Effects of weeding and fertilization on soil biology and biochemical processes and tree growth in a mixed stand of Dalbergia odorifera and Santalum album

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Abstract In southern China, the eucalyptus plantation industry has been severely restricted by government policy over concerns on negative environmental impacts. In its place, large-scale plantations of high-value tropical tree species such as nitrogen-fixing Dalbergia odorifera and hemiparasite Santalum album have been widely cultivated including in mixed-species plantations. However, despite their poor growth, little information is available on suitable silvicultural practices of these plantations. Therefore, we subjected an 8-year-old mixed stand of D. odorifera and S. album to weeding, fertilization, weeding + fertilization, or no (CK) treatments and measured soil microbial biomass, respiration, nutrients, nitrogen mineralization and leaching and tree growth and litter production. Weeding and fertilization decreased microbial biomass but increased soil respiration, inhibited mineralization, had not effect on leaching of soil nitrogen, and improved the nutrient status of plantation soil. All practices improved the growth of D. odorifera. In the mixed plantation, fertilization increased litter production and nutrient content, but weeding and weeding + fertilization decreased growth of S. album and litter production in mixed plantation because weeding decreased the number of S. album haustoria in underground plant roots. In conclusion, fertilization is recommended; however, weeding-related practices are inappropriate for D. odorifera and S. album mixed plantations. These conclusions have important implications for managing other parasite or mixed-species plantations.

Keywords Plantation practices · Microbial biomass · Soil respiration · Mineralization · Leaching · Hemiparasite

Introduction

Management practices are applied to improve the production and quality of plantation forests by increasing soil nutrient availability and regulating competition among plants (Erb et al. 2018; Sida et al. 2018). Weeding and fertilization are the most common of these practices around the world for tree species such as Populus spp. (Pokharel and Chang 2016), Eucalyptus spp. (Carrero et al. 2018), Pinus banksiana (Pokharel et al. 2017), Cunninghamia lanceolata (Wang et al. 2008) and Phyllostachys edulis (Li et al. 2016; Song et al. 2020).

Biological and biochemical processes in the soil such as microbial activity and nutrient transformation contribute to maintaining soil ecological functions. Weeding changes energy and nutrient inputs into the soil by decreasing vegetation cover in plantations (Rey et al. 2011; Zhang et al. 2018). Thus, weed affects not only soil physicochemical properties, such as temperature, water content (Özkan and Gökbulak 2017), pH (Li et al. 2014), and nutrient availability (Rey et al. 2011), but also the quantity and activity of roots and microbes in the soil (Fierer et al. 2012; Allison et al. 2013).
Fertilization is commonly used to increase nutrient content and availability in the soils and thus improve productivity of plantations (Fox 2000). It also can affect soil biological and biochemical properties processes such as soil microbial biomass, nutrient cycling and respiration rate (Lee and Shibu 2003). Numerous studies have explored the effects of fertilizer application, especially nitrogen (N), on soil biological and biochemical processes, but results have been inconsistent; fertilizer application has significantly increased soil microbial biomass (Li et al. 2010; Song et al. 2020), microbial community diversity (Ramirez et al. 2010) and soil respiration rate (Bowden et al. 2004) and also had negative or neutral effects (Samuelson et al. 2009; Sun et al. 2011; Wang et al. 2017). The inconsistencies can be due to differences in tree species, plantation age, site conditions and fertilizer content and dosage (Peng et al. 2008). In addition, little is known about the effects of combined weeding and fertilization on plantation ecosystems. A better understanding of these effects will help better manage plantation ecosystem functions.

