characteristics of the four plots studied are shown in Table 1.  $P.\ nigra$  and  $P.\ aurea$  had a similar DBH (cm), and their culm basal area was 0.42 and 0.35 %, respectively.  $P.\ aureosulcata$  had fewer culms per square meter than the two previous plots, had twice the average DBH (p < 0.05), and the culm basal area was 1.07%. Soil bulk density (kg m<sup>-3</sup>) was the highest for grassland and the lowest for  $P.\ aureosulcata$ . The bulk density of the three bamboo plots

increased with depth and was negatively associated with SOC concentration (gC kg<sup>-1</sup>). However, the grassland's bulk density seemed constant below 10 cm. P. nigra and P. aurea showed similar aboveground biomass and bulk density (p > 0.05). Only P. aureosulcata had significantly higher SOC contents than the grassland in the top 20 cm (p < 0.05). Until this depth, the three bamboo species had a considerable variability for SOC concentrations. Below 20 cm, both P. aurea and P. aureosulcata had significantly higher SOC contents than grassland (p < 0.05), and the SOC contents of the three bamboo species were also comparable.

## 3.2 Organic carbon storage

340 3.2.1 SOC stocks

SOC stocks are shown as boxplots in Fig. 2. Between 0 and 30 cm in depth, the SOC stocks were not statistically different (p > 0.05) in all four plots. In the 30-40 cm layer, the SOC stocks of the three bamboo species remained comparable but were significantly higher in P. aurea and P. aureosulcata than in grassland (p < 0.05). The SOC stocks in the top 40 cm of the grassland, P. nigra, P. aurea and P. aureosulcata were (mean  $\pm$  SD)  $7.92 \pm 1.18$  kgC m<sup>-2</sup>,  $8.28 \pm 1.35$  kgC m<sup>-2</sup>,  $8.87 \pm 2.44$  kgC m<sup>-2</sup> and  $10.33 \pm 2.98$  kgC m<sup>-2</sup>, respectively. Although the three bamboo species' total SOC stocks were higher than the grassland's, no statistical difference was found (p > 0.05). The difference between the top 40 cm SOC stock of P. aureosulcata and grassland was nevertheless close to being statistically significant (p = 0.07). However, when comparing the SOC stocks calculated based on the ESM approach, Fig. 2 shows that P. aureosulcata had a significantly higher SOC stock than grassland (p < 0.001). More precisely, the SOC stocks (mean  $\pm$  SD) considering this approach (i.e. the top 2 kg) of the grassland, P. nigra, P. aurea and P. aureosulcata were  $5.32 \pm 0.88$  kgC m<sup>-2</sup>,  $6.52 \pm 1.00$  kgC m<sup>-2</sup>,  $6.88 \pm 2.33$  kgC m<sup>-2</sup> and  $10.33 \pm 2.98$  kgC m<sup>-2</sup>, respectively. The raw SOC data are available in Supplementary Table 1.

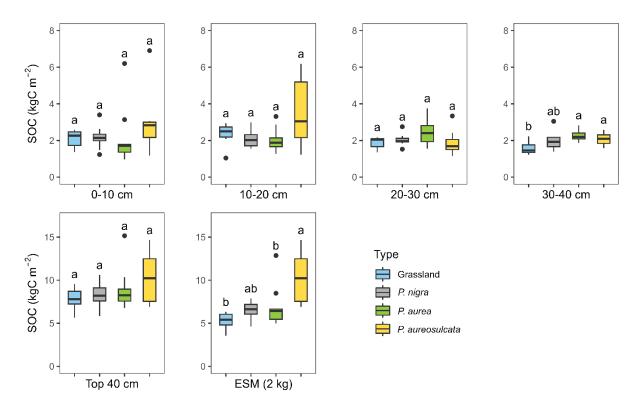


Fig. 2. Boxplots (median, first and third quartiles) of SOC stocks (kgC m<sup>-2</sup>) under grassland, P. nigra, P. aurea and P. aureosulcata (n = 10). Different letters indicate significant differences between vegetation types at p < 0.05.

3.2.2 Root and leaf litter stocks

C stocks in roots (all vegetation types) and leaf litter (bamboo only) are presented in Fig. 3. Roots were present down to 40 cm in depth. The three bamboo species had significantly more root C than grassland at each depth (Fig. 3a). *P. aurea* and *P. aureosulcata* had more root C than *P. nigra*, especially below 20 cm. Larger roots (rhizomes) were generally found in the top 20 cm of all three species, but *P. aureosulcata* was the only one developing rhizomes below 30 cm.

