LETTER

Biodiversity and phytochemical quality in indigenous and state-supported tea management systems of Yunnan, China

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Abstract

The Chinese government initiated one of the world's largest conservation programs involving agricultural ecosystems with the implementation of the 'Grain for Green' (*Tui Geng Huan Lin*) forest policy between 1999 and 2003. This is the first study to systematically quantify multiple dimensions of biodiversity, phytochemical quality and economic benefits associated with (1) the Grain for Green's tea (*Camellia sinensis*; Theaceae) initiative; (2) the state's previous forest policy involving tea populations in protected areas and; (3) the indigenous tea agro-ecosystems replaced or overlooked by this conservation program. There are several novel and unexpected findings. While forest populations contained the greatest ecological diversity, agro-forests and mixed crop plots were associated with the greatest genetic diversity, phytochemical quality and economic benefits. Indigenous management practices should be incorporated into conservation in China in order to create policies that are more aligned towards biodiversity conservation and sustainable livelihoods while allowing local communities to maintain their cultural identity through agrarian practices.

Introduction

The Chinese government initiated one of the world's largest conservation programs involving agricultural ecosystems with the implementation of the "Grain for Green" (*Tui Geng Huan Lin*) forest policy between 1999 and 2003. The Grain for Green program sought to increase forest cover on sloped cropland in order to improve watershed conditions, prevent soil erosion and conserve natural resources (Yan-qiong *et al*. 2003). The state offered subsidies to smallholder farmers to convert certain land uses, such as indigenous agro-ecosystems, to permanent cash cropping schemes classified as forest or 'green' cover. These cash cropping schemes included fast-growing fruit trees, rubber and tea among other plants. Over 30 million smallholder households in more than 27 thousand villages participated in the Grain for Green program by planting an area greater than 22 million hectares of trees and crops (Uchida *et al*. 2003; Liu *et al*. 2008; Xu 2011). Despite the large scale of the initiative, little or no monitoring was undertaken to track changes in the landscapes.

This is the first study to systematically quantify multiple dimensions of biodiversity, phytochemical quality and economic benefits associated with 1) the Grain for Green's tea (*Camellia sinensis* (L.) O. Kuntze; Theaceae) initiative in southwestern Yunnan Province, China; 2) the state's previous forest policy involving tea populations in protected areas and; 3) the indigenous tea agro-ecosystems replaced or overlooked by this conservation program. Biodiversity was measured in tea forest populations, agro-forests, mixed crop fields and terrace gardens on the basis of ecological and genetic diversity. Phytochemical profiles were measured as an indication of tea quality that ultimately impact consumer buying decisions, market prices and farmer income (Ahmed *et al*. 2010a). Phytochemical content of crops has been largely left out of the conservation literature and our study highlights the importance of considering this characteristic when reconciling management practices between biodiversity conservation and crop production. Lastly, we highlight the economic benefits of the various systems.

Yunnan's rich biological and cultural diversity and extensive management history of tea resources, coupled with China's rapid socio-economic change, provide a compelling case study to assess the relationship of agrarian management and conservation. There are over 17,000 vascular plant species in Yunnan (Wu 2006). The province encompasses the center of diversity of the tea plant, including twelve wild relatives (section *Thea* of *Camellia*) and hundreds of cultivars (Ming & Zhang 1996; Xiao & Li 2002; Long *et al*. 2003). Several of the twenty-six state-designated socio-linguistic groups of Yunnan, including those in the Indo-Tibetan and Mon-Khmer language families, have managed tea in montane forests for hundreds of years or more. These indigenous tea management systems are linked to cultural identity, livelihoods and wellbeing of communities (Ahmed *et al*. 2010b).

Forest tea populations consist of uncultivated tea plants sparsely distributed in forests or those once cultivated and now feral and able to interbreed with uncultivated tea plants. Tea plants in forests grow to 15 m or higher and may live for over one hundred years (Xiao & Li 2002). Tea agro-forests are areas where forests have been thinned or where old swidden plots have undergone succession. Agro-forests have a multi-storied vegetative structure with a high canopy layer, mid-level tree layer of tea plants and herbaceous ground layer (Long & Wang 1996). Tea plants in agro-forests are pruned to spread out branch formation. Mixed crop tea fields are created in swidden areas or in tea agro-forests by replacing associated woody plants with grains and maintaining tea plants as shrubs. In contrast to these forest and indigenous tea systems, terrace tea gardens are open fields managed for uniformity, high-yield and efficiency where tea plants are cultivated in compact rows and pruned to waist-high shrubs. This management model usually relies on agrochemical inputs.

