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Alder trees enhance crop productivity and soil microbial biomass in tea plantations

P.E. Mortimer[a,b,1,*], H. Gui[a,b,c,1], J. Xu[a,b], C. Zhang[d], E. Barrios[e], K.D. Hyde[a,c]

[a] Key Laboratory of Plant Biodiversity and Biogeography of East Asia (KLPB), Kunming Institute of Botany, Kunming 650201, China
[b] World Agroforestry Centre, East and Central Asia, Kunming, 650201, China
[c] School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand
[d] Changning Forest Ownership Management Service Center, Baoshang, Yunnan, 678100, China
[e] World Agroforestry Centre, Headquarters, Nairobi 00100, Kenya

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ABSTRACT

Monoculture farming systems lead to soils depleted of nutrients and diminished microbial functional diversity, disrupting processes crucial to maintaining soil health. The planting of trees in these monoculture systems is one way to improve soil nutrition and biodiversity. Therefore, the objective was how planting the N-fixing tree *Alnus nepalensis* (7 years old), into monoculture tea (*Camellia sinensis* var., *assamica*) plantations (32 years old), influences the soil fungal and bacterial communities, and how this impacts on tea productivity. Soil samples (0–15, 15–30, 30–60 cm depths) were collected from plantations of monoculture tea and tea interplanted with *A. nepalensis* trees. The samples were analyzed for basic soil properties and nutrients. Phospholipid fatty-acid analyses were conducted on the soil samples to determine the microbial functional groups and biomass of bacterial and fungal communities. Biomass of soil fungi and bacteria were 41% and 10% higher in the tea + *A. nepalensis* sites than in the tea monoculture sites, respectively. These higher levels were recorded despite there being no changes in the diversity of the soil fungi and bacteria, or the soil nutrition, between the different sites. Tea productivity increased between 52% and 72%, and is attributed to the increases in the soil community biomass. Ectomycorrhizal biomass, as well as Gram-positive, Gram-negative, and actinomycetes bacterial biomass, all increased ranging from 10% to 83%. These groups of organisms have been shown to contain plant growth promoting characteristics, contributing toward increased crop productivity. We provide clear evidence that *A. nepalensis* in tea plantations promotes the growth and development of the soil microbial communities and that this impacts on above ground productivity. This study highlights the benefits of introducing N-fixing tree species, such as *A. nepalensis*, into monoculture systems, and how this relates to soil health and harvest yield.

1. Introduction

Soil plays a crucial role in all terrestrial ecosystems, providing substrate, nutrition, water and a reservoir of organisms upon which the plants rely on for survival. An integral component of soils, and a component which has an important influence on above ground productivity, is the soil biota (Barrios, 2007). The soil microbial community is the largest constituent of soil biota and plays key roles in several ecological processes, such as organic matter decomposition, nutrient acquisition and cycling, and soil formation and aggregation (Zhou and Thompson, 2002). Soil microbes also contribute to plant productivity through the formation of symbiotic relationships (Bainard et al., 2013). Nitrogen-fixing bacteria and mycorrhizal fungi are the most widely known and studied of these symbiotic groups; they have a strong influence on plant productivity through the enhancement of nutrient acquisition and transport (van der Heijden et al., 2006). There are however, many other soil microbial groups that perform vital functions in maintaining above and below ground functions (Gui et al., 2012).

Amongst these microbial groups, soil fungi and Gram positive and negative bacteria play key roles in plant growth and production, eliciting both positive and negative above ground vegetation growth responses (Welbaum et al., 2004). Soil fungi contribute toward soil nutrient cycling, as well as influencing plant

* Corresponding author at: Key Laboratory of Plant Biodiversity and Biogeography of East Asia (KLPB), Kunming Institute of Botany, Kunming 650201, China. Tel.: +86 871 65223599; fax: +86 871 65223014.
E-mail address: P.Mortimer@cgiar.org (P.E. Mortimer).
[1] Joint first authorship, authors contributed equally.

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community composition through the development of symbiotic relationships and pathogenic infections (Bashan et al., 2004; Kernaghan, 2005; Liu et al., 2013; Moore et al., 2004). Gram negative and gram positive bacteria have a wide range of functions within the soil environment, including both free-living, associative and symbiotic N-fixation, antibiotic production, siderophore (iron chelating compounds) production, and sulfur oxidizing capabilities (Bashan et al., 2004; Kishore et al., 2005; Neeno-Eckwall et al., 2001; Welbaum et al., 2004).

Besides bacteria and fungi, actinomycetes, such as *Frankia*, are known to form symbiotic relationships with actinorhizal plants, developing root nodules, where N-fixation takes place (Wall, 2000). Alder trees, such as the Himalayan alder (*Alnus nepalensis*), are well known actinorhizal plants. *A. nepalensis* occurs naturally throughout the eastern Himalayan region and is a fast growing pioneer species. It is widely used in land restoration, reforestation projects, and has a long traditional use as an intercropping tree species (Carlson and Dawson, 1985; Chand et al., 1994; Goldman, 1961; Li et al., 2006).

