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ORIGINAL ARTICLE

Effects of soybean–tea intercropping on soil-available nutrients and tea quality

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Abstract

Plant intercropping is increasing in popularity, is conducive to plant growth and development and can improve plant quality and yield. In this study, we intercropped tea (*Camellia sinensis*) cv. 'Su cha zao' and soybean (*Glycine max*) cv. 'Lamar' in a tea plantation. The chlorophyll content was higher in intercropped tea leaves than in monoculture, and the different phenotypic characteristics of intercropping and monoculture were correlated with chlorophyll and carotenoid content. Our analyses showed that soybean–tea intercropping not only alleviated cold damage, but also influenced tea plant growth. Furthermore, the soil ammonium nitrogen (N) in intercropping mode increased during soybean flowering and mature periods and was highest in the soybean flowering and podding period. Catechin levels in tea leaves significantly decreased, and the amino acid and soluble sugars increased, for intercropped compared with monoculture tea leaves. The analysis of soil fertility and tea leaf physiological indices also indicated that N fertiliser was significantly positively correlated with free amino acids in tea leaves. In conclusion, soybean–tea intercropping affected the effective N content in soil, especially ammonium N, and the formation of the main physicochemical composition of tea leaves, as well as tea taste and aroma. Thus, intercropping can sustainably improve nutrient management and increase crop yield and quality.

Keywords Intercropping soybean · Soil nutrient availability · Tea quality · Tea plantation

Introduction

Agroforestry ecosystems are spatially and temporally complex systems that are motivated by economic returns from two or more crops and have been employed to generate economic value (Liu et al. 2013b; Steffan-Dewenter et al. 2007). Agroforestry is a sustainable plant mode, which can enhance soil fertility and maintain long-term productivity (Power 2010). Intercropping is the practice of growing two or more crop varieties or genotypes in the same area at the same time and can increase yields per area as well as the benefits of mutualism (Baumann et al. 2002; Brooker et al. 2015). There are two main effects of intercropping on the soil environment: full utilisation of soil nutrients and improved soil quality. Intercropping can improve absorption of soil nutrients by crops and significantly increase accumulation of crop biomass (Zhang et al. 2010); for example, intercropping increases soil carbon and nitrogen (N) in agricultural production systems (Cong et al. 2015). Choosing a reasonable intercropping pattern and crop species can effectively reduce fertiliser application and land erosion and further promote moisture and nutrient uptake from soil (Weil et al. 2004). Previous studies have shown that intercropping of annual cereal crops with perennial legumes reduced greenhouse gas emissions and increased deposition of soil N, as well as increasing soil biodiversity (Canfield et al. 2010; Guo et al. 2010). At present, soil acidification and declining soil fertility occur frequently in many tea plantations in China because of non-environmentally friendly management (Jumadi et al. 2008; Zhu et al. 2014). Intercropping tea plantations with aromatic plants has alleviated acidification of the soil, improved soil fertility and increased soil moisture, resulting in promoted growth and development of tea

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plants (Zhang et al. 2016). In addition, for intercropping of Chinese chestnut and tea, organic matter, N, phosphorus (P) and potassium (K) contents were higher in soil, soil enzyme activity was higher, and soil pH increased compared to tea monoculture (Ma et al. 2017). Most research has focused on the effects of the intercropping patterns on soil fertility in tea plantations, but the influence of intercropping on tea components remains unclear.

Tea plants (*Camellia sinensis* (L.) O. Kuntze) prefer warm, moist, shaded and acidic soil (Lin et al. 2009). Fresh tea leaves contain rich secondary metabolites, including polyphenols, caffeine, amino acids and soluble sugars (Chan et al. 2008; Zhang and Ruan 2016), and these components collectively determine the quality characteristics of tea, e.g., sensory quality, economic value and health benefits (Harbowy et al. 1997). Tea polyphenols and soluble sugars are the main carbon compounds in tea leaves, and catechin biosynthesis is closely associated with chlorophyll content (Wei et al. 2011). Caffeine and free amino acids are the main nitrogenous compounds in tea leaves (Li et al. 2016), and N is the most important nutrient for increasing free amino acid levels (Silva et al. 2015). In chestnut–tea intercropping, the length, weight and theanine content of tea leaves all increased, but amino acid and catechin contents decreased, compared with monoculture (Ma et al. 2017). Intercropped plants uptake and consume N by rhizospheres in the legume nodules, which accelerates N fixation (Ehrmann and Ritz 2014; Hauggaard-Nielsen et al. 2001). Intercropping legumes in tea plantations may be beneficial to tea plant growth and development and further improve tea quality. Legumes, as green manure, are very valuable for intercropping because of their ability to fix N, especially in environments with chronic N deficiency (Bedoussac et al. 2015; Hauggaard-Nielsen Henrik et al. 2008). Long-term intercropping and coverage of perennial legumes not only increases effective soil nutrients, but also reduces transpiration and slows down changes in soil moisture, which are beneficial to crops grown in high-temperature- and drought-prone environments (Pang et al. 2013; Seyfried and Wilcox 2006). In wheat–winter pea intercropping, wheat production increased by 20% compared to monoculture, mainly due to N fixation by peas promoting absorption of soil N, and the use of illumination intensity increased by 10% (Bedoussac and Justes 2010a, b). Legume intercropping is common in sustainable agriculture (Rose et al. 2016); however, few studies have examined legume–tea intercropping.