Over the first 40 cm of the soil profile, all bamboo species had significantly higher root C stock than the grassland (Fig. 3b). The root C stocks in *P. aurea* and *P. aureosulcata* were similar and significantly higher than that in *P. nigra*. More precisely, we have found (mean  $\pm$  SD)  $0.35 \pm 0.14$  kgC m<sup>-2</sup> in the roots of the grassland, and  $2.30 \pm 1.09$  kgC m<sup>-2</sup>,  $3.85 \pm 1.43$  kgC

m<sup>-2</sup>,  $3.68 \pm 0.21$  kgC m<sup>-2</sup> in *P. nigra*, *P. aurea* and *P. aureosulcata*, respectively. Finally, the amount of leaf litter was similar between the three bamboo plots and significantly different from 0 (p < 0.001), which can be considered as the grassland condition as no litter was present here (Fig. 3c). Although *P. nigra* tends to have more leaf litter than the two other bamboo species, this difference was not significant.

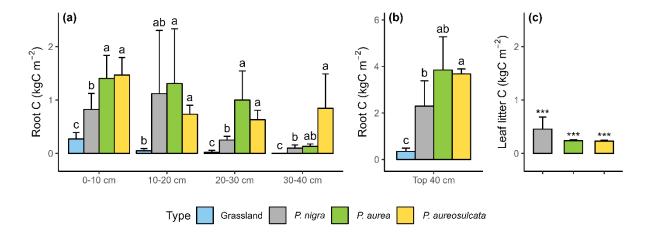


Fig. 3. Dry root carbon stocks (kgC m<sup>-2</sup>) in grassland, *P. nigra*, *P. aurea* and *P. aureosulcata* (a) in each 10 cm soil layer, and (b) in the top 40 cm. Data are presented as the mean + SD (n = 3). The bamboo root system includes fine roots and rhizomes. Different letters within the same depth indicate significant differences between vegetation types when both means fall outside the 95% confidence interval (CI). The bar and SD of the grassland at 30–40 cm in depth are too small to appear on the graph. (c) Dry leaf litter C stock (kgC m<sup>-2</sup>) in the three bamboo species. Data are presented as the mean + SD (n = 3). Stars (\* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001) indicate mean C stock is significantly different from zero (grassland condition).

### 3.2.3 Total carbon storage

Fig. 4 shows the total additional C storage considering the combined effect of the belowground components (i.e. soil and roots) and leaf litter on the soil surface (representing a future C input

in the soil after decomposition) for each of the three bamboo species 12 years after the conversion from permanent grassland. On average, P. nigra, P. aurea and P. aureosulcata had an additional SOC stock in the top 40 cm of 0.35 kgC m<sup>-2</sup> (relative increase of +4 %), 0.95 kgC m<sup>-2</sup> (+12 %) and 2.40 kgC m<sup>-2</sup> (+30 %), respectively. Although none of these increases were statistically significant (Fig. 2), considering all belowground components and the leaf litter, the three bamboo species had highly significant C storage (p < 0.001). The corresponding C stock increases were 2.76 kgC m<sup>-2</sup> (relative increase of +33 %), 4.69 kgC m<sup>-2</sup> (+57 %) and 5.97 kgC m<sup>-2</sup> (+72 %) for P. nigra, P. aurea and P. aureosulcata, respectively (Fig. 4a). However, when the SOC stock was calculated using an ESM, the total C stock increases (p < 0.001) were 3.60 kgC m<sup>-2</sup> (relative increase of +64 %), 5.30 kgC m<sup>-2</sup> (+ 94 %) and 8.57 kgC m<sup>-2</sup> (+151 %), respectively (Fig. 4b).

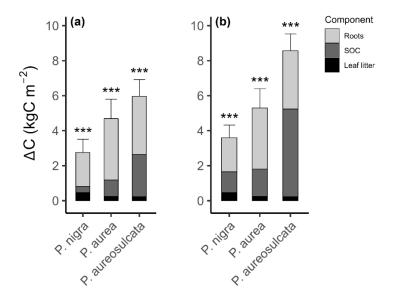


Fig. 4. Total additional C stock (kgC m<sup>-2</sup>) of *P. nigra*, *P. aurea* and *P. aureosulcata* (as compared to grassland after 12 years) when the SOC stocks are calculated using (a) the fixed depth of 40 cm and (b) the ESM of 2 kg. Data are presented as the mean + SE of total storage in the three components. The total error is calculated as the square root of the sum of the squared errors, assuming that the components are independent. Stars (\* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001) indicate C storage is significantly different from zero (grassland condition).