Methods

Study sites

We conducted surveys in three study sites (Figure 1) located in evergreen broadleaf montane forest between 1,600 and 2,000 m in elevation in southern Yunnan Province of Southwestern China in the Indo-Burma biodiversity hotspot (Dijk *et al*. 1999). Study sites include: (1) a forest tea population in Fengshuiling National Nature Reserve in Honghe Prefecture that is harvested by Yao, Yi, and Hmong, (2) an Akha community in Xishuangbanna Prefecture that manages tea agro-forests and, (3) a mixed Lahu and Han community in Lincang Prefecture that manages mixed-crop tea fields. Since 2002, these latter two communities have also managed tea terraces as part of the Grain for Green program (terrace site I and terrace site II).

Farmers at the study sites manage 10 to 30 land-use types in integrated landscape mosaics of forests, fields and home gardens for subsistence farming and foraging, and increasingly for commercialization in national and global markets. The population of each study community is 400–500 inhabitants in family units of 70–85 households that consist of two to four generations. Each community has some specific knowledge and practices associated with their tea management systems but all have the same tea harvest seasons.

Ecological sampling

We sampled twenty 20 m × 20 m plots of forest and indigenous tea systems consisting of five forest plots, five agro-forest plots and five mixed crop field plots. Comparisons were made with four 20 m × 20 m plots of tea terraces managed for the Grain for Green program including two plots near the agro-forest site (terrace site I) and two plots near the mixed-crop fields (terrace site II). No terrace tea gardens were located in neighboring areas to the forest populations for comparison.

The spatial pattern of vegetation in each plot was measured for all tea trees $\geq$1.0 m tall and all associated woody species $\geq$5.0 cm DBH (diameter at breast height). Individual plants of all plant species within the plots were mapped, measured for height and diameter and recorded for local name and use. A horizontal transect of 20 m × 1 m was run through the center of each plot to measure canopy coverage with a spherical densiometer at intervals of 2 m (Lemmon 1956).

Genetic diversity

Genetic differentiation of tea plants within sample plots was measured using amplified fragment length polymorphism (AFLP) based DNA fingerprinting as described by Vos *et al*. (1995). Leaf samples were collected in triplicate from five tea plants from each plot and dried. Genomic DNA was extracted using an SDS/NaCl extraction buffer with 0.2% beta mercaptoethanol. Samples