Agroforestry systems are known to improve soil nutrient availability, soil microbial diversity and above ground productivity (Barrios et al., 2012; Li et al., 2013). More specifically, numerous studies have indicated that planting *Alnus* trees in agricultural settings have positive effects on plant growth, crop production and soil health (Binkley, 1983; Sharma et al., 2009; Vanlalhluna and Sahoo, 2009; Das et al., 2010).

The use of *A. nepalensis* as a shade tree in tea plantations is gaining popularity in Asia (Guo et al., 2006). Tea (*Camellia sinensis*) plantations dominate much of the agricultural landscape in Asia and usually occur as monoculture systems, impacting negatively on local biodiversity and soil health (Bainard et al., 2013). Thus, the incorporation of N-fixing trees into these monoculture systems is highly advantageous in terms of soil health and crop production, both of which have been shown to be negatively affected in monoculture stands (Guihua, 1996; Wang and Li, 2003). The use agroforestry systems for tea production, exemplified by the traditional practice of planting *C. sinensis* into existing forest systems, is an emerging practice in many tea-growing regions, such as northern Thailand and southwestern China (Sysouphanthong et al., 2010). However, the primary method of tea production remains monoculture systems, which are easier to manage and more economically viable for large-scale agriculture, albeit more detrimental to the environment.

Despite the large numbers of studies highlighting the benefits of using agroforestry and incorporating trees into the agricultural landscapes, few studies have focused on the changes brought about in the soil microbial community and the subsequent role that this plays in contributing toward healthier soils and crop production. Thus, the primary objective of this study was to elucidate how soil microbial communities, in particular bacteria and fungi, are influenced by the incorporation of the N-fixing tree *A. nepalensis* into monoculture plantations, and how this impacts on crop productivity.

2. Methods and materials

2.1. Site description and field experiment

This study was conducted in Changning County, Yunnan Province, China. Three tea (*C.sinensis*) plantation sites (Dazhuangwan (T), Xiaoluopo (X) and Ertaipo (E)) were selected. The geographical information for each site is given in Table S1. The three study sites were comparable in terms of climate, soil type, plant age, planting density, and management practices (pruning, weeding). Slope was comparable between the plots within each site. The climate is classified as temperate humid, with an annual rainfall of *ca.* 1268 mm, most of which falls between May and October. The mean annual temperature is approximately 15 °C and the maximum and minimum temperatures are 24.7 °C and 7.7 °C, respectively. The detailed meteorological data in 2012 is given in Fig. 1S according to China Meteorological Data Sharing Service System (2014).

Each study site consisted of a paired comparison between the agroforestry plots, that is tea plants + *A. nepalensis* trees, and the control plots, represented by a tea monoculture. The age (7 years) and planting density (*ca.* 660 trees ha^{-1}) of the *A. nepalensis* trees was similar in the agroforestry plots at all the study sites. The *A. nepalensis* trees were planted into the tea plantation by replacing

Table 1
The soil properties of soils taken from either monoculture (tea) or agroforestry (tea + alder) plots ($n = 3$), from three soil depths (0–15, 15–30, 30–60 cm).

	Sites	0–15 cm			15–30 cm			30–60 cm		
		PLFA	SE	*p* value	PLFA	SE	*p* value	PLFA	SE	*p* value
Organic matter ($g\,kg^{-1}$)	Tea	48.98	2.69	0.245	43.32	2.73	0.933	37.98	3.2	0.22
	Tea + alder	54.88	4.08		43.69	3.29		32.26	3.13	
Total N ($g\,kg^{-1}$)	Tea	2.01	0.13	0.614	1.73	0.13	0.961	1.59	0.14	0.221
	Tea + alder	2.1	0.12		1.72	0.16		1.34	0.14	
Total P ($g\,kg^{-1}$)	Tea	0.98	0.11	0.012*	1.98	0.96	0.18	1.02	0.16	0.083
	Tea + alder	0.65	0.05		0.63	0.08		0.64	0.13	
Total K ($g\,kg^{-1}$)	Tea	12.03	0.31	0.632	11.68	0.39	0.332	11.73	0.21	0.076
	Tea + alder	12.37	0.62		12.42	0.63		12.67	0.45	
Available N ($mg\,kg^{-1}$)	Tea	172.04	9.24	0.116	163.85	9.99	0.793	162.54	13.57	0.184
	Tea + alder	197.21	12.02		159.66	12.06		132.87	16.52	
Available P ($mg\,kg^{-1}$)	Tea	10.67	2.19	0.173	11.01	2.53	0.052	10.48	3.6	0.462
	Tea + alder	7.28	0.91		5.16	1.15		6.84	3.23	
Available K ($mg\,kg^{-1}$)	Tea	116.64	33.27	0.205	72.01	21.97	0.265	51.4	13.73	0.721
	Tea + alder	68.68	14.44		44.09	10.03		42.76	19.42	
pH	Tea	4.94	0.13	0.736	5.33	0.31	0.182	5.1	0.14	0.054
	Tea + alder	5	0.14		4.89	0.05		4.74	0.1	