Because N is an essential element in forming caffeine, amino acids, chlorophyll and other chemical ingredients of tea leaves, intercropping leguminous plants may have an important effect on tea quality. In this study, nutrients in soil and the physiological indices of tea leaves from a tea–soybean intercropping system were determined, correlations between soil nutrients and physiological quality in tea were analysed and metabolites in tea leaves were investigated.

Materials and methods

Experimental design and sampling

The young tea plants were the annual ‘Su cha zao’ cutting seedlings (Nanjing Yarun Tea Industry Co. Ltd., Nanjing, China). The soybean (*Glycine max*) variety ‘Lamar’ was provided by the Nanjing Agricultural University Plant Protection Institute.

To simulate tea–soybean intercropping in tea plantations, we designed a greenhouse experiment (Fig. 1). Approximately 15 cm-long young tea plants, with good growth, were planted in a custom-built box (120 cm × 80 cm × 36 cm) containing nutrient soil (Jiangsu Xingnong Matrix Technology Co. Ltd., Nanjing, China), where the tea plant line spacing was 40 cm, with planting distances of 15 cm. Two weeks after tea planting, soybean seeds were sown, and the planting distance between tea and soybean was 20 cm, with planting distance 5 cm. Soil and tea leaf samples were collected for analysis at the period of soybean seedling (6th October 2017), flowering–podding (5th November 2017) and mature period (18th December 2017).

Chlorophyll fluorescence parameters in tea

Chlorophyll fluorescence parameters in tea plants were measured using a PocketPEA portable fluorescence spectrometer (Handy PEA Fluorometer, Hansatech Instruments Ltd., UK). The three leaves from the top bud were selected for chlorophyll fluorescence determination. All tests were performed three times, in the same way, with independent sample data.

Soil-available nutrients of a tea plantation

Soil samples were collected from a 0–20 cm depth (except for top soil) in a tea plantation. Samples of each sampling point were mixed uniformly, and 1 kg of mixed soil was kept for further analysis, by point-centred quarter method.

Soil organic matter was determined by hydrothermal dichromate potassium oxidation colorimetry (Schmidt and Torn 2012). Nitrate N (NO_3^--N) was measured using ultraviolet spectrophotometry (Koenig and Cochran 1994), and ammonium N (NH_4^+-N) by the indophenol blue colorimetric method. Effective P and K levels were determined by $NaHCO_3$ solution leaching–molybdenum antimony colorimetry and CH_3COONH_4 solution–flame photometric methods, respectively (Carter 1993).