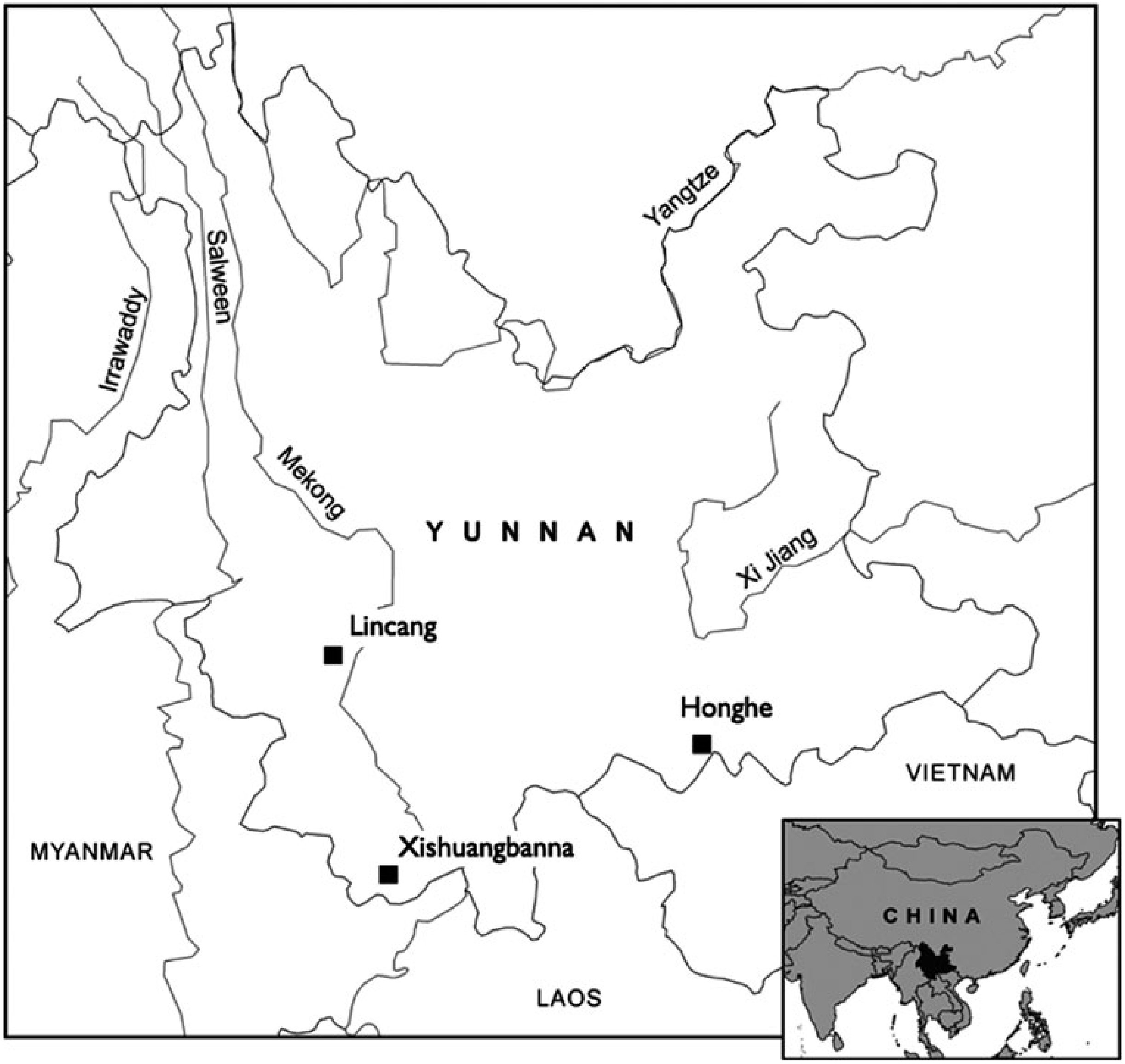

Figure 1 Map of study sites in Xishuangbanna, Lincang, and Honge Prefectures of southern Yunnan Province, southwestern China. Study sites are located in evergreen broadleaf montane forest between 1,600 and 2,000 meters in an area part of the Indo-Burma biodiversity hotspot.

of 300 ng of isolated DNA were digested with MseI and EcoRI restriction endonucleases. Ligation, preselective polymerase chain reaction (PCR) and selective PCR was performed according to Zerega *et al*. (2002) using six primer combinations. Selective D4 dye-labeled (Sigma-Aldrich Corp. St. Louis, MO, USA) primers were based on the EcoRI core sequence with base extensions AGT and GTG. Three unlabeled selective primers were based on the MseI core sequence with base extensions CAA, CGA and CTA. Fragments were analyzed from 3.75 μL of the selective PCR product using a CEQ 8800 (Beckman/Coulter, Brea, CA, USA).

Phytochemical quality

We measured eleven phytochemicals responsible for tea quality including health claims and flavor that ultimately impact consumer buying decisions, market prices and farmer income. Study analytes include eight catechins (epigallocatechin 3-gallate (EGCG), epigallocatechin (EGC), catechin (C), epicatechin (EC), epicatechin 3-gallate (ECG), gallocatechin 3-gallate (GCG), gallocatechin (GC), catechin 3-gallate (CG)) and three methylxanthines (caffeine (CAF), theobromine (TB), and theophylline (TP)). Seventy-five leaves from various positions on tea plants surveyed for genetic analysis were collected for phytochemistry on the basis of preliminary morphological analysis to determine an appropriate sample size. Leaves were preserved as described by Gulati *et al*. (2003). Dried ground leaves were extracted with 80% aqueous methanol (1 g: 10 mL) in a sonicator for 30 min. The supernatant was filtered under vacuum, partially evaporated under reduced pressure, frozen overnight and lyophilized. The dried extract (10 mg) was reconstituted in 1 ml 80% aqueous methanol, centrifuged at 15,000 rpm for 15 min and filtered. High Performance Liquid Chromatography with a photodiode array detector (HPLC-PDA) was performed as described by Unachukwu *et al*. (2010).