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# 3.4 Soil respiration

The comparison among the four plots of the raw  $CO_2$  effluxes ( $\mu$ mol  $CO_2$  m<sup>-2</sup> s<sup>-1</sup>) for the different measurement days is given in Supplementary Fig. 2. The six nested ANOVA statistical results are shown in Supplementary Table 2. The significant F-stastitic (p < 0.001) for the nested factor (i.e. collar) indicated high variance among the three collars within a vegetation type for each measurement day. It also reveals that vegetation type significantly (p < 0.01) impacted soil relative  $CO_2$  efflux (mg $CO_2$ -C g<sup>-1</sup>C h<sup>-1</sup>) during the first four measurement days. During this period, the variability between plots was greater than within plots. This effect reversed from June 5 onwards as the F-statistic showed that the impact of the collar became much more important than that of the vegetation type. Fig. 5 shows that during the first four measurement days, the relative  $CO_2$  efflux of the three bamboo species had a similar trend and was significantly lower than that of grassland. On June 5 and 22, the respiration of the three bamboo species was not significantly different from that of grassland. Moreover, there appear to be two contrasting periods: (i) until May 3, the soil of each vegetation type was characterised by a constant and limited  $CO_2$  efflux; (ii) from May 21 onwards, the  $CO_2$  efflux more or less doubled, except for grassland on June 22.

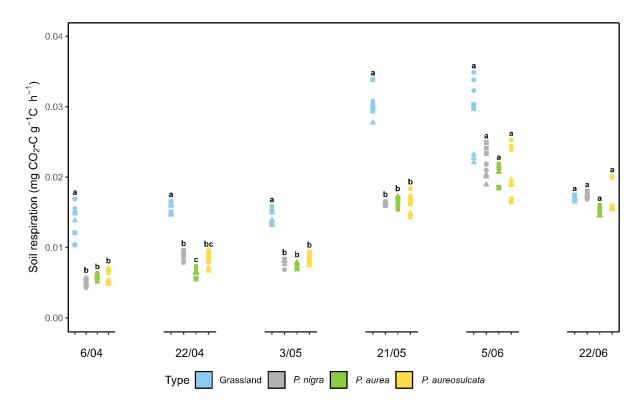


Fig. 5. Comparison of CO<sub>2</sub> efflux (mgCO<sub>2</sub>-C g<sup>-1</sup>C h<sup>-1</sup>) between grassland, P. nigra, P. aurea and P. aureosulcata over six days. Soil respiration is expressed as a relative fraction of the total belowground carbon mass (SOC + root C). Each colour represents a vegetation type (n = 9) within which each symbol (i.e. circle, square or triangle) is associated with a series of 3 repeated measurements of the same collar. Different letters within the same date indicate significant differences between vegetation types at p < 0.05.

 $T_S$  and  $S_w$  data are shown in Supplementary Fig. 2. For the same day,  $S_w$  varied very little between each vegetation type.  $T_S$  between the four vegetation types was similar on April 6 and consistently lower in the three bamboo species than in the grassland on the other days. Moreover, the soil temperature was always similar between the three bamboo species.

The comparison between the univariate and bivariate models is shown in Supplementary Table 3. The univariate analysis (equation 4) shows that  $T_S$  was positively associated with relative  $CO_2$  efflux rate (mg $CO_2$ -C g $^{-1}$ C h $^{-1}$ ) for grassland ( $R^2_{adj} = 0.28$ , p < 0.05). This is even more

