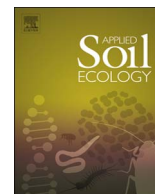




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Short communication

Intercropping improves soil nutrient availability, soil enzyme activity and tea quantity and quality

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ABSTRACT

Intercropping has long been an important agroforestry technique that has been applied worldwide. While intercropping increases soil carbon and nitrogen, an elucidation of its effects on the activities of soil enzymes and product quantity and quality remains elusive. Here we examined the effects of the intercropping of chestnut trees (*Castanea mollissima* Blume) in tea (*Camellia sinensis* L.) plantations on the seasonal dynamics of soil nutrients, soil enzyme activities, and tea quantity and quality in a temperate region in China. We collected soil samples from 0 to 20 cm (topsoil) and 20–40 cm (subsoil) depths in May, July, September, and November 2011, and examined the tea quantity and quality from bud samples collected in late April to early May 2011. We found that the effects of intercropping on soil nutrients and enzyme activities were strongly dependent on the sampling date. Soil pH and organic matter, nitrogen, phosphorus, and potassium content, as well as soil enzyme activities (catalase, urease, dehydrogenase, invertase, and polyphenol oxidase) increased on average with intercropping; however, the effects were more pronounced in the spring and early summer than late summer and fall for most nutrient and enzyme activities. Intercropping also increased the tea length and weight and tea quality by reducing the amino acid and catechin content, while increasing theanine and caffeine. Our analysis suggests that chestnut tree shading, enhanced soil nutrient availability, and the augmented activity of soil enzymes, facilitated the increase of tea quantity and the improvement of tea quality. Our results indicate that intercropping chestnut trees in tea plantations as a diversity treatment improves resource availability, ecosystem function, and product quantity and quality in the agroforestry ecosystems.

1. Introduction

Agroforestry ecosystems are spatially and temporally complex systems that have the potential to generate economic value, enhance biodiversity, while improving soil and water quality (Steffan-Dewenter et al., 2007; Power, 2010; Brooker et al., 2015; Cong et al., 2015). Diverse agroforestry systems, motivated by the economic returns from two or more crops, have been employed for more than 7000 years of agricultural production in China (Liu et al., 2013). The role of agroforestry in enhancing and maintaining long-term productivity and sustainability has been well documented, including the enhancement of soil fertility (Power, 2010; Brooker et al., 2015). For example, intercropping trees into agricultural crop systems increases soil carbon and nitrogen (Brooker et al., 2015; Cong et al., 2015).

The activities of soil enzymes, as an indicator of soil quality, are essential to organic matter synthesis and degradation in soils (Dick,

1994; Burns et al., 2013; Veres et al., 2015). Soil enzymes such as catalase, dehydrogenase, urease, and invertase play critical roles in catalyzing biochemical reactions during the decomposition of microorganisms, plants, and animals, where their debris and subsequent release of nutrients into the soil is made available to plants (Dick, 1994). Polyphenol oxidase has a defensive role against soil pathogens (Mayer, 2006). Intercropping has been reported to augment the activity of soil enzymes such as dehydrogenase and alkaline phosphatase (Chander et al., 1998; Hauggaard-Nielsen and Jensen, 2005); however, others have reported that although intercropping increases crop yields and soil nutrient availability, the activities of soil enzymes differed little from monocultures (Wang et al., 2015). Moreover, the responses of soil enzyme activities to intercropping appear to be enzyme-specific (Udawatta et al., 2008; Wang et al., 2015). Soil enzyme activities are also strongly influenced by soil temperatures associated with growing seasonality (Boerner et al., 2005; Wallenstein et al., 2009). The

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divergent findings associated with the effects of intercropping may be attributable to different sampling seasons. We thus hypothesize that the effects of intercropping on soil enzyme activities are most potent in the summer.

Tea (*Camellia sinensis* L.) is a popular, healthful, non-alcoholic drink that is consumed in many regions of the world (Alcazar et al., 2007). Recent studies revealed that white tea had a discernible anti-mutagenic activity in a *Salmonella* assay (Santana-Rios et al., 2001; Alcazar et al., 2007). Chestnut (*Castanea bungeana* Blume)/tea intercropping is a common practice in tea plantations in China due to the economic value of both chestnuts and tea. Similar to other intercropping trees with agricultural crops, chestnut-tea intercropping mediates changes in the microclimate, reduces soil bulk density and soil erosion (Wan et al., 2009), and improves soil moisture and nutrients (Yang et al., 2012). However, the influence of intercropping on tea quantity and quality remains unclear. Moreover, the relationships between soil nutrients, soil enzyme activities, and tea quantity and quality in chestnut-tea intercropping plantations are poorly understood.