In southern China, concerns about negative environmental impacts from eucalyptus plantation industry have led to severe governmental policy restrictions. Instead, high-value tropical tree species such as Dalbergia odorifera and Santalum album have been widely grown in large-scale mixed-species plantations. Both species are renowned for their valuable heartwood and widely used as religious, cosmetic, furniture and medicinal materials (Dhanya et al. 2010; Cui et al. 2019). Santalum species are hemiparasites that take up water and nutrients from host plants through haustoria in the roots (Lu et al. 2014). In addition, D. odorifera is a good host for S. album because it is strong nitrogen-fixer (Lu et al. 2017). However, little information is available on suitable cultivation practices for these plantations (Cui et al. 2017). Furthermore, herbicides must be replaced by manual weeding to avoid harming S. album because of its parasitic characteristics, and intensive farming and fertilization must be implemented in place of extensive traditional management practices. Weeding promotes growth of trees by minimizing neighboring competition, or it may inhibit the growth of S. album by decreasing the number of S. album haustoria in plant roots. However, little is known about the mechanism by which weeding and fertilization regulate ecosystem functions in mixed stands of D. odorifera and S. album. A better mechanistic understanding of these management practices will help manage forest plantations more effectively.

A weeding and fertilization experiment was conducted in a mixed-species plantation of D. odorifera and S. album to study changes in soil microbial biomass, respiration, nutrients, tree growth and litter production. We examined (a) whether weeding decreases and fertilization increase soil microbial biomass and soil respiration, (b) whether weeding and fertilization improve soil nutrients and N transformation, and (c) whether a combined treatment of weeding and fertilization promotes growth of trees over the single and the control treatments.

**Materials and methods**

**Site description**

At the experimental plantation in Foshan City, Guangdong, China (22°47′N, 112°32′E), mean annual precipitation is 1681 mm and mean air temperature is 23.4 °C. The dry season from October to March received 18% of the annual precipitation during the study period (Fig. 1). The mixed-species plantation of D. odorifera and S. album in the study was established in 2009 with a species ratio of 1:1, alternately planted with a spacing 2.5 m × 2.5 m. At the beginning of treatments in April 2017, the survival rate was ~94% (750 trees/ha) for D. odorifera and ~88% (700 trees ha⁻¹) for S. album. At 8 years of age, D. odorifera had a mean DBH of 7.63 ± 1.46 cm and mean height of 6.29 ± 0.87 m and S. album had a mean DBH of 7.04 ± 1.51 cm and mean height of 5.53 ± 0.86 m.

The soil in the mixed plantation is classified as haplic acrisols according to the FAO soil classification. Initial data for soil status are shown in Table 1.

**Experimental design and sampling**

The experiment was laid out in a randomized complete block design with four treatments (control [CK], weeding [W], fertilization [F], and weeding + fertilization [W + F]) and...
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four replicates. Each of the 16 replicate treatment plots was 400 m². For the weeding treatment, ground vegetation was removed manually with a spade and spread evenly on the ground. For the fertilizer treatment, about 0.5 kg of Norwegian compound fertilizer (N15P15K15, Yara International, Oslo, Norway) was applied to each hole, dug at the center point between two trees. All treatments were carried out twice a year (in April and August). Soil samples were collected from all treatment plots one time per season (summer: June, autumn; September, winter: December, spring: March) in 2017 and 2018. Soil samples were collected at 0–10 cm depth in each plot using a five-point sampling method and then stored on dry ice and transported to a laboratory for analysis.

Soil microbial carbon and nitrogen analysis

The chloroform fumigation extraction method (Brookes et al. 1985) was used to analyze soil microbial biomass carbon (MBC) and nitrogen (MBN). A fresh soil sample of 20 g was fumigated with alcohol-free trichloromethane for 24 h, then extracted in 0.5 M K₂SO₄ (1:2.5 w/v). C and N contents were obtained using a TOC analyzer (multi N/C 3100, Analytik Jena, Germany).