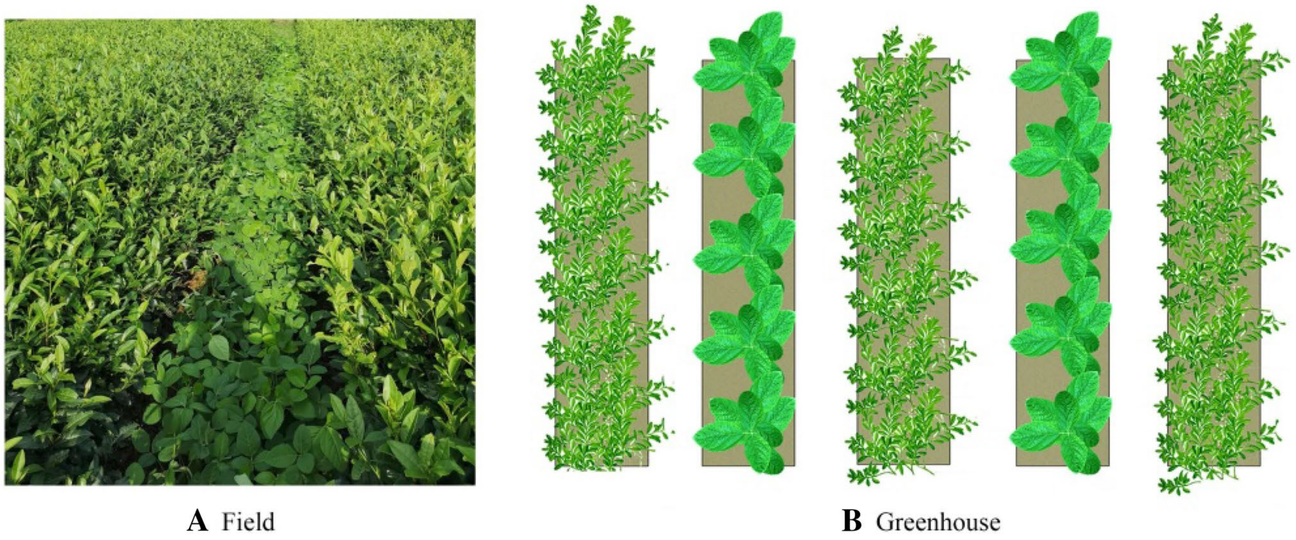

Fig. 1 Diagram of tea–soybean intercropping experiment in field (**a**) and greenhouse (**b**)

Chemical constituents in tea leaves

The preparation of tea samples was based on national standards (GB/T8303). Catechin and caffeine contents were determined based on national standards (GB/T8313-2008) using HPLC (Waters, USA) (Ning et al. 2016). Amino acids were measured using the ninhydrin colorimetry method, according to national standards (GB/T8314-2013). Soluble sugars were determined using the anthrone colorimetric method (Redillas et al. 2012). According to Porra's method (2002), chlorophyll content was extracted and quantified using absorbance at 647 and 664 nm, and carotenoids at 470 nm.

The extraction and detection of differential metabolites in tea plants

Fresh tea leaves, at three different soybean development stages, were collected, washed in distilled water and stored at –80 °C for metabolite extraction.

Liquid chromatography–mass spectrometry (LC–MS) analysis was performed with the assistance of the Guangzhou Genedenovo Biotechnology Co. Ltd. Briefly, freeze-dried samples were crushed using a mixer mill (MM 400, Retsch) with zirconia beads for 1.5 min at 30 Hz. Then 100 mg of powder was extracted overnight at 4 °C with 1.0 mL of 70% aqueous methanol, containing 0.1 mg L^{-1} lidocaine as an internal standard. Following centrifugation at 10,000g for 10 min, the supernatant was absorbed and filtrated (SCAA-104, 0.22-μm pore size; ANPEL, Shanghai, China, http://www.anpel.com.cn/) before LC–MS/MS analysis. Quality control samples were used with all samples to determine reproducibility of the whole experiment. Extracted compounds were analysed using an LC–ESI–MS/MS system (UPLC, Shim-packUFLC Shimadzu CBM20A, http://www.shimadzu.com.cn/; MS/MS Applied Biosystems 4500 QTRAP, http://www.appliedbiosystems.com.cn/).

Statistical analysis

All data were analysed using Excel 2010 and SPSS Statistics 22.0 software. Duncan's multiple comparisons were used. The lowercase letters indicate significant differences between data at $P < 0.05$; and for Pearson's correlation coefficient, ** represents significance at $P < 0.01$ and * represents significance at $P < 0.05$.

Results

Effects of intercropping soybean on soil nutrients in tea

For the different soybean growth periods, soil N from both the intercropped and monoculture tea plantation is shown in Fig. 2. In the soybean seedling stage, the NH_4^+-N, NO_3^--N and effective N of soil showed no significant differences when compared with monoculture tea. In all soybean growth periods, the NO_3^--N levels in soil were significantly higher, when compared with the monoculture (Fig. 2a)—this showed no obvious changes with time under intercropping, but decreased and then increased for monoculture. During soybean flowering–podding and maturity periods (Fig. 2b, c), NH_4^+-N and effective N levels were clearly higher in intercropped than monoculture soil. In addition, in the flowering–podding period, NH_4^+-N levels were highest and effective N levels were relatively high, indicating that the

