Forest Research
Open Access

Tian et al., Forest Res 2012, 1:2
DOI: 10.4172/2168-9776.1000107

Research Article Open Access

Soil Microbiological Properties and Enzyme Activities in Ginkgo-Tea Agroforestry Compared to Monoculture

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Abstract

Studies on enzyme activities and microbiological properties in the soil were very important as they may give indications of the potential of the soil to support biochemical processes which were essential for maintaining soil fertility. The objective of this study was to investigate the difference of soil quality parameters, including organic carbon and nitrogen in the soil, microbial biomass, basal respiration, and the activities of soil enzymes (Catalase, polyphenoloxidase, dehydrogenase, urease, protease and invertase), between monoculture system and agroforestry system. Three management treatments were studied in this work: pure tea system (G_0), intercropped with grafted ginkgo seedlings (G_1 and G_2), which were in a twenty years old tea orchard. Three kinds of depths of soil (0-10 cm, 10-20 cm and 20-30 cm) were used for all treatments. All the parameters except polyphenoloxidase in soil surface in the agroforestry system showed significantly higher values as compared to those in G_0. The contents of soil organic C, total N, microbial biomass and the activities of enzymes were higher in the surface soil as compared to the soil from middle and lower layers. The activities of soil enzymes, such as catalase, dehydrogenase, urease, protease and invertase, and soil organic carbon, total nitrogen were significantly positively correlated. The results of this study suggested that growing teas in combination with ginkgo could be considered as a good forest management practice, which would enhance organic matter accumulation in the soil and improve the activities of soil enzymes, furthermore, could maintain soil productivity and sustainability.

Keywords: Agroforestry system; Ginkgo-tea; Enzyme activities; Soil biological properties

Introduction

Combining trees and crops in spatial or temporal arrangements had been used to improve the safety of food and nutrition and mitigate the pressure of the environment by offering sustainable and alternative products instead of monoculture production. In practices, agroforestry could improve soil quality, carbon sequestration, and water quality in cropping systems [1-3]. In fact, agroforestry could offer lots of social and environmental benefits to human beings, not only in landscapes but also in economies [4].

Tea (*Camellia sinensis*) was an important economic crop and planted widely on acid red soils in the tropical and subtropical regions in China [5]. Camellia sinensis needed full sun to part shade. They prefered a well drained, neutral to slightly acidic soil rich in organic matter (2 parts peat moss or compost to 2 parts loam to 1 part sand or perlite). Gingko (*Ginkgo biloba L.*) was a traditional economic tree species in China, and usually cultivated in agroforestry systems [6]. The practice of ginkgo agroforestry had been adopted in order to obtain more economic benefits during the initial stages of establishment.

Soil quality refereed to the capacities of soil to sustain biological productivity, maintain environmental quality, and promote plant and animal health [7-8]. Trees would uptake nutrients from deep soil layers and maintain soil fertility through the litter fall and other parts of plants [9]. The microbial parameters were usually used as indicators to evaluate the soil quality and the effects to the environment [10].

Soil microorganisms were involved in many soil biological processes [11], which played a crucial role in the circles of most major plant nutrients in soils [5]. Soil microbial biomass was not only the labile nutrient pool but also the medium for the transformation and cycling of organic matters and plant nutrients in soils [5].

In the soil system, soil enzymes had important biochemical functions in the whole process of organic matter decomposition. Enzyme could rapidly response to the changes in soil management practices [12], provide quantitative information on functional diversity of microbial activities [13], such as soil chemical processes, mineralization rates, and organic matter accumulation [11]. The measurement of soil enzyme activities in the ecosystem would help quantify and evaluate specific biological processes in the soil [8]. The combined measurements of enzyme activities and microbial biomass had been widely used over the last 10 years in the study of the microbiological responses to agricultural management [14-17].

Although there had been extensive studies on soil microorganism and soil enzymes [11,18-21], little had been reported on their roles in ginkgo-tea agroforestry system. Meanwhile, how to understand and maintain biodiversity had become an increasingly important issue for researchers, which was also the goal for resource management [8]. The objective of this research was to compare the effects of agroforestry and monoculture systems on soil organic carbon, total nitrogen, microbial biomass, basal respiration and enzyme activities.