Soil respiration measurement

Three polyvinyl chloride (PVC) collars (20 cm diameter and 10 cm height) were inserted 7 cm into the soil on the diagonal of each plot. Soil respiration rates were measured monthly from October 2017 to September 2018, using an LI-8100 automatic soil CO₂ flux system (LI-COR, Lincoln, NE, USA). Measurements of soil respiration in all PVC collars were completed between 09:00 and 11:00 h on a sunny day. At the same time, soil temperature (T) and soil moisture (W) at 10-cm depth were measured by a TRIME-PICO TDR probe (IMKO, Ettingen, Germany). The calculation to determine annual soil CO₂ fluxes was described by Inoue and Koizumi (2012).

| Soil layer (cm) | pH    | Organic matter (g kg⁻¹) | Total N (g kg⁻¹) | Total P (g kg⁻¹) | Total K (g kg⁻¹) | Hydrolyzed N (mg kg⁻¹) | Available P (mg kg⁻¹) | Available K (mg kg⁻¹) |
|----------------|-------|-------------------------|-----------------|-----------------|-----------------|-----------------------|-----------------------|-----------------------|
| 0–10           | 4.09 ± 0.71a | 27.59 ± 3.37a            | 1.11 ± 0.47a    | 0.42 ± 0.08a    | 5.12 ± 0.72ab   | 140.59 ± 17.53a       | 38.84 ± 22.33a         | 83.41 ± 22.68a        |
| > 10–20        | 4.35 ± 0.15a | 17.38 ± 3.12b            | 0.89 ± 0.26c    | 0.28 ± 0.09b    | 4.82 ± 0.82c    | 84.78 ± 15.98b        | 19.43 ± 15.27b         | 39.63 ± 9.50b         |
| > 20–40        | 4.29 ± 0.13a | 11.97 ± 2.44c            | 0.59 ± 0.14c    | 0.21 ± 0.08c    | 5.56 ± 1.00b    | 55.72 ± 9.10c         | 6.40 ± 9.63c           | 24.04 ± 5.33c         |
| 40–60          | 4.32 ± 0.16a | 8.25 ± 1.06d             | 0.46 ± 0.07c    | 0.18 ± 0.06c    | 6.32 ± 1.24a    | 41.14 ± 5.27d         | 4.45 ± 9.00c           | 21.64 ± 4.61c         |

Notes: Different letters in the same column indicate significant differences between different soil layers in a one-way ANOVA and least significant difference test. Data are means ± standard deviation (n = 5)

Soil nutrient, nitrogen mineralization and leaching determination

Soil samples were homogenized and fine roots, stones and other materials (> 2 mm) were discarded. Soil pH was measured using a glass electrode pH meter. Ammonium and nitrate nitrogen concentrations were measured using ion-selective electrodes (Greenberg et al. 1985). Available phosphorus was extracted using ammonium hydrochloride and determined using the molybdenum antimony colorimetric method (Olsen and Sommers 1982). Available potassium was determined using atomic absorption spectroscopy (Bahr et al. 2018).

Net N mineralization and leaching rates were measured in situ using a sequential coring technique (Adams and Attiwill 1986; Raison et al. 1987). Five sampling points were selected randomly along the diagonal line of each plot. At each sampling point, three PVC collars (4.6 cm in diameter and 15 cm in height) were hammered into the soil to a depth of 10 cm, and one of the three collars with soil (S₀) was collected and taken to a laboratory for the various measurements of the soil. The other two collars were left in situ for 30 days. Another collar had an open top to allow rain to pass through (S₁), and the other collar had a covered top and perforations in the upper 5 cm of the sidewall (S₂) for ventilation. Net N mineralization was defined as the increase in ammonium plus nitrate N between (S₀) and (S₂), and net leaching was calculated between S₀ and S₁. Initial soil samples were collected in June (summer), September (autumn), December (winter) and March (spring) in the following year. Each soil sample was oven-dried, and the moisture content was measured by weighing method. A fresh soil sample of 10 g was mixed with 50 mL of 2 M KCl, shaken for 1 h, and filtered through filter paper. NH₄-N and NO₂-N + NO₃-N concentrations were then measured using automated colorimetry.

Tree growth and haustorial number

Height and DBH of all trees in the 16 treatment plots were measured using a height meter and caliper, respectively.
at the start of the experiment and 1 year later. Four
20 cm × 20 cm subplots were selected randomly in each plot
within the vertical projection of the crown of S. album. The
roots of all underground plant parts within each subplot were
dug out, and the haustoria were counted.