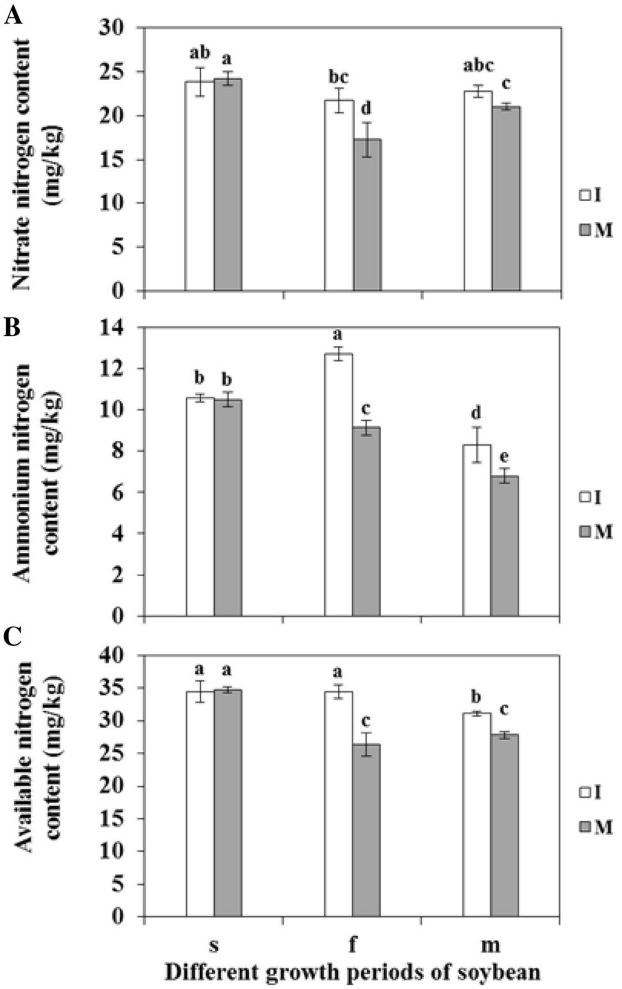

Fig. 2 The effects of intercropping on **a** nitrate N **b** ammonium N and **c** available N levels. *I* intercropping, *M* monoculture, *s* seedling period of soybean, *f* flowering–podding period of soybean, *m* mature period of soybean. Error bars show the standard deviation based on three replicates. Different letters present significant difference ($P<0.05$)

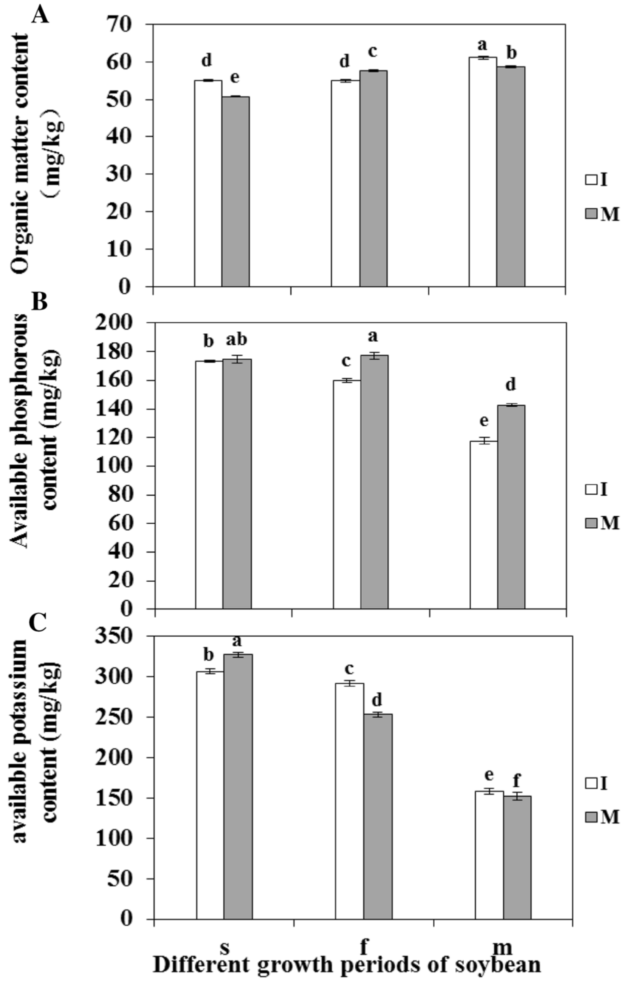

Fig. 3 Effects of intercropping on **a** soil organic matter **b** available P and **c** available K contents. *I* intercropping, *M* monoculture, *s* seedling period of soybean, *f* flowering–podding period of soybean, *m* mature period of soybean. Error bars show the standard deviation based on three replicates. Different letters present significant difference ($P<0.05$)

change trends of NH_4^+-N and effective N were similar, and intercropping with soybean improved soil nutrients.