* $p < 0.05$.

one tea plant with an *A. nepalensis* sapling, thus having a minimal effect on the density of the tea plants. For the control and experimental plots, both tea and *A. nepalensis* trees (only in experimental plots) were planted in contour rows, with a row spacing of 1m. The tea plants were terraced, with a space of approximately 1 m between plants and the *A. nepalensis* trees were planted every 5 m in the experimental plots. Tea plants within the control and the experimental plots were of the same age (32 years). Management practices mainly consisted of weeding the monoculture plots; dead weeds were left on top of the soil between the rows of tea plants. According to interviews with the farmers and the extension officers from the Baoshan Forestry Bureau, no pesticides were applied in the plots, however, fertilizer was applied 3 times per year. Weed biomass, as well as seasonal pruning of the *A. nepalensis* trees, was left on top of the soil between the planting rows at all plots. Each site was treated as 1 replicate ($n = 3$). Within each site, five subplots (20 m × 20 m) were randomly selected and marked out.

2.2. Soil sampling and chemical analyses

The soil at the sites is classified as Torrents Vertisols according to the Soil Survey Staff (2010). Soil samples were taken with an auger from the corners and center point of each sub-plot. Samples were then bulked into a composite sample. Samples were taken at 3 depths, *viz.* 0–15 cm; 15–30 cm; 30–60 cm. All the soil sampling was carried out in April 2012. A soil subsample was freeze-dried for PLFA analysis at a later date. The remainder of the soil sample was stored at 4 °C for subsequent soil chemical analysis (Table 1). Soil pH was determined in 1:1 water extract and measured using a pH meter (FE-20, FiveEasy Plus™, Mettler-Toledo, Germany). Soil organic matter was determined by Dumas combustion (White et al., 1997), and total nitrogen (N) by a semi-micro Kjeldahl apparatus (Yuen and Pollard, 1953). Total phosphorus (P) and potassium (K) were measured spectrophotometrically after digested with a mixture of concentrated H_2SO_4 and H_2O_2 (Murphy and Riley, 1986). Hydrolysable N was analyzed by reaction with iron(II) sulfate and sodium hydroxide by a diffusion procedure (Mulvaney and Khan, 2001). Available P and K were determined using ammonium fluoride and ammonium acetate respectively (Hedley et al., 1982). All the chemical analyses were conducted in Yunnan Agriculture Academy, Yunnan, China.

2.3. Tea yield

Yield data for each site was obtained from the Baoshan Forestry Bureau for the period 2008–2010. Yield is shown as the total weight of dried leaves harvested per year in kg ha^{-1}. Furthermore, the average tea yield for the agroforestry sites (1997–2007), prior to the planting of *A. nepalensis* trees in these sites, was obtained from the farmer's records. For 2012, tea picking was conducted twice in the spring, three times in the summer, and three times in the autumn. For each picking, 5–10 fresh tea leaves were collected from the growing tips of each tea plant.

2.4. Lipid extraction and PLFA analysis

Lipid extraction and PLFA analyses were performed in the laboratories of the South China Botanical Gardens, Chinese Academy of Sciences, using the modified Bligh and Dyer-method (Bligh and Dyer, 1959; Peacock et al., 2001). Briefly, a subsample (1 g) of the freeze-dried soil sample was extracted with a chloroform–methanol–citrate buffer mixture (1:2:0.8), and the phospholipids were separated from other lipids on a silicic acid column. The phospholipids were subjected to a mild alkaline methanolysis and the resulting fatty acid methyl esters (FAMEs) were analyzed on a gas chromatograph/mass spectrometry (GC–MS) system. The specific signatures derived from the mass spectra for each of the individual FAME were identified by the MIDI Sherlock™ Microbial Identification System (MIDI, Newark, DE).

Standard nomenclature for fatty acid characterization was used (Frostegard and Baath, 1996). Individual fatty acids were designated according to convention by the total number of carbon atoms; number of double bonds, followed by the position of the double bond from the methyl-end of the molecule. For unsaturated fatty acids, ωn follows, where n indicates the position of first carbon of the double bond from the aliphatic end of the molecule. The prefixes *i* and *a* indicate iso- and anteiso- branching, respectively, and *cy* indicates cyclopropane fatty acid. *Me* refers to the position of the methyl group from the carboxyl-end of the chain. Fatty acids were classed into different groups (bacterial, fungal and actinomycete), and used to indicate their respective biomass estimates, according to Leckie (2005).

2.5. Statistical analysis

The treatment (agroforestry and control plots) effects were tested using a one-way ANOVA, and significant differences ($p \leq 0.05$) for these factors, between the monoculture and agroforestry sites, analyzed using least significant difference (LSD), using SPSS 21.0 for Windows (IBM Inc.). The ANOVA analysis was run separately for each soil depth between treatments. Variability within the PLFA profiles was determined using principal component analysis (PCA) based on the content of each of the detected fatty acids (Statsoft, 1995).