Materials and Methods

Study site and soil sampling

The study was conducted in Sanfeng farm (31°40' N, 120° 42' E), located at the foot of Yu mountain in Changshu, Jiangsu Province,

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Received May 04, 2012; **Accepted** May 29, 2012; **Published** May 31, 2012

Citation: Tian Y, Cao F, Wang G, Zhang W, Yu W. (2012) Soil Microbiological Properties and Enzyme Activities in Ginkgo-Tea Agroforestry Compared to Monoculture. Forest Res 1:107. doi:10.4172/2168-9776.1000107

China. The area was characterized by a subtropical wet monsoon climate with mean annual temperatures of 15°C and mean annual rainfall of about 1,500 mm, most of the rain falling between April and August.

This tea plantation was established in 1990 and three systems with different management treatments were employed in the study (Figure 1): (1) pure tea system (G_0), (2) intercropped with grafted ginkgo seedlings at a spacing of 10 m × 10 m (G_1), (3) intercropped with grafted ginkgo seedlings at a spacing of 6 m × 8 m (G_2). No inorganic nutrient amendments were applied, but herbicide and glyphosate were occasionally used. All the tea branches were pruned after tea picking seasons annually, with the tea rows 1.5 m width and 1m height.

The experimental design was completely randomized with a split plot for soil depths (0-10, 10-20 cm and 20-30 cm). There were two replicates for treatments and three sampling locations per treatment plot. Soil samples were collected with a soil auger and were placed in labeled plastic bags, which were sealed and transported to the laboratory in a cooler. All samples were maintained at field moist condition and were stored at 4°C until be analyzed. Three separate sub-samples were taken from each sample. One sub-sample was air-dried for soil carbon and nitrogen analysis. The second sub-sample was used to determine soil microbiological properties. The third sub-sample was used for enzyme assays after passing through a 2 mm sieve.

Soil analysis

Soil pH was determined in a 1: 2.5 soil/water ratio by a combination glass electrode [5]. Soil organic C was measured by oxidation with potassium dichromate. Total nitrogen was extracted with perchloric-concentrated sulphuric acid and determined by Kjeldahl digestion [6].

Soil microbial biomass C and N was determined by the chloroform fumigation extraction method with extraction coefficient of 0.45 and 0.54 for biomass C and N, respectively [22,23]. The K_2SO_4-extracted C of both fumigated and unfumigated samples was analyzed using a total organic C analyzer (Elementar liqui TOC, Germany). Basal respiration was determined by measuring CO_2 evolution. Twenty gram (oven-dry basis) of field-moist soil was incubated in 250 ml airtight glass vessels at 25° for 1 day. Vials containing 10 ml of 0.1 M NaOH were placed inside the flasks and the CO_2 evolved was determined by titration of carbonates with 0.1 M HCl [6,24]. Microbial quotient was defined as the ratio of microbial biomass C to soil organic C. The metabolic quotient was defined as the ratio of basal respiration to microbial biomass, i.e., the amount of CO_2-C produced per unit of microbial biomass carbon.

The activities of urease, sucrase, catalase, polyphenol oxidase, dehydrogenase and protease were determined by sodium phenolate colorimetry, sodium thiosulfate titration, potassium permanganate titration, iodimetry, TTC colorimetry and ninhydrin colorimetry, respectively [25].

Statistical analysis

Differences in soil properties for different soil depths and different systems were analyzed using one-way ANOVA. The least significant difference tests (Duncan's LSD) were used for pair-wise comparisons of treatment means. Differences were declared significant at the five percent level of significance ($p \leq 0.05$).

Figure 1: Satellite imagery obtained from Google Earth of the three sites in Sanfeng farm

Results

Soil chemical properties

Soil samples collected from the three systems varied in chemical properties. As shown in Table 1, pH in upper layer (0-10 cm) of G_0 was significantly lower than others and increased with the soil depths.

There were significant differences among the three systems for organic C. Organic C was highest in G_2 of each layer, lowest in G_0 of upper layer and lowest in G_1 of both middle (10-20 cm) and lower layers (20-30 cm). Total N in upper layers showed significant differences between pure and agroforestry systems, which was 1.1 times higher in G_2 than that in G_0. There were no significant differences in middle layer but in lower layer, the orders of the differences in lower layer were: $G_2>G_1>G_0$. The accumulation for either organic C or total N was decreased with soil depths.

Microbial biomass C, N and microbial quotient

G_0 had the lowest soil microbial biomass C (C_{mic}) in comparison with G_1 and G_2 in both upper and middle layers, and there were no significant differences between G_1 and G_2 in the two layers. Meanwhile, in lower layer, soil microbial biomass C was significantly higher in G_2 than in G_0 (Table 2).