During the flowering–podding period of intercropped soybeans, the soil organic matter (SOM) content was relatively low, but during the seedling and mature periods, the SOM content was relatively high (Fig. 3a). Under intercropping, the soil-available P was much lower, and the content of soil-available P tended to decrease with tea plant growth and development (Fig. 3b). For both intercropping and monoculture, the soil-available K gradually decreased during the whole experimental period, and the content was lower for intercropping than monoculture, during the soybean seedling period. However, during the soybean flowering–podding and mature periods, soil effective K was higher for intercropping than monoculture (Fig. 3c).

Effects of intercropping on chlorophyll fluorescence and pigments in young tea leaves

Chlorophyll fluorescence kinetic curves of tea plants are shown in Fig. 4. In the soybean seedling and flowering–podding periods, both intercropping and monoculture tea plants showed typical OJIP chlorophyll fluorescence-induced kinetic curves, indicating that the tea plants grew normally. However, in the soybean mature period, the chlorophyll fluorescence curve of tea plants under monoculture was not a typical OJIP dynamic curve (Fig. 4).

The chlorophyll content of tea leaves was significantly higher for intercropping, with values 1.2 and 1.6 times those of monoculture in the soybean flowering–podding and mature periods, respectively (Fig. 5a). There were significant differences in carotenoid levels in tea leaves, between

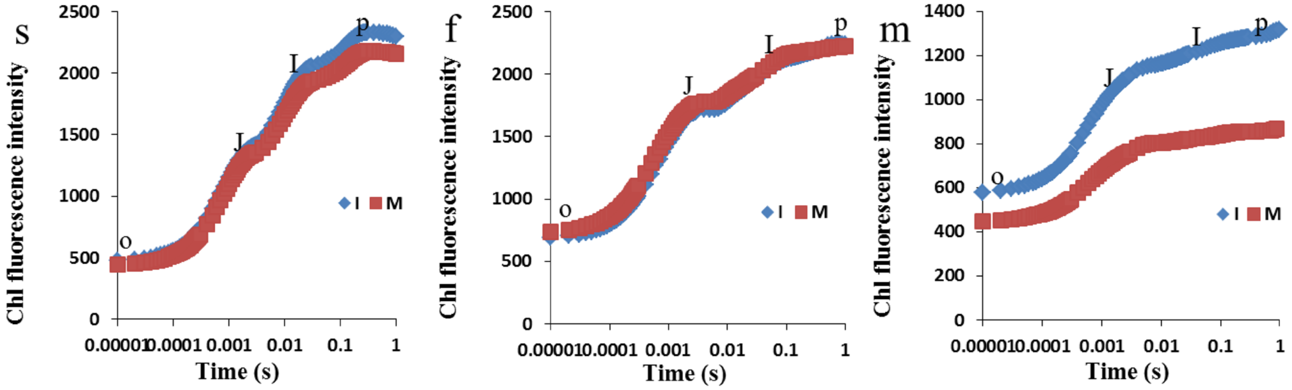

Fig. 4 Effects of intercropping on chlorophyll fluorescence intensity in tea leaves. *I* intercropping, *M* monoculture, *s* seedling period of soybean, *f* flowering–podding period of soybean, *m* mature period of soybean

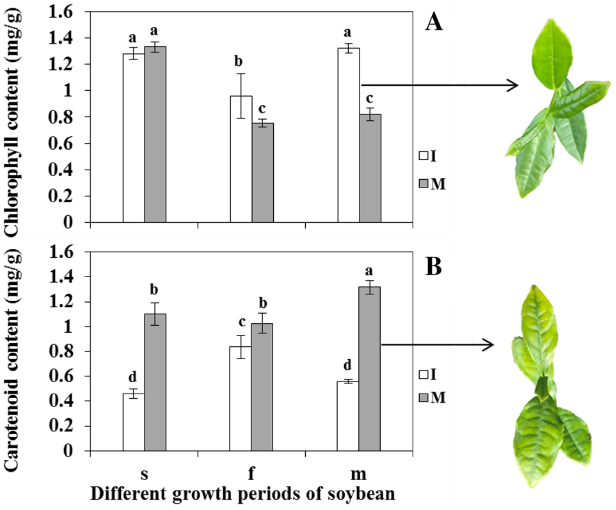

Fig. 5 Effects of intercropping on **a** chlorophyll and **b** carotenoid levels in tea leaves. *I* intercropping; *M* monoculture, *s* seedling period of soybean, *f* flowering–podding period of soybean, *m* mature period of soybean. Error bars show the standard deviation based on three replicates. Different letters present significant difference ($P < 0.05$)

intercropping and monoculture during the three different soybean growth periods (Fig. 5b).