Soil microbial community diversity, based on the PLFA profiles, was determined using the Shannon–Weaver Index (H') (Shannon and Weaver, 1998), and calculated using the following formula:

$$H' = -\sum \mathrm{Pi} \times \ln \mathrm{Pi}$$

where Pi refers to the ratio of the content of each fatty acid to the total content of one soil sample. Each fatty acid was considered as representative of one species (Frostegard et al., 2011).

Redundancy analysis (RDA) was carried out in order to determine the relationship between the soil community structure and soil properties. Positions of samples along the axes are determined by loading scores. In this analysis, each study site is represented by a point in the ordination space, and the PLFA response variables and environmental variables (soil properties) are represented by arrows projecting from the origin (McKinley et al., 2005).

3. Results and discussion

3.1. Tea yield

The mean tea yield was higher under agroforestry incorporating *A. nepalensis* (Fig. 1). The relative tea yield, representing the difference between the two tea production systems, ranged between 52 and 72% and did not change significantly between the three recorded years. Furthermore, the average yield for the tea plantations, prior to the introduction of *A.* nepalensis, was significantly lower than the yield of the tea plants in the agroforestry system (Fig. 1). Numerous reports have indicated that the intercropping of various crops with *A. nepalensis* has positive effects on crop production (Sharma et al., 2009; Vanlalhluna and Sahoo, 2009; Das et al., 2010). Few studies, however, have investigated the affect of shade trees on the biodiversity and productivity of tea plantations. Guo et al. (2006) noted that the agroforestry system of *C. sinensis* and *Hevea brasiliensis* is more economically viable than monoculture

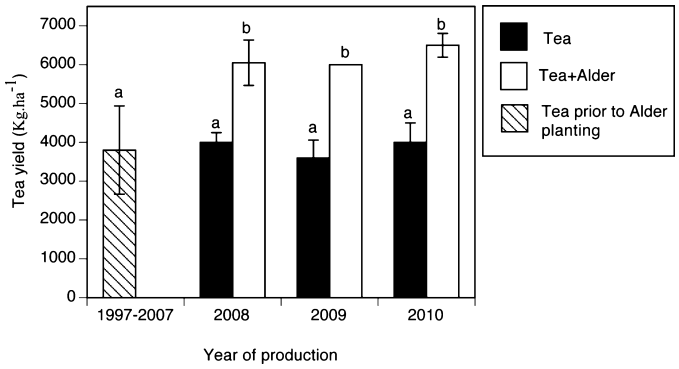

Fig. 1. Tea yield in kg ha^{-1}. Tea was grown either in monoculture stands (tea) or in agroforestry systems (tea + alder). Bars within a year followed by the same letter are not significantly different at $p < 0.05$.

practices, and Sysouphanthong et al. (2010) found increases in the macro-fungal diversity of tea plantations, when tea was grown under tree canopies. Our findings not only indicate a large percentage increase in the productivity of monoculture tea plantations as a result of adding *A. nepalensis,* but also how the microbial community biomass and diversity is affected in this agroforestry system.

3.2. Soil nutrients and environmental factors influencing soil community structure

Except for total P in the topsoil layers, no differences were found between the soil properties of the different sites (Table 1). The RDA analysis indicated that, of the environmental variables tested, soil organic matter, total N, available N and available P most strongly influenced microbial community biomass (Fig. 2). This is in partial

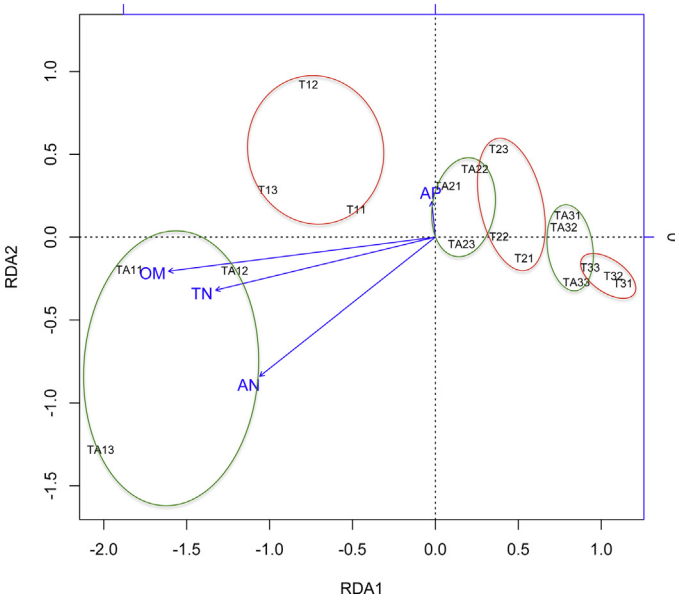

Fig. 2. Redundancy analysis (RDA) of the distribution of the PLFA profiles, across the different soil depths (0–15, 15–30, 30–60 cm) for the PLFAs, taken from the three study sites. The sites included either monoculture plantations (tea) or agroforestry systems (tea + alder). Each site is clustered as one circle, red circles for the monoculture stands (tea) and green circles for agroforestry systems (tea + alder). Figure codes: T = tea, TA = tea + alder, eg: TA11: tea + alder, the first number represents the replicate (1 = first replicate; 2 = second replicate; 3 = third replicate) and the second number represents the soil depth (1 = 0–15 cm; 2 = 15–30 cm; 3 = 30–60 cm). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