Economic benefits

Ten household structured interviews were conducted at each study site for a total of 30 interviews to determine economic benefits associated with each type of tea management system.

Data analysis

We calculated Importance Values (IVs) as the sum of relative density, frequency and dominance (Kent & Coker 1992) to rank species within each plot. Shannon–Weiner and Simpson indices values were calculated to describe plant species alpha-biodiversity (cf. Magurran 2004). We scored AFLP fragment data prior to statistical analysis with GeneMarker software (SoftGenetics, State College, PA, USA). Total Catechin Content (TCC; expressed as mg/g dry tea) and Total Methylxanthine Content (TMC) was calculated from HPLC data by the addition of individual amounts of catechin and methylxanthine compounds. JMP 7.0 software (SAS) was used for statistical analyses (significance level of $\alpha = 0.05\%$).

Results

Ecological sampling

Analysis of Variance (ANOVA) of woody species height, DBH and over-story density showed significant variation of means ($P < 0.0001$) on the basis of type of tea management system (Figure 2). Tukey–Kramer pairwise comparisons of all pairs of tea management systems showed significant variation of means ($P < 0.0001$) for woody species height, DBH and over-story coverage except between terrace site I and terrace site II. Tea forests had the greatest mean plant height (7.53 m ± 4.28), DBH (17.75 cm ± 17.72) and over-story coverage (93.01% ± 2.16) followed by agro-forests (3.93 m ± 3.34 mean height; 13.31 cm ± 10.02 mean DBH; 34.95% ± 5.99 over-story density) and mixed crop fields (2.24 m ± 2.12 mean height; 7.46 cm ± 6.70 mean DBH; over-story coverage 25.83% ± 17.89). Terraces contained the lowest mean height (1.02 m ± 0.13 for terrace site I and 0.70 m ± 0.39 for terrace site II) with no DBH and over-story coverage.

Mean Shannon-Weiner Index ($H' = -\Sigma_i[p_i{}^*\ln(p_i)]$) values of plant species in forest, agro-forest, and mixed crop plots were 2.24 ± 0.55, 0.49 ± 0.22, and 0.17 ± 0.24, respectively. Mean Simpson Dominance Index ($D = 1 - \Sigma_i\ p_i{}^*\ p_i$) values were 0.85 ± 0.12, 0.21 ± 0.10, and 0.30 ± 0.41, respectively. *Camellia sinensis* var. *assamica* was the dominant species in two out of five forest plots with Importance Values (IVs) ranging from 7.4% to 32.4%. Forest plots contained four other *Camellia* species locally used similarly to tea and as breeding sources for adding diverse traits in tea systems including *Camellia crassicolumna*, *Camellia taliensis*, *Camellia tsaii* and *Camellia caudata*. Other dominant species in the forests with IVs greater than 10% included *Acer oblongum* (Sapindaceae), *Castanopsis platyacantha* (Fagaceae), *Dalbergia henryana* (Fabaceae), *Quercus glauca* (Fagaceae), *Eurya groffii* (Pentaphylacaceae), *Eurya loquaiana* (Pentaphylacaceae), *Ilex godajam* (Aquifoliaceae), *Radermachera pentandra* (Bignoniaceae), and *Viburnum pyramidatum* (Adoxaceae). *Camellia sinensis* var. *assamica* was the dominant species in all agro-forests with IVs from 43.96% to 74.67%. Other dominant species in agro-forests include *Calophyllum polyanthum* (Calophyllaceae), *Docynia delavayi* (Rosaceae), *Erythrina indica* (Fabaceae), *Koelreuteria bipinnata* (Sapindaceae), *Prunus majestica* (Rosaceae), and *Schefflera* sp. (Araliaceae). In mixed crop fields, *C. sinensis* var. *assamica* was the dominant species in four out of five plots with IVs from 43.95% to 100%. *Pinus kesiya* var. *langbianensis* (Pinaceae) had the highest IV in one of the mixed crop plots. In terraces, *Camellia sinensis* var. *assamica* was the only species.