Effects of intercropping on chemical components of young tea leaves

The catechin content in tea leaves was significantly lower for intercropping when compared to monoculture, during the soybean flowering–podding period, but significantly higher during the mature period (Fig. 6a). There was no significant difference in caffeine levels in tea leaves during the soybean seedling and flowering–podding periods, between intercropping and monoculture (Fig. 6b). However, in the mature period, the caffeine content was lower for intercropping than for monoculture. Amino acids in tea leaves were significantly higher for intercropping than monoculture during all soybean growth periods, and soluble sugars were higher for intercropping than monoculture, during the soybean flowering–podding and maturity periods (Fig. 6c, d).

Correlation analysis of soil fertility indices and physiological ecological indices of tea plants

Correlation analysis between soil fertility and quality components was performed by multiple regression methods (Table 1). The available K in the soil was positively correlated with catechin, caffeine and amino acid levels in tea leaves, but negatively correlated with soluble sugars. Furthermore, available K in the soil was positively correlated with N, NH_4^+-N and available P in soil. Soil-available P was positively correlated with catechin and caffeine levels in tea leaves, as well as with soil NH_4^+-N and available K, but negatively correlated with soluble sugars. There were significant positive correlations of soil NO_3^-N levels with amino acids and chlorophyll levels in tea leaves, as well as with soil-available N; the level of soil NH_4^+-N was positively correlated with caffeine and amino acids levels in tea leaves and with available N, P and K. The effective N was positively correlated with amino acids and chlorophyll in tea leaves. In addition, SOM was negatively correlated with caffeine and amino acid levels in tea leaves and with soil NH_4^+-N levels and available N, P and K, but there were no significant correlations with catechins and soluble sugars in tea leaves. The chlorophyll in tea leaves was positively correlated with amino acids and soluble sugars, as well as with soil-available N and NO_3^--N levels.