agreement with the findings reported in past studies, however, these studies also highlighted the importance of soil moisture and pH on community structure, which were not found to have a significantly impact on community structure in our study (Brockett et al., 2012; Prescott and Grayston, 2013; Wakelin et al., 2008). The reason no major differences were found in the soil nutrient profiles between the monoculture tea and agroforestry sites, is likely due to the fertilization treatments added by the farmers to both sites. Past studies have shown that young (below 10 years old) *A. nepalensis* trees, comparable in age to those used in this study, can contribute *ca.* 71 kg N ha^{-1} year^{-1} and *ca.* 2.9 kg P ha^{-1} year^{-1} and between 2850–3200 kg litter ha^{-1} year^{-1}, to an agroforestry system (Sharma et al., 1994, 2002; Sharma and Ambasht, 1991).

3.3. Soil microbial diversity

The Shannon index indicated that diversity between the soil depths was fairly stable, with significant differences found between the topsoil layers of both the monoculture and agroforestry sites, and the 30–60 cm layer of the monoculture site (Table 2). These findings are in agreement with the work of Pansombat et al. (1997), who reported that the microbial community structure changed across soil depths in tea plantations, and that these changes were related to substrate availability.

No differences however, were found in diversity of the soil communities between the agroforestry and monoculture systems, for any of the depths evaluated (Table 2). Past research on the impact of trees on soil communities has shown that the presence of trees do not always lead to an increase in soil diversity, but more frequently result in an increase in microbial biomass (Huang et al., 2013), which is congruent with our results. The absence of changes in community diversity between the agroforestry and monoculture sites would imply that the soils from monoculture tea plantations were not conducive to the establishment and maintenance of a larger microbial community. These findings are in agreement with previous studies showing that the soil microbial biomass of monoculture tea plantations decreased with age and the intensification of tea production (Han et al., 2007). In the current study, in the absence of any differences between the soil pH, nutrients and water status between the agroforestry and monoculture tea sites (Table 1), the increases in microbial biomass in the agroforestry systems can only be attributed to *A. nepalensis* trees.

3.4. Changes in soil microbial biomass

The PCA plots of the first two principal components explained 71.6% and 7.9% of the total variance, respectively (Fig. 3a and b). The soil fungal and bacterial samples, from the monoculture and agroforestry plots were significantly separated along axis 1 and 2. Furthermore, samples taken from the different sites were clustered together (Fig. 3 a and b). Thus indicating that the respective microbial groups were distinctly separated according to the type of land use system (agroforestry or monoculture).

This separation in the soil microbial groups, between the monoculture and agroforestry systems, is further evidenced by the PLFA results. The PLFA profiles of the soil microbes consisted of saturated, unsaturated, methyl-branched, and cyclopropane fatty acids, and 20 PLFA signatures were detected as biomarkers for specific microbial groups (Table 3). A number of microbial groups, including fungal, bacterial and actinomycetes, differed significantly between the monoculture and agroforestry systems. The biomass of the following groups was higher in the agroforestry systems: Gram-positive bacteria (a15:0; a17:0; i15:0), Gram-negative bacteria (cy17:0; 16:1 ω7c; 18:1ω7c), actinomycetes (10Me16:0), ectomycorrhiza (18:2 ω 6,9c) and some non-specific

Table 2
Phospholipid fatty acid (PLFA) composition of soils taken from either monoculture (tea) or agroforestry (tea + alder) plots ($n = 3$), from three different depths (0–15, 15–30, 30–60 cm). The different PLFA profiles have been grouped together according to relevant functional groups.