Similar significant differences were observed in microbial biomass N (N_{mic}) in upper layer. Microbial biomass N was significantly higher in G_2 than in other two systems both in middle and lower layers, and there were no significant differences between G_0 and G_1 (Table 2).

Systems	pH			Organic C (g•kg^{-1})			Total N (g•kg^{-1})		
	0-10cm	10-20cm	20-30cm	0-10cm	10-20cm	20-30cm	0-10cm	10-20cm	20-30cm
G0	5.21b	5.97	6.02	14.98c	11.26b	6.94a	1.68b	1.1	0.31c
G1	5.86a	6.12	6.37	16.5b	10.1c	5.02b	2.64a	1.4	0.82b
G2	5.86a	6.18	6.36	17.88a	12.08a	6.98a	2.84a	1.62	1.09a

Means within a column of the same position followed by a different lower-case letter were significantly different at p≤0.05.

Table 1: Soil pH, organic C and total N of the three systems

Systems	C_{mic} (μgCg^{-1}soil)			N_{mic} (μgNg^{-1}soil)			C_{mic}/C_{org}(%)		
	0-10cm	10-20cm	20-30cm	0-10cm	10-20cm	20-30cm	0-10cm	10-20cm	20-30cm
G0	264.2b	143.3b	66.7b	40.6b	27.4b	20.6b	1.75	1.28b	0.98b
G1	287.5a	161.8a	75.1ab	48a	30.3b	22.2b	1.74	1.60a	1.50a
G2	295.8a	166.8a	82.6a	52.4a	36.4a	26.5a	1.65	1.38b	1.21ab

Means within a column of the same position followed by a different lower-case letter were significantly different at p≤0.05.

Table 2: The contents of microbial biomass C, N and microbial quotient of the three systems

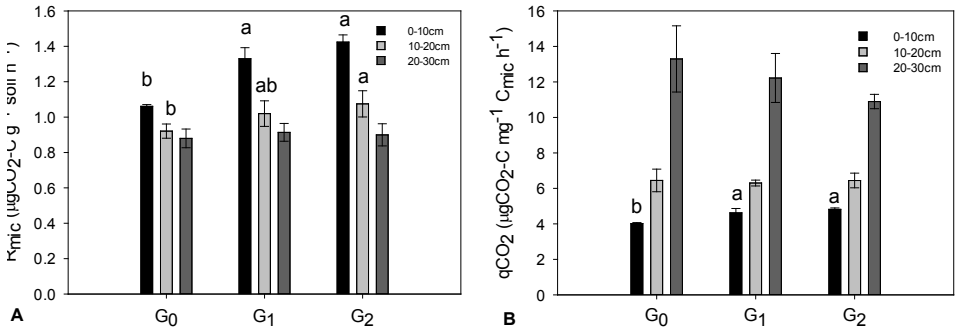

Figure 2: Basal respiration (A) and metabolic quotient (B) for the soil samples of three depths in the three systems. Different letters indicated significant differences at p ≤ 0.05. Bars represented standard errors.

Microbial quotient, the ratio of microbial biomass C to soil organic C (C_{mic}/C_{org}) was observed no significant differences among the three systems in upper layer. G_1 showed the highest amount of microbial quotient in middle layer. While in lower layer microbial quotient in G_1 was significantly higher than that in G_0, and there were no differences between G_0 and G_2 (Table 2).

Similar to organic C and total N, microbial biomass C and N and microbial quotient decreased with soil depths (Table 2).

Basal respiration and metabolic quotient

Basal respirations (R_{mic}) in upper layers of G_2 and G_1 were 1.31 and 1.25 times higher than those of G_0, respectively. And in middle layer, basal respiration of G_2 was 1.17 times higher than that of G_0. No significant differences were observed in lower layer among the systems (Figure 2A).

Similarly, metabolic quotient (qCO_2) in upper layers of G_2 and G_1 were 1.2 and 1.15 times higher than those of G_0, respectively. There were no significant differences in both middle and lower layers. Contrary to basal respiration, metabolic quotient showed a decreasing trend with soil depths (Figure 2B).