Associated woody plant species inventoried in forests and agro-forests had multiple local uses for medicine, food, construction material, dye, fodder, fuelwood, ritual, and tools. Medicine and food were the most documented uses in forest and agro-forest plots. Twenty-seven percent of species were used medicinally in forest plots and 20% in agro-forest plots. Twenty-one percent of species were used for food in forest plots and 60% in agro-forest plots. Documented medicinal uses included treatments for gastro-intestinal, skin and respiratory conditions.

Genetic diversity

Dendrogram analysis did not distinctly cluster tea samples on the basis of type of tea management system and

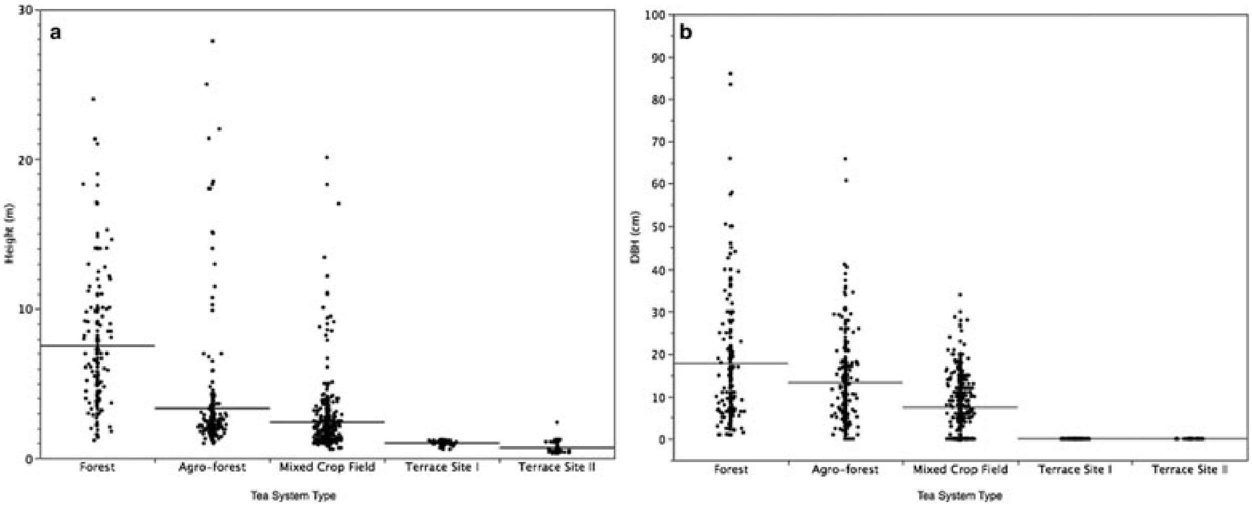

Figure 2 Analysis of Variance (ANOVA) for vegetative structure characteristics of (a) plant height and (b) diameter breast height (DBH) showed significant variation of means ($P < 0.0001$) of plants tallied ($n = 1197$) within sample plots on the basis of type of tea management system. Tukey–Kramer pairwise comparison of all pairs of tea management systems showed significant variation of means ($P < 0.0001$) for plant height and DBH except between terrace site I and terrace site II. Horizontal bar lines represent mean values for each management system.

suggests that tea from the study populations are not genetically distinct. A K-means cluster plot of polymorphisms (Figure 3) showed that mixed crop tea samples have the greatest dispersion around their means as indicated by their confidence ellipses and suggests the greatest genetic diversity of these tea samples. Tea samples from terraces showed the least dispersion of genetic diversity based on distance between plot points. Confidence ellipses for forest and agro-forest samples showed overlap on the K-means cluster plot, indicating closer genetic similarity of these samples compared to samples from other systems. Alternatively, confidence ellipses for terrace and mixed crop samples showed the greatest distance between any two groups and points to the greatest genetic differentiation between these tea sample groups.