Litter collection and analysis

Litter was collected monthly from October to September
during 2017–2018. Three 1 m × 1 m litter traps 50 cm tall
were placed across the diagonal of each plot. Litter from
the three traps was mixed into one sample, oven-dried and
weighed using an analytical scale (0.0001 g) (Souza et al.
2019). Total N, P and K of the litter were measured using the
Kjeldahl method (Vanlauwe et al. 1996), phosphovanado-
molybdate method of Hanson (1950) and flame photometry
(Herrera et al. 2008), respectively.

Statistical analyses

A one-way ANOVA and least significant difference tests
were used to determine the statistical significance of dif-
ferences at the 0.05 level in mean soil microbial biomass,
soil respiration, soil nutrient, nitrogen mineralization and
leaching, litter production and growth increment in height
and in DBH (Increment in height or DBH = Height or DBH
1 year after treatment – Height or DBH before treatment)
in response to the weeding or fertilization treatments for
each sampling date. All data were tested for homogeneity of
variance and normality of residuals before conducting the
ANOVA. Data analyses were performed using SPSS 22.0
(SPSS Inc., Chicago, IL, USA).

Results

Soil microbial biomass

Microbial biomass showed strongly seasonal variations
with the highest in the summer and the lowest in the winter
(Fig. 2). MBC and MBN contents differed depending on
the treatment. In the spring after 1 year of treatment, MBC
with weeding, fertilization and weeding + fertilization treat-
ments decreased significantly by 34.1%, 27.8%, and 11.3%,
respectively, compared with the control treatment (Fig. 2a).
MBN in the weeding and fertilization treatments decreased
significantly by 37.7% and 47.8% compared with the control
(Fig. 2b). In general, all the treatments significantly reduced
the soil microbial biomass compared to the controls.

Soil respiration

Soil respiration rate fluctuated from month to month dur-
ing the experiment; the unimodal curve for each treatment
showed a maximum from May to November and minimum
from December to April (Fig. 3a). CO2 flux values peaked
in October and ranged from 7.28 to 10.48 μmol m−2 s−1. The
CO2 flux ranged from 2.29 to 2.79 μmol m −2 s−1 and was
lowest in February. During each month, the weeding + ferti-
lication samples generally had the maximum soil respiration
rate.

Fig. 2 Mean (±SD) soil microbial carbon (a, MBC) and nitrogen
(b, MBN) in each season after different cultivation practices. Different
letters above the histobars denote significant (p < 0.05) differences
among treatments in the same season as determined by a one-way
ANOVA and least significance difference test; blue lines represent
standard deviations (n=4). Control (CK: no weeding or fertilization),
weeding (W), fertilization (F), and weeding + fertilization (W + F)
Compared with the CK, the weeding, fertilization and weeding + fertilization treatments increased the annual flux in soil respiration by 22.2%, 17.1% and 45.5%, respectively (Fig. 3b). Thus, compared to CK, all the cultivation practices in this study significantly increased the soil respiration.

**Soil nutrient, nitrogen mineralization and leaching**

Soil pH (4.59–5.03) varied little among treatments throughout the year (Fig. 4a). The ammonium nitrogen content in the soil peaked in autumn for the three treatments and the CK (Fig. 4b). Compared with CK, ammonium nitrogen contents in the weeding and weeding + fertilization treatments increased significantly by 65.3 and 75.1%, respectively. Unlike ammonium nitrogen, nitrate nitrogen was highest in the spring (Fig. 4c). Nitrate nitrogen contents for all cultivation treatments were significantly greater than for the CK. Over the year, the nitrate nitrogen content for the CK was minimal compared to the three cultivation practices. Available phosphorus was highest in summer (Fig. 4d), and was significantly increased by weeding + fertilization over the four seasons. Available potassium for the three treatments was significantly greater than for the CK and was highest with weeding + fertilization (Fig. 4e). These results indicated that all the cultivation in this study significantly improved the nutrient levels of the plantation soil compared to the CK.