clear for P. nigra (adj.  $R^2 = 0.91$ , p < 0.001), P. aurea ( $R^2_{adj} = 0.76$ , p < 0.001) and P. 444 aureosulcata ( $R^2_{\text{adj}} = 0.74$ , p < 0.001).  $S_w$ , however, did not predict soil respiration (p > 0.05). 445 446 The bivariate model (equation 5) was significant and explained a higher percentage of the grassland's soil respiration variance than temperature alone ( $R^2_{\text{adj}} = 0.48$ , p < 0.01). In the 447 448 bamboo plantations, the performance of the bivariate models was higher than in grassland, though adding the  $S_w$  resulted in no or minimal improvement of  $R^2_{\text{adj}}$  (i.e.  $R^2_{\text{adj}} = 0.90$ , p < 0.001; 449  $R^{2}_{\rm adj} = 0.80, \, p < 0.001; \, R^{2}_{\rm adj} = 0.75, \, p < 0.001 \, \, {\rm for} \, P. \, \, nigra, \, P. \, \, aurea \, \, {\rm and} \, \, P. \, \, aureosulcata,$ 450 451 respectively). Fig. 6 shows that *P. nigra* and *P. aurea* have similar R<sub>10</sub> (mgCO<sub>2</sub>-C g<sup>-1</sup>C h<sup>-1</sup>) and Q<sub>10</sub> values. 452 453 Their  $R_{10} \pm SE$  (i.e.  $0.007 \pm 0.001$  and  $0.006 \pm 0.001$ , respectively) were remarkably lower than 454 grassland (0.012  $\pm$  0.003), though this difference was only significant for *P. aurea*. This result 455 indicated a more stable total belowground C under these bamboo types than grassland. 456 However, their  $Q_{10}$  value (i.e.  $4.78 \pm 0.80$  for *P. nigra* and  $4.18 \pm 1.03$  for *P. aurea*) was 457 significantly higher than that of grassland (1.84  $\pm$  0.43), indicating a greater temperature 458 sensitivity. These results of P. aureosulcata, both in terms of  $R_{10}$  (0.008  $\pm$  0.001) and  $Q_{10}$  (2.89 459  $\pm$  0.58), were in between those of grassland on the one hand, and P. nigra and P. aurea on the 460 other hand.

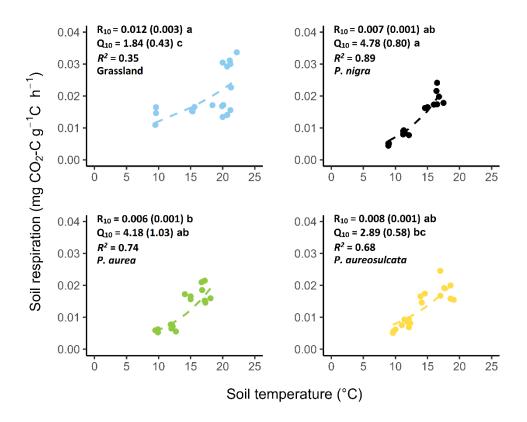


Fig. 6. Soil temperature dependence on relative soil CO<sub>2</sub> efflux (mgCO<sub>2</sub>-C g<sup>-1</sup>C h<sup>-1</sup>) for grassland, P. nigra, P. aurea and P. aureosulcata. Each point represents the mean of the three repeated measures on one collar. The dashed lines correspond to the Q<sub>10</sub> function fit. Model parameters are presented with their corresponding SE in brackets. Different letters indicate significantly different parameter values between vegetation types at p < 0.05.

### 4. Discussion

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### 4.1 Sampling depth

To examine the study site's pedological context and identify an adequate sampling depth, we initially conducted a 1-meter depth survey. The first key element was the absence of visible roots below 40 cm under all the vegetation types. Moreover, a paleosol was observed below 50 cm in each soil profile (Supplementary Fig. 3a). It corresponds to an ancient black horizon enriched with organic matter compatible with the geological context of the region. This paleosol was also observed in a quarry 900 meters southeast of the botanical garden, with a slight slope of c. 1 or 2%, corresponding to the paleosol's depth at the study site. The podzol profile was consistent in the upper part between the permanent grassland and the three bamboo plots (Supplementary Fig. 3b). Below 50 cm, however, the potential differences between them were not related to the change in land use but rather to the paleosol. Consequently, we have limited the study depth to 40 cm. This has been considered a valid reference depth for studying current C dynamics affected by the recent land use change from grassland to bamboo and associated biological activity. Furthermore, 40 cm is a common reference depth for assessing SOC stocks in bamboo plantations (Chen et al., 2009). For example, Yang et al. (2019) compared SOC stocks in Moso bamboo (P. pubescens) stands with evergreen broadleaf forest in China following the ESM approach after taking samples up to 40 cm, corresponding to the present methodology.