Here, we examined how chestnut-tea intercropping affects soil nutrient properties, enzyme activities, and tea quality by sampling tea plantations under three levels of intercropping intensities: no intercropping (pure tea plantation), low intercropping (tea intercropped with chestnut trees at a 4.0 × 8.0 m spacing), and high intercropping (tea intercropped with chestnut trees at a 4.0 × 4.0 m spacing). All of the sampled tea plantations were established 36 years ago, and after 18 years of pure tea plantation management, the intercropping treatments were applied. We hypothesized that 1) intercropping of chestnut trees into tea plantations increases soil carbon and nutrient availability due to positive diversity effects (Hooper and Vitousek, 1998; Zhang et al., 2012; Brooker et al., 2016); 2) soil enzyme activities increase with intercropping more strongly in the summer than in winter due to the strong seasonality of soil microbes (Boerner et al., 2005; Wallenstein et al., 2009); 3) intercropping improves tea quantity and quality. Furthermore, we examined the linkages between soil nutrients, soil enzyme activities, and tea quantity and quality. We measured the enzymatic activities of the soil encompassing catalase, dehydrogenase, urease, polyphenol oxidase, and invertase, which comprise soil quality indicators (Ndiaye et al., 2000). As soil nutrients and enzyme activities are highly seasonally dynamic (Boerner et al., 2005; Wallenstein et al., 2009), these parameters were measured in May, July, September, and November 2011. For soil nutrients and enzyme activities, soil samples were extracted from two soil depths (topsoil at 0–20 cm and subsoil at 20–40 cm). We assessed the quantity and quality of the white tea by measuring the length, weight, and the contents of amino acid, catechin, caffeine, and theanine (Ning et al., 2016) of tea samples collected between late April and early May 2011. Unlike black tea, oolong tea, and green tea, white tea has a plucking season of approximately 20 days (from late April to early May), making it quite rare and highly valuable.

2. Methods

2.1. Study area

This study was conducted at the Experimental Station of the Forestry Research Institute in South Anhui Province, China (31°01'N, 115°53'E, and an altitude of 400–600 m). The mean annual temperature ranged from 12.5 °C, the mean annual rainfall is 1500 mm, and the annual frost-free period was from 161 to 176 days, between 1980 and 2010. The rock base is mainly granite gneiss, and the soil is granite yellow brown soil (Luvisols by IUSS Working and Group WRB (2014)), with a pH of ~4.5. The soil is typically light loamy with a depth of 100 cm.

2.2. Sampling design

In 2011, we sampled a 36-year-old white tea (*Camellia sinensis* L.)

garden, which had an area of approximately 20 ha. The spacing of the tea trees was 0.5 × 1.5 m. After 18 years of pure tea management, chestnuts (*Castanea mollissima* Blume) trees were intercropped in 1993. Hence our experimental plots encompassed a control (pure tea without chestnut trees), low-density intercropping (the spacing of chestnut trees was 4.0 m × 8.0 m), and high density intercropping of chestnut and tea (the spacing of chestnut trees was 4.0 × 4.0 m).

We randomly established three spatially interspersed sample plots, for each of the intercropping treatments. Each sample plot was 10 × 10 m² in area, located at a distance of at least 10 m between adjacent plots. Other than intercropping treatments, all plots underwent identical weeding treatments and other standardized management practices. During the growing season, all plots were pruned once or twice and manually weeded. Over February and March, all plots received the same compound fertilizer (NPK No. 1, 375 kg/ha).

For each sample plot, 100 sample points were systematically located at distance intervals of 1 m. The location of each sample point was examined vertically to determine whether the sample point was enshrouded by the chestnut canopy. We determined the degree of chestnut canopy shading on tea trees using the following formula: The degree of shading = Sample points shaded by chestnut/Total number of sample points.

To account for the spatial heterogeneity of soils (Chen et al., 1998; Carter and Gregorich, 2007) in each sample plot, we collected soil samples at ten random locations from two depths, i.e., 0–20 cm (topsoil) and 20–40 cm (subsoil) in May, July, September, and November 2011. For each sample plot, the samples from each soil layer were combined into one composite sample, resulting in a total of 18 soil samples (two soil layers for each of the nine sample plots), for the analyses of soil nutrients and enzyme activities in the laboratory.

2.3. Soil nutrients

In the laboratory, samples were initially air-dried, with coarse fragments being removed using a 2 mm sieve, then finely ground and sifted through a 100-mesh (0.15 mm) sieve, to ensure a uniform sample. Following the methods described by Carter and Gregorich (2007), the soil pH was determined in water using a PHM82 pH meter (Radiometer, Copenhagen, Denmark). Soil organic matter (SOM) was determined following the recommended Walkley-Black method (Nelson et al., 1996). Total nitrogen (N) concentration was analyzed via the flash dynamic combustion method, using a high temperature reactor to fully combust each sample, and gas chromatographic separation and thermal conductivity detection systems, to precisely quantify the elemental gases per 2 g sample (Carter and Gregorich, 2007). Total phosphorus (TP) and total potassium (TK) were determined by the NaOH molten-molybdenum antimony colorimetric method. Hydrolysable nitrogen (HN) was determined by the alkali solution diffusion method. Available phosphorus (P) was determined using the ascorbic acid reductant method on Bray P-1 (dilute acid ammonium fluoride) extract. Available (extractable) potassium (AK) was assessed through atomic absorption (Carter and Gregorich, 2007).

2.4. Soil enzyme activities

Following the methods for soil enzyme activity analysis (Guan et al., 1986), catalase (CA) activity was estimated by the Johnson-Temple method, using one gram of air-dried soil, which was titrated with 0.1 mol/L KMnO₄; the volume of each titration was measured and the activity was calculated. Dehydrogenase (DE) activity was determined using the 2,3,5-triphenyltetrazolium chloride method, based on the hydrogen ion content of the soil. Urease (UR) activity was estimated via the sodium phenolate method, subsequent to incubating one gram of air-dried soil for 24 h, whereas the urease content was determined based on the weight of soil resident NH₃-N. With one gram of air-dried soil incubated at 30 °C over 24 h, soil polyphenol oxidase (PO) activity

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