Nitrogen nitrification was higher in the spring and autumn, and nitrification rate was lowest in winter (Table 2). In addition, the nitrogen nitrification rate was lowest in the weeding treatment (1.39 mg kg⁻¹ month⁻¹). The CK group had the highest net nitrification rate in each season. The ammonium rate was highest in the fertilization treatment in the winter and lowest in the weeding treatment in the autumn. The net nitrogen ammonium rate had significant seasonal variation. Soil ammonia rates were lower in the autumn and spring. Ammonium nitrogen accumulated in the summer and winter and was highest in the winter (Table 2). Rates of nitrogen mineralization were significantly greater than for the CK. The net nitrogen ammonium rate had significant seasonal variation. Soil ammonia rates were lower in the autumn and spring. Ammonium nitrogen accumulated in the summer and winter and was highest in the winter (Table 2). The ammonium rate was highest in the fertilization treatment in the winter and lowest in the weeding treatment in the autumn. The net nitrogen ammonium rate had significant seasonal variation. Soil ammonia rates were lower in the autumn and spring. Ammonium nitrogen accumulated in the summer and winter and was highest in the winter (Table 2).

Compared with CK, the weeding + fertilization, fertilization, and weeding treatments significantly reduced annual mineralization by 7.5%, 20.3% and 22.9%, respectively (Fig. 5a). The annual nitrogen leaching in the weeding treatment was significantly lower than in the other treatments; the fertilization and weeding + fertilization treatments did not differ significantly from the CK (Fig. 5b). For all four treatments, the annual mineralization was greater than the annual nitrogen leaching. In brief, all cultivation practices significantly inhibited mineralization but did not increase leaching of soil nitrogen.
No statistically significant differences were found among the four treatments with regard to mean height and DBH for either species before treatments started (Table 4).

In the evaluation of increment change in height and DBH (Fig. 6), the greatest increase for *D. odorifera* (Fig. 6a) was found in the weeding + fertilization treatment (49.50 cm), followed by fertilization (42.40 cm), weeding (33.65 cm) and CK (27.90 cm). All cultivation treatments significantly increased the height increment compared to the CK. The DBH increment varied between 5.55 and 9.11 mm (Fig. 6c) and was significantly higher for the weeding + fertilization and fertilization treatments than in the CK; weeding did not significantly affect the DBH increment. Compared to the CK, all cultivation treatments increased the growth of *D. odorifera*.

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**Table 2** Effects of different cultivation practices and seasons on nitrogen nitrification and ammonium

| N transformation          | Treatment | Summer         | Autumn        | Winter          | Spring         |
|---------------------------|-----------|----------------|---------------|-----------------|----------------|
| Net nitrification rate (mg kg⁻¹ month⁻¹) | CK        | 12.39 ± 2.62Ba | 21.22 ± 2.00Aa | 7.32 ± 1.54Ca   | 21.29 ± 3.28Aa |
|                           | W         | 12.22 ± 2.65Ba | 17.45 ± 1.27Ab | 1.39 ± 0.40Cc   | 16.90 ± 1.30Ab |
|                           | F         | 10.71 ± 3.13Ba | 17.14 ± 1.54Ab | 5.57 ± 0.80Cb   | 16.18 ± 0.89Ab |
|                           | W + F     | 13.65 ± 1.98Ca | 16.70 ± 1.87Bb | 5.91 ± 0.71Db   | 21.31 ± 2.19Aa |
| Net ammonium N accumulation (mg kg⁻¹ month⁻¹) | CK        | 0.08 ± 0.84Ab  | 0.66 ± 0.54Aa  | 0.54 ± 0.45Ab   | −0.04 ± 0.63Bb |
|                           | W         | 0.33 ± 0.25Ab  | −3.38 ± 2.29Cc | −0.89 ± 0.76Abd | −2.22 ± 0.65Cc |
|                           | F         | −0.67 ± 0.28Bc | 0.25 ± 0.15Aa  | 1.69 ± 0.47Aa   | −2.03 ± 0.40Bc |
|                           | W + F     | 1.13 ± 0.30Aa  | −1.51 ± 0.33Cb | −0.29 ± 0.23Bc  | −0.45 ± 0.17Ba |