Community	PLFA	0–15 cm		15–30 cm		30–60 cm	
		Tea	Tea + alder	Tea	Tea + alder	Tea	Tea + alder
Actinomycetes[a]	10Me16:0	2.48	2.774	1.424	1.91*	0.314	1.026
	10Me17:0	0.307	0.345	0.164	0.161	0.023	0.071
Fungi[b]	18:1 ω9c	2.207	2.904	0.942	1.075	0.443	0.514
Ectomycorrhiza[g]	18:2 ω6,9c	1.098	1.743*	0.377	0.605	0.394	0.316
AMF[f]	16:1 ω5c	0.799	0.929	0.425	0.564	0.19	0.251
Gram-negative Bacteria[c,e]	16:1 ω9c	0.281	0.824	0	0.121	0	0.07
	16:1 ω7c	1.248	1.478*	0.725	0.978*	0.29	0.459*
	18:1 ω9c	2.207	2.904	0.942	1.075	0.443	0.514
	cy17:0	0.714	0.854	0.393	0.581	0.232	0.275
	cy19:0	2.16	2.13	1.087	1.337	0.471	0.717
	18:1 ω7c	1.865	2.326	0.988	1.395*	0.421	0.583*
Gram-positive Bacteria[d,f]	i14:0	0.124	0.161	0.023	0.114	0	0
	i15:0	3.082	3.446*	1.598	2.131*	0.589	1.135*
	a15:0	1.13	1.345	0.687	0.962*	0.219	0.579*
	i16:0	1.651	1.158	0.859	0.915	0.536	0.657
	i17:0	1.26	1.407	0.681	0.767	0.457	0.527
	a17:0	0.651	0.736*	0.36	0.456*	0.225	0.312*
Non specific	14:00	0.243	0.303	0.116	0.161*	0.41	0.055
	15:00	0.222	0.26	0.081	0.126	0	0.019
	16:00	5.256	8.655	2.323	3.164*	1.335	1.619*
	17:00	0.175	0.223	0	0	0	0
	18:00	1.083	1.353	0.551	0.709*	0.329	0.422
	a17:1 ω9c	0.421	0.266	0	0	0	0
	17:1 ω8c	0.148	0.161	0	0	0.044	0
	11Me18:1 ω7c	0.31	0.337	0	0	0	0

Citations: [a](White et al., 1997), [b](Zelles et al., 1992), [c](Zogg et al., 1997), [d](Steinberger et al., 1999), [e](Frostegard and Baath, 1996), [f](Frostegard et al., 1993), [g](Olsson, 1999).
* $p < 0.05$.

groups (14:0; 15:0; 16:0; 18:0) (Table 2). These findings are confirmed by past studies showing changes in soil microbial biomass related to different species of *Alnus* trees (Golinska and Dahm, 2011; Prescott and Grayston, 2013). Selmants et al. (2005) found that the soils found beneath stands of *Alnus rubra* had greater microbial biomass and activity than soils where *A. rubra* was absent. In order to sustain larger soil communities, there needs to be enough substrate on which these organisms can feed. Plant derived C acts as a primary source of substrate for soil microbes (Denef et al., 2009), thus it is likely that the incorporation of *A. nepalensis* in the tea fields led to an increase in available substrate, forming an environment capable of sustaining larger soil microbial communities.

Based on the PLFA profiles obtained from the respective soil plots, it is evident that there were no changes in the biomass of *Arbuscular mycorrhiza* (16:1ω5c) (Table 3). This is in contrast to the work of Šnajdr et al. (2013) who found that beneath stands of *A. glutinosa* and *A. incana* there was an increased presence of *A. mycorrhiza*. However, for the current study, the ectomycorrhizal biomass (18:2ω6, 9c) was found to be greater beneath the *A. nepalensis* trees. This is in agreement with the work of Sysouphanthong et al. (2010) who found that there was greater fungal (including ectomycorrhizae) biomass and diversity in tea plantations grown beneath a tree canopy layer. The higher levels of ectomycorrhizal fungi would further contribute to improving plant growth within these systems. Furthermore, a recent study has shown that ectomycorrhizal hyphae provide networks along which *Pseudomonas* are "farmed" (Pion et al., 2013), thus the observed increase in ectomycorrhizal biomass may be linked to the increases witnessed in the Gram-negative bacteria (Table 3).

The biomass of a number of Gram-negative bacterial groups was found to increase significantly (16:1ω9c; 16:1ω7c; 18:1ω9c, 18:1ω7c; cy17:0; cy19:0). Gram-negative bacteria have been associated with a number of plant growth-promoting attributes, such as the inclusion of groups responsible for siderophore production (pseudomonas) as well as sulfur oxidizing bacteria (*Acidithiobacillus*) (Scher and Baker, 1982); (Stamford et al., 2008), all of which assist in nutrient cycling and plant growth. In addition to the observed increases in Gram-negative bacteria, there were also significant increases within the Gram-positive bacterial groups (i14:0; i15:0; a15:0; i16:0; i17:0; a17:0), under the *A. nepalensis* trees. Gram-positive groups include *Bacillus*,*Brevibacterium*, *Sarcina* and *Paenibacillus*, all of which are known to have high phytase activity, which allows these bacteria greater access to P sources that would otherwise be unavailable to the affiliated plants, and contribute to overall nutrient cycling (Jorquera et al., 2008; Vazquez et al., 2000). Furthermore, we also recorded increased levels of actinomycetes (10Me16:0, 10me17:0) in the soils surrounding *A. nepalensis*. These findings are congruent with those of past studies, which compared the soil communities associated with *A. rubra* to other tree species, and found that the soils surrounding the *A. rubra* trees hosted higher amounts of actinomycetes (Golinska and Dahm, 2011; Prescott and Grayston, 2013). Apart from their N-fixing ability (*Frankia*), actinomycetes have also been associated with phosphate solubilization, plant growth promotion, and bacterial and fungal pathogen resistance. These factors were associated with improved plant growth and above ground productivity in previous studies (Francis et al., 2010; Hamdali et al., 2008).