Enzyme activities

Catalase activities in G_1 and G_2 were significantly higher than G_0 in upper layer. Soil of G_1 in middle layer had 1.1 and 1.13 times the catalase activity as compared to that of G_0 and G_2, respectively. There were no significant differences in lower layer (Figure 3A). Polyphenoloxidase activities varied significantly among the three systems in upper layers, the order was: $G_1>G_0>G_2$. In addition, polyphenoloxidase activities showed the same trends in middle and lower layers, which were that G_1 had the highest activities and there were no significant differences between G_0 and G_2 (Figure 3B). Dehydrogenase activity was observed significantly higher in G_2 than in G_0 in upper layer, and with no significant differences between G_0 and G_1. There were no significant differences in middle layers among the three systems for dehydrogenase activity. G_2 showed the highest activity of dehydrogenase in lower layer compared to other systems (Figure 3C).

The trends of urease activity were observed similarly in upper and middle layers, with the orders: $G_2>G_1>G_0$. The differences in lower layers among three systems were not significant (Figure 3D). G_2 system had the highest protease activity in upper and middle layers, which were 1.56 and 1.40 times higher than G_0 and G_1, respectively. There were no significant differences in middle layer. Protease in G_1 showed the lowest activity than others in lower layer (Figure 3E). Invertase activities were significantly higher in G_1 and G_2 systems. G_1 had the lowest activity of invertase compared to G_0 and G_2 in middle layers. Significant differences were observed in lower layers, with the order: $G_2>G_0>G_1$ (Figure 3F).

Discussion

Chemical properties

The role of agroforestry in enhancing and maintaining long-term soil productivity and sustainability had been well documented [26]. Trees could enhance soil physical, chemical and biological properties by adding significant amount of above- and below-ground organic matters, and releasing and recycling nutrients in agroforestry systems [26,27]. Lee and Jose demonstrated that alley cropping systems involving pecan and cotton *(Gossypium hirsuitum)* in the southern United States had higher soil organic carbon and microbial biomass compared to monoculture cotton [28]. This study found that soil organic C and total N in ginkgo-tea agroforestry systems were significantly higher than that in pure tea system. The build-up of soil organic matter and nutrient turnover was affected by the input of litter fall. Soil organic matter held basic cations and was the source of energy for decomposers, which contributed to the increasment of the nutrients supply, such as N and K, in soil [29]. The higher organic carbon content of the upper layer in agroforestry systems could due to higher inputs of organic residues from ginkgo litter fall. The soil in tea orchard was very different than other soils [5]. Long-term root exudates and leaf litter could result in the decline of pH and the accumulation of Al [30,31]. In this study, as