Notes: Means ± standard deviation (n=4) are given and were analyzed with a one-way ANOVA and least significant difference test. Different capital letters in the same row indicate significant seasonal differences at P<0.05 level; different lowercase letters in the same column mean significant treatments difference at 0.05 level Control (CK, no weeding or fertilizer), weeding (W), fertilization (F)
Unlike *D. odorifera*, *S. album* attained the greatest height increment in the fertilization treatment (Fig. 6b). Compared with the CK, the weeding and weeding + fertilization treatments led to significantly lower changes in height increment (30.03 and 23.6%, respectively). However, fertilization increased the height increment by 23.2%. The DBH increment with weeding was significantly lower than that in the CK, whereas fertilization and weeding + fertilization treatments did not differ significantly from the CK (Fig. 6d). Compared to the CK, fertilization significantly increased and weeding significantly reduced the growth of *S. album*.

### Table 3 Effects of different tending treatments and seasons on nitrogen mineralization and leaching

| N transformation                  | Treatment | Summer     | Autumn     | Winter     | Spring     |
|-----------------------------------|-----------|------------|------------|------------|------------|
| Net N mineralization rate         | CK        | 12.47 ± 2.59Bab | 21.88 ± 1.81Aa | 7.86 ± 1.76Ca | 20.25 ± 3.62Aa |
|                                  | W         | 12.55 ± 2.83Aab | 14.06 ± 2.22Ac | 0.50 ± 0.87Bc | 14.68 ± 1.64Ab |
|                                  | F         | 10.03 ± 2.97Cb | 17.39 ± 1.45Ab | 7.26 ± 0.72Da | 14.15 ± 1.15Bb |
|                                  | W + F     | 14.78 ± 2.21Ba | 15.19 ± 1.80Bbc | 5.62 ± 0.70Cb | 20.86 ± 2.11Aa |
| N leaching rate                   | CK        | 7.99 ± 1.09Ba  | 18.24 ± 2.65Aa | -0.30 ± 3.67Db | 2.70 ± 0.98Ca |
|                                  | W         | 9.40 ± 1.22Ba  | 15.81 ± 1.61Ab | -4.24 ± 1.89Dc | 0.63 ± 0.31Cc |
|                                  | F         | 8.98 ± 1.77Ba  | 11.36 ± 0.64Ab | 4.36 ± 0.62Ca | 1.69 ± 0.52Db |
|                                  | W + F     | 7.82 ± 1.39Ba  | 18.24 ± 1.34Aa | -1.25 ± 0.48Db | 2.32 ± 0.64Cab |

Notes: Means ± standard deviation (*n* = 4) are given and were analyzed with a one-way ANOVA and least significant difference test. Different capital letters in the same row indicate significant seasonal differences at *P* < 0.05 level; different lowercase letters in the same column mean significant treated difference at 0.05 level. Control (CK), weeding (W), fertilization (F)

### Table 4 Initial mean height and diameter at breast height of individuals of *Dalbergia odorifera* and *Santalum album* when treatments started

| Tree              | Growth | CK        | W         | F          | W + F      |
|-------------------|--------|-----------|-----------|------------|------------|
| *D. odorifera*    | Height | 6.23 ± 0.76a | 6.18 ± 0.86a | 6.48 ± 1.02a | 6.26 ± 0.76a |
|                   | DBH    | 7.77 ± 1.76a | 7.89 ± 1.33a | 7.38 ± 1.45a | 7.45 ± 1.17a |
| *S. album*        | Height | 5.69 ± 0.92a | 5.67 ± 0.84a | 5.38 ± 0.76a | 5.39 ± 0.85a |
|                   | DBH    | 6.99 ± 1.52a | 7.06 ± 1.64a | 6.96 ± 1.74a | 7.14 ± 1.02a |