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## 4.2 Grassland SOC

For a Belgian sandy mowed grassland in another study, Mestdagh et al. (2006) measured a soil bulk density (mean  $\pm$  SD) of  $1.26 \pm 0.33$  g cm<sup>-3</sup> between 0 and 10 cm depth, which is very similar to our value ( $1.30 \pm 0.03$  g cm<sup>-3</sup>). However, Mestdagh et al. (2006) obtained for the 10-30 cm depth increment a bulk density of  $1.42 \pm 0.11$  g cm<sup>-3</sup>, which is somewhat lower than the

value we found  $(1.95 \pm 0.05 \text{ g cm}^{-3})$ . This rather high soil bulk density might be an indication of soil compaction on our study site. Our top 30 cm SOC stock (mean  $\pm$  SE) equals  $6.35 \pm 0.31$  kgC m<sup>-2</sup>, which aligns with Meersmans et al. (2009), reporting a SOC stock of  $6.37 \pm 0.78$  kgC m<sup>-2</sup> for moderately wet sandy grassland soils in Belgium. However, when taking the SOC contents (gC kg<sup>-1</sup>) and bulk densities of Mestdagh et al. (2006) into account, their top 30 cm stock is somewhat lower (5.98 kgC m<sup>-2</sup>) but falls within our confidence interval.

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## 4.3 Bamboo SOC and potential carbon inputs

Our results showed that all bamboo species had higher mean SOC contents and lower bulk density than grassland (Table 1). Kaushal et al. (2020) reported that bamboo's lower soil bulk density could be due to increased SOC contents, which are added by the formation and turnover of fine roots, leaf litter, dead branches, and culm sheaths. Generally, the two primary sources of C inputs to the soil are root remains, and C exudates from roots (Kuzyakov & Domanski, 2000). Fine-roots turnover (year-1), defined as the number of times the fine root system dies and grows back each year (Lukac, 2012), is generally rapid and provides considerable C inputs to the soil (Kaushal et al., 2020). We did not calculate species turnover rate in this work, but we have tried to find consistent values in the literature. Gill & Jackson, (2000) reviewed multiple root turnover rates for terrestrial ecosystems and reported a value of about 0.52 year<sup>-1</sup> for temperate grassland. A previous study by Xue et al. (2015) found a turnover of the fine roots of *P. pubescens* in China that ranged between  $0.68 \pm 0.19$  and 1.37 $\pm$  0.32 year<sup>-1</sup>, depending on the forest health condition. To the best of our knowledge, the turnover rate of soil C for P. nigra, P. aurea and P. aureosulcata has not been studied yet. These values should be interpreted cautiously for several reasons. As highlighted by Gill & Jackson (2000), root turnover depends on abiotic parameters, i.e. soil temperature, moisture content and nutrient availability, as well as the definition of fine roots and the climatic region.

Moreover, the plant species examined in prior studies differ from those in our investigation. Nonetheless, Qin et al. (2017) argued that root systems with high turnover rates and healthy rhizomes may have a better potential to increase C inputs in the soil. In addition, Kawakami et al. (2022) showed that bamboo (*Phyllostachys pubescens*) had a very high annual production of C exudates thanks to their considerably greater fine-root biomass, which significantly determined stand-scale root exudation. For example, they showed bamboo stands have substantially higher exudation than temperate forests due to this greater root biomass. We found that P. aurea and P. aureosulcata had many fine roots in the deeper layers, with P. aureosulcata still having a consequent rhizome in the 30-40 cm layer. Hence, the significantly higher SOC stock below 30 cm in P. aurea and P. aureosulcata compared with the grassland probably came from a higher amount of C inputs through turnover and exudation. While it remains a hypothesis due to the lack of direct C input measurements, substantial evidence supports its validity. The C mineralization rate should be lower in those deeper layers, resulting in a more stable SOC (Lin et al., 2018), which could explain the significant SOC storage after 12 years in these two species between 30 and 40 cm (Fig. 2). Additionally, P. nigra, P. aurea and P. aureosulcata are all perennial plants and, therefore, can maintain soil organic matter supply over the year through leaf litterfall and root residues (Liese & Köhl, 2015). It has also been demonstrated that bamboo (P. pubescens) stimulates the presence of arbuscular mycorrhizal fungal (AMF) communities in the soil, which, in addition to boosting soil nutrients and water uptake by the plant, also help to enhance SOC storage (Qin et al., 2017). No mycorrhizae were visually identified in the bamboo plantations in our study. Given the extensive root development of the three bamboo species, it is very likely that the mycorrhizae were microscopic or visually confused with the very fine roots. The highest SOC was found in *P. aureosulcata*. This species also had the highest culm basal area (Table 1) but had similar leaf litter C stock (Fig. 3c). Several authors have reported the