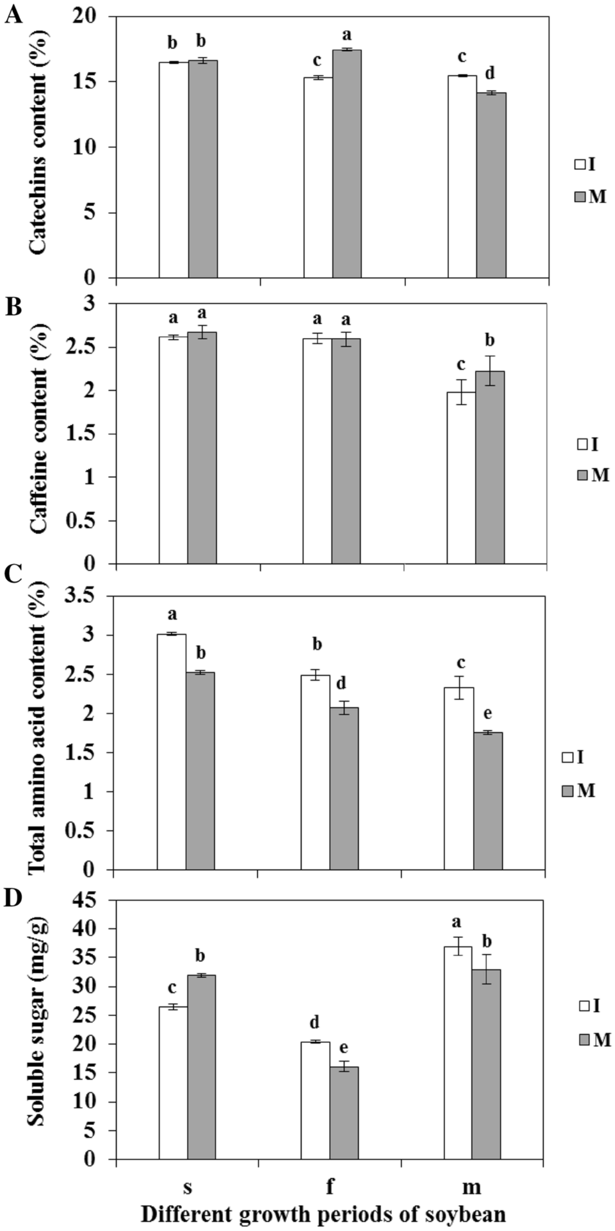

Fig. 6 Effects of intercropping on secondary metabolites in tea leaves: **a** catechin **b** caffeine **c** amino acid and **d** soluble sugar contents. *I* intercropping, *M* monoculture, *s* seedling period of soybean, *f* flowering–podding period of soybean, *m* mature period of soybean. Error bars show the standard deviation based on three replicates. Different letters present significant difference ($P < 0.05$)

Secondary metabolites in tea plants under intercropping and monoculture

The VIP value of multivariate statistical analysis OPLS-DA, combined with univariate statistical analysis *t* test *P*-values, were employed for metabolites screening (Saccenti et al. 2014) using threshold values of significant difference of VIP > 1 and *t* test $P < 0.05$. There were different levels for ten metabolites between intercropping and monoculture tea plants, during the soybean seeding period (six upregulated and four downregulated metabolites), 16 in the flowering–podding period (five upregulated and 11 downregulated metabolites) and 16 in the mature period (four upregulated and 12 downregulated metabolites) (Fig. 7); the main differential metabolites are shown in Tables S1–S3. In addition, the differential metabolites in tea plants, whether intercropping or monoculture, were relatively greater in the three soybean growth periods; the amino acids in the different comparison groups were all down-regulated, while most of the flavonoids were upregulated (Additional Tables S4–S9).

Discussion

Effects of intercropping on tea plant growth and development

Chlorophyll is the basis of plant photosynthesis and is very important for plant growth and metabolism (Wei et al. 2011). In this study, in the soybean flowering–podding and mature growth periods, the chlorophyll content in tea leaves was higher for intercropping when compared with monoculture, but the carotenoid content had an opposite result. This may have been due to shading from soybean growth, which may have promoted chlorophyll synthesis in tea leaves (Mauro et al. 2011). Under intercropping and monoculture, the chlorophyll and carotenoid levels in young tea leaves were correlated with phenotypic characteristics during the soybean maturity period. Low temperatures have been shown to inhibit the enzymatic activity of chlorophyll biosynthesis, and further inhibit its synthesis (Liu et al. 2013a), indicating that soybean–tea intercropping aids tea plant growth and development.

The chlorophyll fluorescence curve of the young tea plants under monoculture did not conform to the typical OJIP shape in the soybean mature period, similar to the chlorophyll fluorescence curve of *Zoysia japonica* at low temperature (Gururani et al. 2015). In addition, the pigment levels in tea leaves for intercropping and monoculture were closely related to the phenotypic characteristics (Figs. 1, 5). According to the trend in temperature change (Fig. 1 s), the low temperature led to the abnormal transfer of electrons (Strasserf et al, 1995) and the effects on the growth and metabolism of young tea plants can be alleviated by soybean intercropping under low-temperature conditions.

Our results indicate that intercropping soybeans in tea plantations can effectively counteract the low-temperature inhibition of tea plants and may be beneficial for their growth and development in agroforestry ecosystems.

Table 1 Correlation analysis between the secondary metabolites in *C. sinensis* leaves and soil fertility index

	Catechin	Caffeine	Amino acid	Soluble sugar	Chlorophyll	Organic matter	Available nitrogen	Ammonium nitrogen	Nitrate nitrogen	Available phosphorus	Available potassium
Catechin	1	.581*	.419	-.510*	.129	-.387	.097	.252	-.032	.679**	.628**
Caffeine		1	.421	-.614**	-.060	-.797**	.307	.718**	-.052	.918**	.884**
Amino acid			1	-.063	.664**	-.501*	.808**	.612**	.655**	.351	.707**
Soluble sugar				1	.572*	.202	.120	-.446	.431	-.672**	-.470*
Chlorophyll					1	-.270	.657**	.166	.739**	-.150	.270
Organic matter						1	-.619**	-.625**	-.404	-.754**	-.895**
Available nitrogen							1	.646**	.880**	.177	.644**
Ammonium nitrogen								1	.206	.484*	.776**
Nitrate nitrogen									1	-.074	.343
Available phosphorus										1	.838**
Available potassium											1

* indicated a significant correlation at the 0.05 level.