3.5. Changes across the soil depths

The abundance of the PLFAs varied significantly in response to the soil depths and sites (Fig. 3 a and b). The greatest number of PLFAs were located in the top soil layer (0–15 cm) for both the monoculture and agroforestry plots (Fig. 3a). Terminally branched, saturated PLFAs (i14:0, i15:0, a15:0, 16:0, i16:0, 17:0), representative of Gram-positive bacteria, and middle-chain branched saturated PLFAs (10Me16:0, 10Me18:0), representing the actinomycetes, had loading weight values larger than 0.8. The highest value was achieved by the PLFA 18:0 (Fig. 3b). The biomass of soil fungi was significantly greater in soils under agroforestry at a

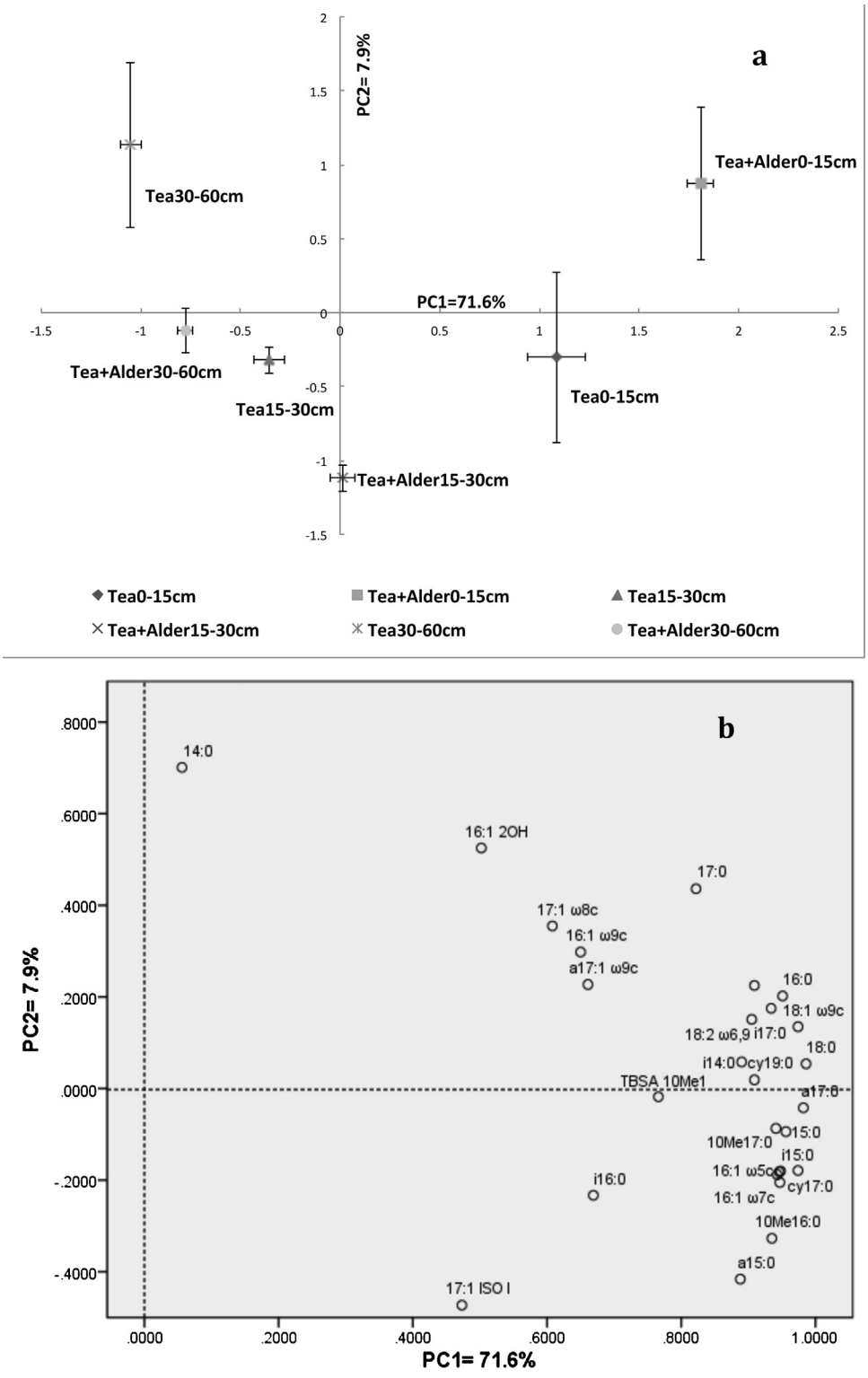

Fig. 3. Principle component analysis (PCA) (a) of the distribution of the PLFA profiles, across the different soil depths (0–15, 15–30, 30–60 cm), and eigenvector loading of PLFAs (b) contributing to the microbial communities ordination patterns.

depth of 0–15 cm, bacteria biomass greater at all depths, and the biomass of actinomycetes was greater at 15–30 cm and 30–60 cm (Table 4). This variation in microbial abundance according to soil depth, including a decreasing trend in biomass in relation to increasing soil depth, is supported by past studies on this topic (Liu et al., 2013).