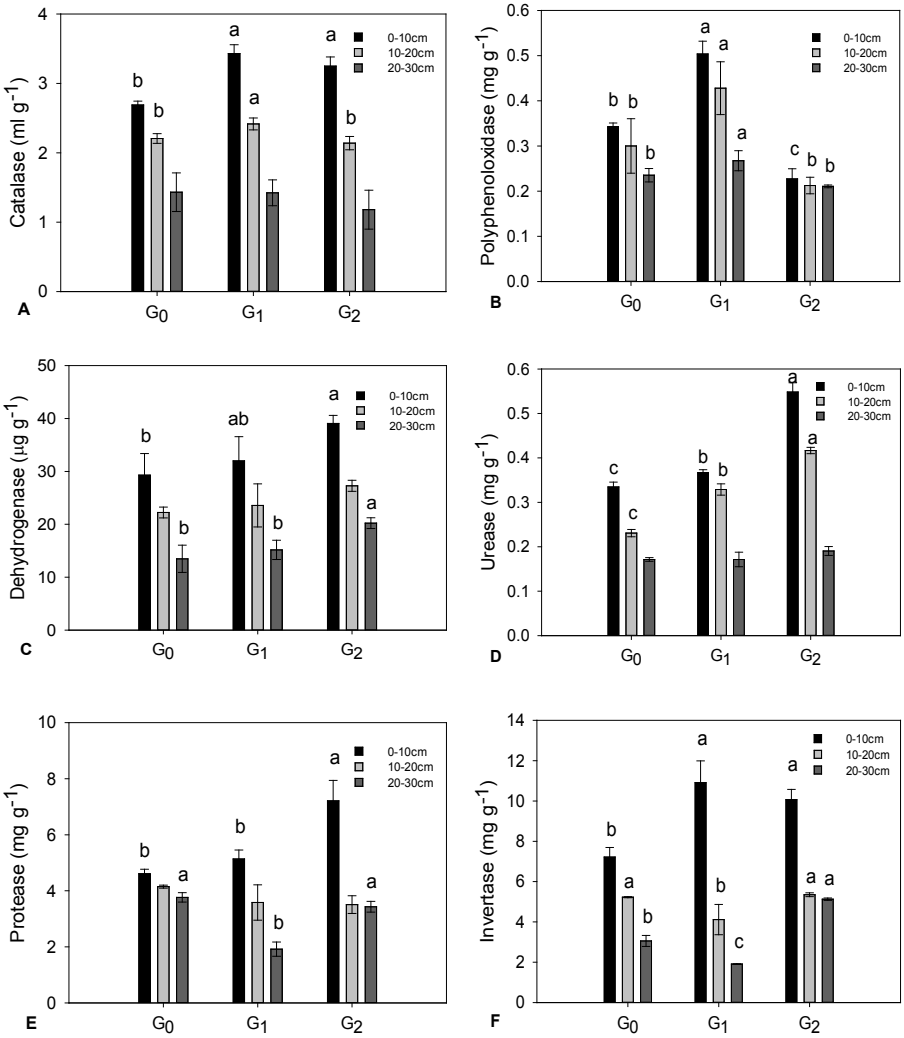

Figure 3: Enzyme activities of catalase (A), polyphenoloxidase (B), dehydrogenase (C), urease (D), protease (E) and invertase (F) for the soil samples of three depths in the three systems. Different letters indicated significant differences at $p \leq 0.05$. Bars represented standard errors.

intercropped with ginkgo, soil pH was significantly higher compared to monoculture, which suggested that ginkgo-tea agroforestry systems could improve soil pH, avoid soil exorbitance acidification and sustain soil productivity.

Microbiological properties

Soil microorganisms were important media in nutrient cycling and energy flow, and they were extremely sensitive to environmental changes [32]. The accumulation of soil organic C enhanced both the microbial biomass C and the proportion of microbial biomass C to soil organic C [5]. Fernandes found that the C biomass values ranged from 99 to 809 mg kg-1 in the top 10 cm and from 71 to 577 mg kg-1 in the 10-20 cm layer, whereas the N biomass values ranged from 11 to 101 mg kg-1 soil in the 0-10 cm layer and from 8 to 84 mg kg^{-1} in the 10-20 cm layer. These data indicated that C and N in the microbial biomass were concentrated in the first 10 cm of soil [33]. Our results provided additional evidence, which was that soil microbial biomass N decreased gradually with soil depth. The differences in soil microorganisms in upper layer among systems could be the results from different factors combined together, such as soil structure composition, root biomass and turnover, lignin content in crop residues, root and litter fall and microclimatic environment of community [34].

Microbial quotient had been used as an indicator of conversion efficiency of organic C into microbial C and losses of soil C during decomposition; it was also proposed to be used as a soil quality indicator to allow comparisons across soils with different organic matter contents [35]. In this study, there were no significant differences of microbial quotient among these systems. Meanwhile, it was found that microbial quotient of G_2 was lower than G_0 in upper layer, although the G_2 system had higher organic C, significantly. The reason for the phenomena should be that the decomposition of ginkgo leaf litter resulted in a large amount of organic C accumulated in soil of G_2 system and, consequently, lower microbial quotient in this soil. Microbial quotient represented between 0.82 and 1.77% in the areas under study. These percentages were in agreement with the results obtained by Wardle [36], and Anderson and Domsch [37].

Soil basal respiration was a widely used parameter for measuring

the microbiological activity [5,34]. In this study, R_{mic} was significantly greater in G_2 system than that in pure tea system. A higher rate of R_{mic} may be due to the existence of a large pool of labile C substrates [38]. Metabolic quotient defined as the respiration produced per unit of microbial biomass, expressed as mg CO_2-C h^{-1} g^{-1} biomass-C, indicated the energy optimization as ecosystems develop, also used as a sensitive indicator of activity of microorganism, low values being presented in stable and mature systems [34]. Our results showed that there were significant differences between agroforestry systems and pure tea system. High soil basal respiration and low consumption of organic C in G_2 system could bring high metabolism efficiency and ample available organic C, and maintain soil fertility and sustainability, eventually.