** indicated a significant correlation at the 0.01 level.

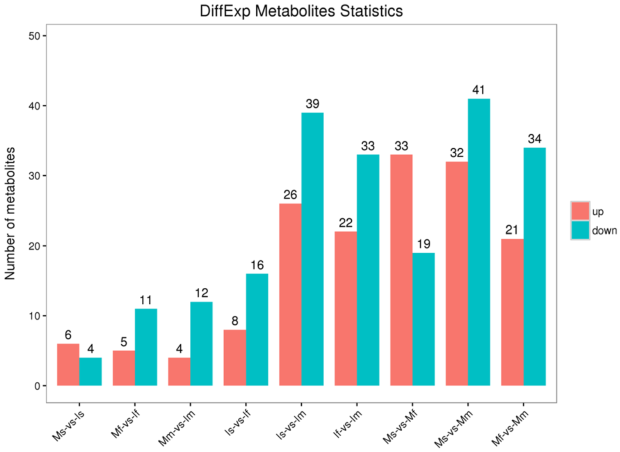

Fig. 7 Differentiated metabolites between intercropping and monoculture, in three soybean growth periods. *I* intercropping, *M* monoculture, *s* seedling period of soybean, *f* flowering–podding period of soybean, *m* mature period of soybean

Effects of intercropping on tea plantation soil N and tea quality

Nitrogen is in great demand for tea plant growth and development and is also one of the key factors contributing to tea quality. During plant growth and development, to maintain normal growth metabolism, tea plants constantly absorb soil N, P, K and other nutrients, which are essential for growth and tea quality. Most leguminous plants prefer to take up NO_3^--N, and tea plants absorb more NH_4^+-N from soil than NO_3^--N (Ishigaki 1974; Ruan and Sattelmacher 2007). Soil NH_4^+-N levels were higher for intercropped tea than monoculture during the soybean flowering–podding and mature periods and was at its highest level during flowering–podding (Fig. 2). In addition, in the process of soybean intercropping, the variation trends of NH_4^+-N and effective N of soil were the same. Therefore, N fixation by soybean was beneficial for preservation of soil effective N in intercropped tea as also found by Koenig and Cochran (1994). In summary, soybean–tea intercropping significantly affected the retention of soil N, with the most obvious influence being on soil N in the soybean flowering–podding period.

Catechin content decreased significantly for intercropping in young tea leaves during the soybean flowering–podding period, while amino acid and soluble sugar levels increased. The amino acid content in tea leaves was higher for intercropping than monoculture, which is beneficial to green tea fresh taste and aroma formation (Alcázar et al. 2007; Lee et al. 2013). Combining the correlation analysis of soil fertility and tea quality components, generated similar results to previous studies, in that N fertiliser was significantly positively correlated with free amino acids, only NH_4^+-N was significantly positively correlated with caffeine, and soil N affected the formation of amino acids and caffeine.

Soybean–tea intercropping not only affected the N content of soil, especially NH_4^+-N, but also affected the formation of the main physicochemical composition in tea leaves, further influencing tea quality.

Differences in tea plant metabolites under intercropping and monoculture

Chemical components in tea leaves are very important for tea quality; thus we investigated the influence of intercropping on chemical component synthesis. The differentiated metabolites showed that soybean intercropping affected tea plant growth and development. In addition, in the three soybean growth periods, the differentiated metabolites clearly increased either under intercropping or monoculture with temperature change (Fig. 1 s), with amino acids down-regulated in all soybean growth periods (Tables S4–S9). Most flavonoid compounds were upregulated during all soybean growth periods (Tables S4–S9), possibly due to lower temperatures.

Our study showed that soybean–tea intercropping mainly enhanced soil NH_4^+-N levels and low-temperature resistance via formation of the main physicochemical composition of tea leaves. Moreover, soil nutrient availability was highly correlated with tea quantity and quality, under intercropping. Intercropping is a low-input management practice and will result in better regional ecological services due to more efficient soil fertiliser applications, which will improve the environment and tea quality.

Author contribution statement Yu Duan and Jiazhi Shen had the original idea for this research. Xiaolei Zhang and Bo Wen were responsible for collected experimental samples. Yuanchun Ma and Yuhua Wang gave a lot of advice to analyze all data. Yu Duan analyzed the data and wrote the paper. All authors have read and approved the final manuscript.

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Compliance with ethical standards

Conflicts of interest The authors declare no conflict of interest.

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