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mean culm diameter for *P. nigra* and *P. aurea*. Those values were typically equal to 2-4 cm for *P. nigra* (Kleinhenz & Midmore, 2001; Liese & Köhl, 2015; Inoue et al., 2018) and 5 cm for *P. aurea* (Liese & Köhl, 2015), which are higher than our mean values (1.57 and 1.64 cm, respectively). We did not find any DBH value of *P. aureosulcata* in previous research. Even though the highest DBH values we measured for *P. nigra* fell within the range of the literature, it seems that our average value appears smaller. Therefore, the temperate climate or the management of the bamboo plantations in Merksplas might limit their growth. In another study where an evergreen broadleaf forest was converted into a Moso bamboo plantation in China, Lin et al. (2018) suggested that a significant SOC stock reduction (after 11 years) could be explained by the annual litterfall decrease associated with understory vegetation removal. Finally, Fu et al. (2014) also argued that the decomposition of the bamboo litter and the fineroots turnover enhance the total C storage in the top 40 cm, which aligns with our findings.

### 4.4 Carbon storage potential

Our results suggested that bamboo can store SOC after only 12 years. Bamboo had many roots and rhizomes, sometimes up to a diameter of 30 mm, which decreased the number of soil particles per volume unit (i.e. soil bulk density). Therefore, we compared SOC stocks by considering the fixed depth and ESM approaches (Fig. 2). As mentioned by Rovira et al. (2022), comparing C stocks by fixed depth overestimates actual storage when the soil has been compacted (i.e. an increase in bulk density) and underestimates it in the case of a decrease in soil bulk density after extensive root development. Given the characteristics of our study site, this supports the comparison by ESM. This method detected a significant SOC storage (12 years after converting the grassland) of +5.01 kgC m<sup>-2</sup> (relative increase of +94 %) in *P. aureosulcata*, and a mean (non-significant) SOC stock increase of 1.20 kgC m<sup>-2</sup> (+22 %) and 1.56 kgC m<sup>-2</sup> (+29 %) in *P. nigra* and *P. aurea*, respectively. By comparison, after thoroughly reviewing and

modelling, Poeplau et al. (2011) found that afforestation of grassland in temperate climates often results in a slight decrease in SOC stock (after considering the same time period as in the present study). Only after decades (even up to 100 years) does afforestation of temperate grassland sometimes result in a net SOC storage, highlighting the remarkably rapid C storage potential of bamboo detected in this research (i.e. only after 12 years). This is particularly true given the pedological context of the present study, where the podzol had a very low clay content, resulting in a limited capacity to store additional C (Meersmans et al., 2008; Boitsova et al., 2023). In addition, it is essential to bear in mind that, in the fixed depth approach (i.e. top 40 cm), the bamboo SOC stock was greater than its C stock in the root biomass. Still, the significant total C storage was mainly due to the remarkably well-developed root system of the studied bamboo species (Fig. 4). The SOC stocks of P. nigra, P. aurea and P. aureosulcata fell within the range of values reviewed by Yuen et al. (2017). However, the SOC storage of each of the three bamboo species calculated in this study does not correspond to their total C storage potential, as we only considered a timeline of 12 years after planting. When adequate conditions are met, SOC stock generally increases in a non-linear way until it reaches a new equilibrium, defined as the total storage potential (Poeplau et al., 2011; Chenu et al., 2019). Therefore, it is very likely that the bamboo SOC stocks will still increase in the upcoming years, and the storage in the top 40 cm may become significant. Nonetheless, we are also aware that northern Belgium's permanent grasslands, which already store a relatively important quantity of SOC, may be close to equilibrium, thus limiting the potential for additional SOC storage. However, the C storage would have probably been larger if the conversion was from cropland to bamboo (Meersmans et al., 2008; Poeplau et al., 2011).