Enzyme activities

Soil enzyme activity was critically important for soil quality and could provide indications of changes in metabolic capacity and nutrient cycling due to management practices [39]. Activities of soil enzyme under different vegetation types were significant different [40]. Numerous studies reported significantly higher activities of these enzymes in intercropping systems compared to monocultures [6,34,41]. In this study, the ginkgo-tea agroforestry systems revealed significantly higher enzyme activities compared to pure tea system.

Continuous monoculture would be detrimental to soil enzyme activities, for example, the catalase activities in the soil from a cucumber continuous monoculture system decreased significantly [39]. This study found that catalase activity was significantly higher in agroforestry systems than in pure tea system, which confirmed the previous studies. Polyphenoloxidase was an important oxidase in aromatic compounds cycling [42]. It was found that polyphenoloxidase activity of G_2 system was significantly lower than others, which disagreed with the studies showing that agroforestry system had higher activity [6,39]. But Zhou found that polyphenoloxidase activity was negatively related with soil humification [41], it could be hypothesized that soil in G_2 had the highest degree of humification. Dehydrogenase activity was considered as the reflection for the total range of oxidative activity of soil microflora and, consequently, may be assumed to be a good indicator of microbiological activity [43]. In our study, G_2 system showed relatively high values in dehydrogenase activity as compared to pure tea system.

Urease was the enzyme that catalyzes hydrolysis of urea to CO_2 and NH^3, which was a vital process in the regulation of N supply to plants after urea fertilization [35]. The results of this study were therefore consistent with other studies reporting that urease activity was significantly affected by different soil management systems [44,45]. Understanding of urease activity dynamics could reveal more effective ways of managing N fertilizers [35]. Reductions in urease activity of G_0 system might negatively affect tea growth and yield. Protease and urease were involved in the N cycle [5,34]. Similarly, protease in G_2 was significantly higher than in other systems. Soil invertase catalyzed the hydrolysis of sucrose to glucose and fructose, and was linked to the soil microbial biomass [42].

Relationships between soil properties and soil enzyme activities

In this study, enzyme activities strongly followed the distribution of soil carbon and nitrogen among the systems. The organic C content was highly correlated with catalase, dehydrogenase, urease, protease and invertase enzyme activities (r=0.95, 0.95, 0.87, 0.87, and 0.93, respectively). The greater correlations between enzyme activity and organic carbon were consistent with previously published results [19,46-48]. It could be hypothesized that perennial vegetation provided environmental conditions suitable for greater accumulation of organic C and total N. Increased enzyme activities were attributed to the increasing of organic matter and litter quality and quantity as well as the improvement of soil physical parameters. Increased enzyme activity was proportionally linked to microbial function leading to improved nutrient cycling and availability, which favored root growth, promotes beneficial plant–microbial interactions, and eventually increased the total soil carbon pool [11].

Depth effects

Soil organic C, total N, microbial biomass and enzyme activities were greater in the surface soil as compared to sub-surface soil, which agreed with published results [8,49]. These differences were attributed to the higher organic matter accumulation, favorable moisture and temperature in the surface soil as compared to sub-surface soil [8]. In addition, in this study, metabolic quotient in upper layer was significantly lower than middle and lower layers, which might be due to the relatively high level of organic C on the soil surface.

Conclusions

The objective of this study was to evaluate the changes of soil quality parameters including soil organic C, total N, microbiological properties and enzyme activities in pure tea system in comparison with ginkgo-tea agroforestry systems. In the study, three soil depths (0-10 cm, 10-20 cm and 20-30 cm) and two densities of ginkgo trees were investigated. The results obtained in this study showed that adoption of a ginkgo-tea combination could lead to the increased long-term sustainability of soil fertility, although the benefits to some soil properties may not be apparent immediately. All parameters decreased gradually with soil depths, except metabolic quotient. The contents of soil organic C, total N, microbial biomass and the enzyme activities in ginkgo-tea agroforestry systems were significantly higher than those in a pure tea system. Soil enzyme activities (i.e., catalase, dehydrogenase, urease, protease and invertase) were highly correlated with soil organic C and total N. Higher soil enzyme activities and the content of microbial biomass were enhanced by agroforestry that may lead to the increasing of other soil quality parameters such as organic matter content, soil sustainability and productivity, so that the soil and ecosystem functions would be improved.

Acknowledgment

The authors thank the staff of Sanfeng farm in Changshu, Jiangsu Province, China, for providing experiment sites, helping and supporting during the study. Also thanks to all the postgraduate students in the laboratory of silviculture in Nanjing Forestry University.

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