Phytochemical quality

Phytochemical analyses found that samples from agro-forests and mixed crop fields had greater TCC (addition of the individual amounts of 8 catechin compounds) and TMC (addition of the individual amounts of 3 methylxanthine compounds) compared to samples from forests and terraces (Figure 4). ANOVA of TCC and TMC showed significant variation of means ($P < 0.0001$) on the basis of type of tea management system. Tukey–Kramer pairwise comparison for mean TCC for each pair of tea management systems revealed statistically significant differences ($P < 0.0001$) except between terrace site I and terrace site II.

Economic benefits

Producers estimated tea harvest yields in the following order: terrace site I (64,000 kg per ha) > mixed crop plots (18,000 kg per ha) > terrace site II (16,000 kg per ha) > agro-forests (13,500 kg per ha) > forests (6,000 kg per ha). Forests had the lowest amount of tea harvested due to state harvesting restrictions based on an annual limit. Terrace site I had the highest yields but farmers received the lowest income from these systems because of the perception by consumers that this tea is of lower quality. Average income received by farmers per kilo of dried tea in spring 2010 was in the following order: agro-forests ($219.65 USD) > mixed crop fields ($157.26 USD) > forests ($12.10 USD) > terrace site II ($8.53 USD) > terrace site I ($3.52 USD).

Discussion

Ecological sampling

Findings provide evidence of ecological simplification with increased density of tea cultivation that has occurred with a shift from indigenous tea management to the state-supported terrace gardens. This ecological simplification reflects trends of landscape simplification occurring regionally as well as globally in response to state conservation and development projects (Fox 2009; Zeigler *et al.* 2009). The multiple plant species and use in forest and agro-forest plots is concordant with previous research in Yunnan's indigenous tea systems (Long & Wang