Notes: Means ± standard deviation (*n* = 4) are given and were analyzed with a one-way ANOVA and least significant difference test. Different lowercase letters in the same row mean significant difference before treatment application at 0.05 level. Control (CK, no weeding or fertilizer), weeding (W), fertilization (F)

### Haustorium number

The number of *S. album* haustoria in plant roots after the various treatments is shown in Fig. 7. Significantly more haustoria formed in the fertilization treatment than in the other treatments, up to 2.94, 9.65, and 181.83 times as much as CK, weeding + fertilization, and only weeding, respectively. However, the haustorial number was much lower in the weeding and the weeding + fertilization treatments compared to CK. Thus, fertilization increased haustorial production, but weeding reduced haustorial production.
Litter production

Total litter biomass differed significantly among treatments, ranging from 6.71 to 10.38 t ha$^{-1}$ a$^{-1}$ (Fig. 8A). Compared with levels in the CK, total litter biomass increased by 26.9% with fertilization, but decreased by 6.6% with weeding + fertilization and 18.0% with weeding.

Annual total nutrient content of litter varied among treatments (Fig. 8b). On average, the annual litter nutrient content was in the order nitrogen (142.10–246.45 kg ha$^{-1}$ a$^{-1}$) > potassium (65.62–127.61 kg ha$^{-1}$ a$^{-1}$) > phosphorus (8.38–14.99 kg ha$^{-1}$ a$^{-1}$). The highest content of total litter nutrient was in the fertilization treatment (389.04 kg ha$^{-1}$ a$^{-1}$), whereas values in the fertilization and weeding + fertilization treatments did not differ significantly compared to the CK (Fig. 8b). Thus, compared to the CK, fertilization significantly increased the amount of litter and nutrient return, whereas weeding reduced the litter production of the mixed plantation.

Discussion

Effects of weeding and fertilization on soil biology process

In this mixed-species plantation, cultivation practices decreased microbial biomass, but increased soil respiration, indicating that both weeding and fertilization significantly affected soil biological processes. Soil microbial biomass has an important role in nutrient cycling and is therefore essential for plant growth, which is very sensitive to environmental factors (Fliessbach et al. 1994). Compared with CK, weeding and fertilization decreased soil microbial biomass in line with previous findings (Stewart et al. 2018; Sun et al. 2018). The effects of fertilization on soil communities depend heavily on the contents of soil nutrients (Allison and Martiny 2008). In N-limited soil, N addition will directly increase the microbial populations and activity (Hobbie and Vitousek 2000; Compton et al. 2004). On the contrary, N addition will decrease soil microbial biomass in the soils of N saturation and even N inhibition (Guo et al. 2017). Additionally, weeding reduced microbial biomass as a result of lower input of organic matter into the soil (Wardle et al. 1999).

Soil respiration rates in the different cultivation practices measured in this study were greater than in the CK,
and weeding + fertilization generally yielded the highest rate. Total soil respiration consists of autotrophic and heterotrophic components (Wang et al. 2017). Autotrophic respiration is mainly from plant roots, and heterotrophic respirations is primarily from decomposition of organic matter by soil microbes (Zhao et al. 2018). Weeding and fertilization affect soil respiration through stimulating plant growth and altering microbial biomass and activity (Olsson et al. 2005; Allison and Martiny 2008). After our weeding treatments, the plant residues were spread evenly on the surface of the plantation soil, which allowed more organic matter to be returned to the soil and degraded. Because fertilization accelerates nutrient availability and improves root growth, the rate of root respiration increased in this study. These results are in line with those reported by Zhu et al. (2016), Nguyen and Marschner (2017) and Spohn and Schleuss (2019). Therefore, cultivation practices decreased soil microbial biomass, but increased soil respiration. They also promoted root growth and microbial activity and degradation ability, ultimately increasing root and microbial respiration.