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As Europe is emerging as a progressively significant trade region for bamboo products (INBAR, 2021), and given the nature-based solution that bamboo represents in C sequestration and environmental sustainability (Pan et al., 2023), we also encourage the estimation of the storage potential in the aboveground biomass of *P. nigra*, *P. aurea* and *P. aureosulcata*. The latter will help to carry out a complete LCA regarding the net CO<sub>2</sub> balance of bamboo-derived products, such as those used as building materials, e.g., flooring.

While our study presents valuable results for the SOC storage of temperate bamboo plantations, we must acknowledge the limitations of our experimental design. The absence of true replicates for each species and the limited extent of the botanical garden might affect the reliability of extrapolating our findings to larger geographical entities. Hence, an interesting future research avenue could be sampling across larger areas covered by temperate bamboo species in order to facilitate the extrapolation of results.

## 4.5 C respiration

4.5.1 CO<sub>2</sub> efflux variability

In theory, the impact of soil moisture must be removed to compare soil respiration results throughout the analysis of the differences in  $R_{10}$  and  $Q_{10}$ . In this study, for each vegetation type,  $S_w$  did not impact the residuals between measured  $R_S$  and the  $Q_{10}$  function (i.e. there was no clear under- or overestimation of  $R_S$  using the  $Q_{10}$  function). In addition, the wilting point (i.e. the matric potential limit below which the herbaceous vegetation of the grassland and the bamboo no longer have access to water, which sometimes reduces respiration rates) was considered. In sandy soil, the wilting point is about 0.05 m<sup>3</sup> m<sup>-3</sup> (Pardossi et al., 2009). No  $S_w$  measured during the study was below this value. Occasionally,  $S_w$  was close but always above 5%. Moreover, to our knowledge of the Belgian Kempen region, even if the sandy surface horizon is dry, relatively wet conditions are not uncommon underneath this dry surface. This

trend was confirmed when we calculated  $S_w$  from the gravimetric water content of the root auger samples. As a result, no soil  $CO_2$  efflux was discarded because  $S_w$  was not considered a limiting factor for  $R_S$ . We showed that  $T_S$  was a good predictor of  $R_S$ , even better in the bamboo plantations than in grassland. In the grassland, the combination of  $T_S$  and  $S_w$  was better at predicting CO<sub>2</sub> efflux than temperature alone (Supplementary Table 3). However, this was not the case in the bamboo plantations as  $S_w$  (i.e. the second independent variable) was not significant and the  $R^2_{adj}$  showed insignificant improvement in the  $R_S$  prediction performances. Moreover, supplementary Fig. 4 shows the temporal evolution of the average daily temperature and precipitation during the experiment. Overall, the air temperature rose during May, and the Q<sub>10</sub> law correctly simulated the two periods of contrasting respiration intensity. The variability can also be analysed in light of Supplementary Table 2, which showed a very high F-statistic value on 5 June, and Supplementary Fig. 4, indicating heavy rainfall on the same day. Therefore, rain might activate the soil microbial system, increasing within-plot variability. Given the collar variable was always significant (p < 0.001), and the within-plot variability was higher in June, we suggest that the experimental design could be improved by increasing the number of collars per plot, particularly during summer. On the other hand, the results indicated that increasing the number of replicates per collar is less important. The relatively high within-plot variability of  $R_S$  could be partly explained by a variability in sun radiation due to a fairly high heterogeneity in the bamboo canopy's cover. This can lead to differences in  $T_S$  or  $S_w$  (i.e. through precipitation reaching the ground) between collars installed within the same plot. Finally, the phenology must be taken into account. Indeed,  $R_S$  data are often separated into different seasons (Tan et al., 2013) or phenological stages (Curiel Yuste et al., 2004). Kleinhenz & Midmore (2001) explained that the new rhizomes of a typical monopodial bamboo in a

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temperate climate begin to grow in June, reaching maximum growth in July. The other roots then start to grow in September-October. Therefore, the rhizome development might also explain the remarkable increase in  $R_S$  (from the fourth measurement day onwards). In the  $Q_{10}$  function calculation, it was nonetheless decided to keep the dataset in one part (instead of two) to allow a sufficient number of measurements.