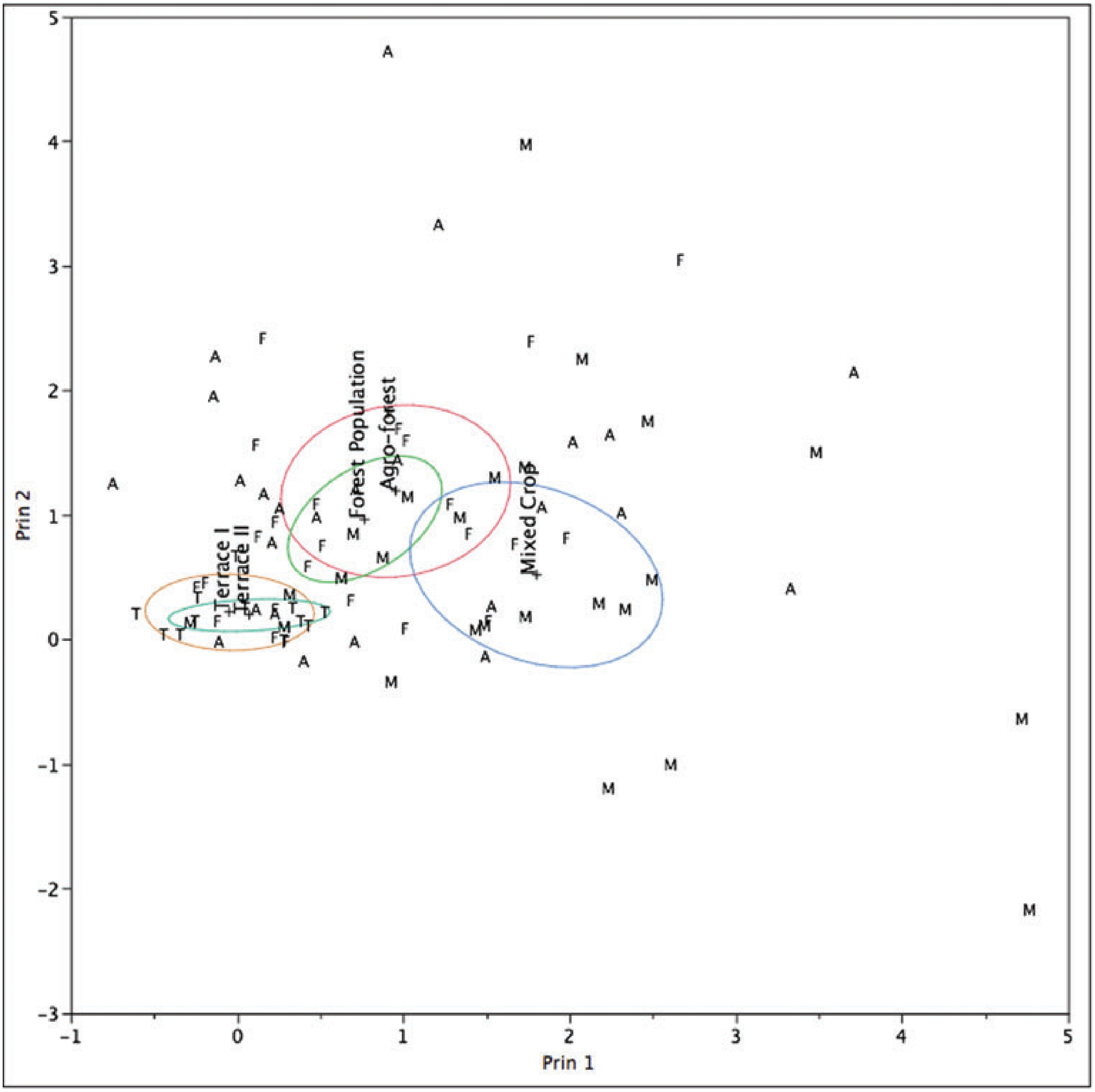

Figure 3 K-means cluster plot of AFLP data matrices for samples from forests (F), agro-forests (A), mixed crop fields (M), and terrace sites I and II (T). The distance between two points indicates genetic relatedness on the basis of polymorphisms. The ellipses represent the means confidence level interval of each group of points for each type of management system.

1996; Qi *et al*. 2005; Sturgeon 2005). Numerous studies have found that indigenous agro-ecosystems are valuable models for biodiversity conservation and contribute to environmental services, economic risk reduction, food security and human health (Padoch and Peters 1993; Brush and Meng 1998; Potvin *et al*. 2005). In some cases, indigenous agro-ecosystems have been shown to harbor greater biodiversity than state-supported agro-ecosystems of the same crop, such as shade-grown coffee systems in Mexico (Moguel and Toledo 1999). The maintenance of agro-ecosystems with diverse vegetative composition, structure and phenotypes is relevant for conservation and development because these systems provide many of the same environmental services and habitats as forests (Schroth *et al*. 2004; Jose 2009; Liu *et al*. 2011) while meeting livelihood and food security needs.