Effects of weeding and fertilization on soil biochemical processes

In our study, all cultivation practices basically improved plantation nutrients. Soil nitrogen mineralization was inhibited, and leaching was not increased. Zhang et al. (2017) concluded that carbon/energy resources decrease with loss of plant diversity, thus leading to reduced soil microbial diversity. Therefore, in this study, weeding could inhibit soil nitrogen processes by reducing the microbial population. Furthermore, weeding + fertilization inhibited soil nitrogen processes less than weeding did because the combined practice added more nutrients required for microbial process.

The variations in soil mineralization and nitrification rates were consistent with the temperature variations throughout the year. Soil temperature is positively correlated with total nitrogen mineralization (Li et al. 2020). When the soil is full of water in the wet season, net nitrogen mineralization decreased. Therefore, soil mineralization rates were higher in the spring and autumn.

Overall, cultivation practices inhibited mineralization but did not increase nitrogen leaching from the soil, and the nutrient status in the plantation soil improved. The cultivation practices were thus beneficial for preserving soil fertility and accumulating nitrogen.

Effects of weeding and fertilization on tree growth of D. odorifera and S. album

For D. odorifera, all cultivation practices promoted higher growth compared to CK. Generally, weeding and fertilization can increase growth rates by controlling competing vegetation and increasing inputs to raise the availability of nutrients (Fox et al. 2007; Campoe et al. 2014). In many of these stands, fertilization will increase plant growth by increasing leaf area. For S. album, unlike for D. odorifera, growth was significantly increased by fertilization and decreased by weeding. These results are consistent with fertilization increasing and weeding decreasing the number of S. album haustoria in plant roots in this study. Although weeding removed nutrient-competing vegetation, it also
reduced the nutrient sources in the host. Weeding promoted the growth of *D. odorifera*, while reducing the growth of *S. album*, perhaps due to the decrease in the number of haustoria that resulted in the weeding + fertilization and weeding treatments. Thus, the combined treatment of weeding and fertilization did not promote the expected higher growth compared to either of the single treatments or the lack of treatments (CK).

Inputs from litter, a major carbon and nutrient source, represent important components in the biogeochemistry in forest ecosystems (Attiwill et al. 1978). Changes in plant growth can also lead to the change in litter; the faster a plant grows, the more litter it produces (Belovsky and Slade 2000). In our study, fertilization significantly increased the amount of litter and nutrient return. The growth of trees increased rapidly with increased soil nutrients after fertilization as did the herbaceous shrubs; thus, *S. album* obtained more nutrients from these herbaceous hosts for its growth (Xu et al. 2011). Conversely, the weeding treatment significantly reduced the growth of *S. album*, resulting in decreased litter production.

In summary, the effects of the cultivation practices on the growth and litter production of *D. odorifera* and *S. album* were inconsistent. Fertilization significantly increased the growth of trees and the amount of litter and nutrient return, improving production in the mixed plantation of *D. odorifera* and *S. album*. However, weeding-related practices decreased the growth of trees and litter production in the mixed plantation by reducing the number of *S. album* haustoria in roots. Considering the short duration of the study, we need to continue monitoring tree growth for a few more years to determine whether these relationships continue over time.

**Conclusion**

In this mixed plantation, the effects of weeding and fertilization on soil biological processes are reflected in the reduction of microbial biomass and the increase of soil respiration. Weeding and fertilization inhibited mineralization but did not increase leaching of soil nitrogen, and nutrient status of the soil improved. Thus, these cultivation practices will help preserve soil fertility and the aid nitrogen accumulation. Inconsistent with non-parasitic plantations, the combined treatment of weeding and fertilization did not promote better growth compared to the single and control treatments. Cultivation practices improved the growth of *D. odorifera*, but weeding and weeding + fertilization decreased the growth of *S. album* and litter production in the mixed plantation because weeding decreased the number of *S. album* haustoria in roots. In conclusion, fertilization is recommended, but weeding practices are inappropriate for *D. odorifera* and *S. album* mixed plantations. These findings hold important implications for management practices for other parasite or mixed plantations.

**Author contributions** PZ wrote the manuscript. XFL and SYX collected data. ZJY and DPX developed the study design. ZYC provided expert knowledge used in the writing and revision of the manuscript.

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