Supplementary Fig. 2 indicated that each bamboo plot's top 10 cm of soil was always colder

# 4.5.2 Basal respiration

than the grassland. This finding was expected as the aboveground bamboo biomass provides shadow, thus preventing the soil from heating. We calculated a basal respiration rate at  $10^{\circ}$ C expressed in mgCO<sub>2</sub>-C g<sup>-1</sup>C h<sup>-1</sup>. This allowed us to compare the SOC mineralization rate and the root respiration of the four vegetation types independently of the differences in total C stocks and soil temperatures. Since the average annual temperature at the study site is  $10.8^{\circ}$ C, calculating R<sub>10</sub> provides a reliable proxy for total belowground C stability. Hence, the lower the basal respiration, the lower the relative soil respiration, indicating a greater total belowground C stability.

P. aurea had a R<sub>10</sub> value significantly lower than the grassland (Fig. 6). The results also showed no significant difference between the grassland, *P. nigra* and *P. aureosulcata*. Still, they must be interpreted cautiously as the measured  $R_S$  of the grassland was greater than these two bamboo species when  $T_S$  was close to  $10^{\circ}$ C (Fig. 5). More winter measurements should be performed to potentially detect a significantly higher basal respiration rate of grassland as

### 4.5.3 Temperature sensitivity

compared to P. nigra and P. aureosulcata.

Our temperate permanent grassland had a Q<sub>10</sub> of 1.84. In comparison, Meyer et al. (2018) measured Q<sub>10</sub> between 1.4 and 2.3 in grasslands from the Rur River catchment in Germany, close to Belgium. In Moso bamboo (Phyllostachys pubescens), Li et al. (2013) and Hsieh et al. (2016) have respectively measured a  $Q_{10}$  value of 4.44 and 4.09, which is similar to the values we obtained for P. nigra (4.78) and P. aurea (4.18) (Fig. 6). The soil of two bamboo species (i.e. *P. nigra* and *P. aurea*) had a significantly higher temperature sensitivity than the grassland. According to Tang et al. (2016), the Q<sub>10</sub> parameter reflects changes in carbohydrates, root biomass, root activity, soil nutrient availability, and litter biomass. Hsieh et al. (2016) highlighted that the specific root growth of bamboo can increase its annual Q<sub>10</sub> value. Given the difference in root systems between grassland and bamboo, the change in temperature sensitivity induced by the land use conversion of grassland to bamboo could originate from a change in root type, root system architecture or microbial community. Schindlbacher et al. (2009) also argued that, when analysing autotrophic respiration, high temperature sensitivity can reflect labile C inputs to the roots and rhizosphere or root growth. After the land use conversion, the labile C inputs in the bamboo plantations could originate from fresh litterfall or root exudates. In addition to a potential increase in C inputs and the development of an extensive root system, bamboo's belowground C storage could also come from a lower relative CO2 efflux through soil respiration. Given bamboo's greater sensitivity to temperature, future studies will have to analyse, for P. nigra, P. aurea and P. aureosulcata, the C dynamics and its sensitivity to temperature in warmer climates (i.e. in future climate change conditions).

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#### 4.5.4 other carbon outputs

As the soil of the study area was very sandy, Dissolved Organic Carbon (DOC) can easily migrate downwards and add C content to the lower horizons, which has probably been

accumulating in the Bh horizon of the podzol. The latter was not considered in the different comparisons (because we restricted our sampling depth to 40 cm), nor was the DOC. Still, the latter could be an extra pool to measure in order to obtain a more complete C balance assessment of the studied systems.

#### Conclusion

To our knowledge, the present study is the first to unravel C dynamics and C storage of *P. nigra*, *P. aurea* and *P. aureosulcata* in temperate climate (after converting a permanent grassland in this case). When considering the SOC stock on an equivalent mass basis, only *P. aureosulcata* had a significantly higher SOC stock than the grassland. Based on the total C stock in the soil, including SOC, roots and leaf litter, we found that all three bamboo species have a highly significant C storage 12 years after the conversion, which was mainly due to the development of an extensive root network. We argue that this net C storage was also a consequence of (1) a significant increase in C inputs through litterfall, root turnover and exudation and (2) an enhanced total belowground C stability. The present research provides a valuable reference for future studies on belowground C storage in bamboo, particularly in temperate climates. Hence, our work underlines the potential of bamboo as a nature-based solution, especially in light of the expanding European bamboo markets.

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/19	Conflict of Interest statement
720	The authors declare that they have no known competing financial interests or personal
721	relationships that could have influenced the work in this paper.
722	
723	Data availability statement
724	The data from this study are available from the corresponding author upon reasonable request.
725	
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