No changes were observed in the ratio of total fungi to total bacteria, between the monoculture and agroforestry sites, at all soil depths (Table 4). Past studies have indicated that increased levels of soil organic matter (SOM) are associated with a higher ratio of fungi to bacteria, which may explain the lack of differences in the SOM between monoculture and agroforestry soils in this study (Jastrow et al., 2007; Six et al., 2006).

Table 3
The total amount of PLFAs, total bacterial, Gram-positive bacterial (GP), Gram-negative bacterial (GN), actinomycetes, and fungal PLFAs, as well as the ratio of GP/GN and fungi/bacteria under the monoculture (tea) or agroforestry (tea + alder) systems ($n = 3$). Values followed by the same letter within a functional group and soil depth are not significantly different at $p \leq 0.05$.

Functional group	Sites	0–15 cm		15–30 cm		30–60 cm	
		PLFA	*p*-value	PLFA	*p*-value	PLFA	*p*-value
Total PLFAs (nmol g^{-1})	Tea	20.27	0.05	15.68	0.004**	8.27	0.002**
	Tea + alder	25.61		20.25		10.78	
General bacteria (nmol g^{-1})	Tea	30.59	0.017*	10.07	0.002**	5.31	0.002**
	Tea + alder	39.48		13.19		7.17	
GP (nmol g^{-1})	Tea	10.68	0.036*	5.79	0.007**	2.36	0.006**
	Tea + alder	11.37		7.36		4.31	
GN (nmol g^{-1})	Tea	9.27	0.02*	4.56	0.005**	2.05	<0.001***
	Tea + alder	11.45		6.05		2.87	
GP/GN	Tea	1.15	0.06	1.28	0.507	0.24	0.146
	Tea + alder	1		1.22		0.52	
Fungi (nmol g^{-1})	Tea	3.3	0.001**	1.32	0.155	0.84	0.955
	Tea + alder	4.65		1.68		0.83	
Actinomycetes (nmol g^{-1})	Tea	2.79	0.069	1.59	0.004**	0.34	0.015*
	Tea + alder	3.12		2.07		1.1	
Fungi/bacteria	Tea	0.11	0.312	0.13	0.81	0.16	0.185
	Tea + alder	0.12		0.13		0.12	

* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

Table 4
The Shannon index (H') for the monoculture (tea) or agroforestry (tea + alder) plots ($n = 3$), and the three different soil depths (0–15, 15–30, 30–60 cm). Values followed by the same letter are not significantly different at $p \leq 0.05$.

Soil sample	PLFA	
	H'	SE
Tea 0–15 cm	2.83a	0.066
Tea + alder 0–15 cm	2.83a	0.035
Tea 15–30 cm	2.75ab	0.083
Tea + alder 15–30 cm	2.66ab	0.03
Tea 30–60 cm	2.51b	0.106
Tea + alder 30–60 cm	2.69ab	0.023

4. Conclusions

Tea production significantly increased as a result of planting *A. nepalensis* in the tea plantations. The mean yield of the agroforestry systems was 65% higher than in monoculture plantations. Thus, these agroforestry systems are capable of maintaining higher plant productivity, supporting the growth of the trees as well as increased tea growth, in similar soils. The lack of significant differences in soil nutritional status under both monoculture and agroforestry plots suggests that differences observed may be biologically driven as a result of increased microbial biomass in the soils under agroforestry. A clear increase in the presence of certain functional groups known to aid plant growth, nutrient cycling, and disease resistance provide evidence for this. Therefore, the incorporation of *A. nepalensis* into tea plantations can improve above ground productivity and below ground dynamics that ultimately results in greater tea yield and profitability. This data contributes to the growing list of studies that recommend the use of this N-fixing tree species as an economically viable choice for agroforestry systems, yet provides novel insight into how this occurs.

Promoting diverse agroforestry systems including tree species with different functional traits has been highlighted as a strategy to foster the sustained provision of soil-mediated ecosystem services (Barrios et al., 2012). Further studies should explore ways to minimize competition and enhance complementarities through spatial arrangements and management of the agroforestry tree species and associated crops. These studies should account for biomass production, nutrient and water use efficiency, and how these in turn influence the abundance, diversity, and activity of key soil biota.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.apsoil.2015.05.012.

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