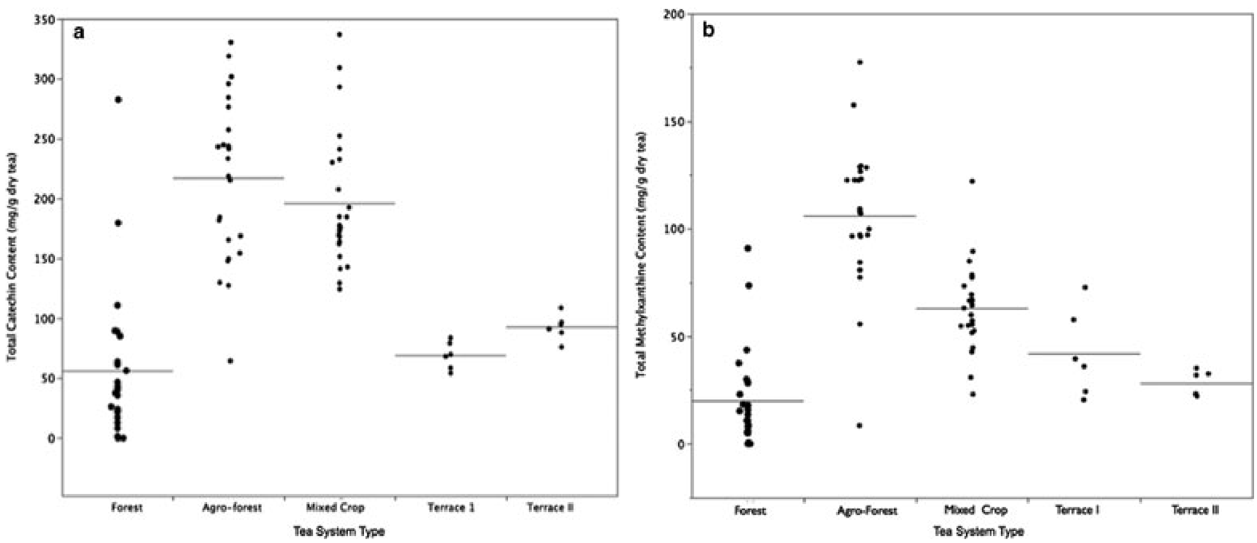

Figure 4 ANOVA of (a) Total Catechin Content (TCC) and (b) Total Methylxanthine Content (TMC) showed significant variation of means ($P < 0.0001$) within each type of tea management system. Tukey–Kramer pairwise comparison for mean TCC for each pair of tea management systems revealed statistically significant differences among management systems ($P < 0.0001$) except between terrace site I and terrace site II.

Genetic diversity

Findings provide evidence for genetic simplification that has occurred with a shift from indigenous tea management to the state-supported terrace gardens and reflect the trend of monoculture production that characterizes global food production. The genetic diversity found in indigenous tea systems should be conserved in situ for its ecological, cultural and commercial values. The lack of genetic distinction between tea populations found in this study is in line with previous tea studies employing AFLP and isozyme analyses showing greater genetic diversity within populations than between populations (Paul *et al*. 1997; Chen *et al*. 2005). However, results deviate from some studies that sampled from a wider geographic range in other regions where tea populations were distinctly clustered (Rajashekaran 1997; Balasaravanan *et al*. 2003). The lack of genetic distinctiveness of tea populations in the present study is possibly the result of greater germplasm exchange in the study area compared to studies sampling from a wider geographic area. It is common practice for farmers at the study sites to exchange tea germplasm between communities and to experiment and select germplasm with desired traits.

Phytochemical quality

The greater phytochemical variation from agro-forest and mixed crop plots is relevant for conservation. This is because not only plant species richness, but also variation in genetic and trait diversity influences the delivery of many essential ecosystem services (Cardinale *et al*. 2012). As tea samples from different sites were not genetically distinct, the significant variation found in phytochemical profiles between tea systems suggests influences of the biophysical environment and farmer management and selection practices. Previous studies have highlighted the role of environmental and human influences on plant characters (Simpson and Ogorzaly 2001; Venkatesha *et al*. 2010) including variation of phytochemical content resulting from variable environmental stress factors (Coley & Barone 2001). The higher phytochemical content in tea agro-forests and mixed crop fields is associated with higher tea quality and consumer preferences (Ahmed *et al*. 2010a). Previous research in coffee agro-forests and open coffee fields found the former to have greater phytochemical content that was associated with reduced sensory quality (Bosselmann *et al*. 2009).

Economic benefits

Smallholders' decisions to participate in conservation programs are determined by multiple variables, with market-incentives as a key driver to invest in biodiversity (Pascual & Perrings 2007). Markets offer farmers of tea agro-forests greater economic incentives for maintaining canopy cover and plant species richness than China's national Grain for Green program. While the highest yields were from Grain for Green terraces, these systems were associated with the lowest quality tea and smallest economic benefits for producers. Farmers were offered annual grain subsidies to participate in this program, but these economic incentives were less than market benefits for managing tea agro-forests. Farmers managing agro-forests receive price premiums through niche market networks that value "organic" production without agrochemicals and value specific phytochemical taste profiles; this price premium is not offered for tea from the state-initiated terrace tea gardens (Menzies 2008).

Conservation and policy implications

State-protected forest tea populations had the greatest ecological diversity of systems examined, while indigenous tea management systems contained the greatest genetic diversity, phytochemical quality and economic benefits for farmers. If economic incentives for managing tea agro-forests and mixed crop plots continue, policy is needed to prevent conversion of forests into more intensified systems while also encouraging the restoration of terrace fields into agro-forests. China's scientific and policy guidelines have been criticized for their "one-size-fits-all" management targets that fail to adequately address the country's diversity of landscapes and ecosystems (Xu 2011). Conservation policy founded on such a framework also fails to adequately address the diversity of indigenous agrarian practices that interact with and manage diverse landscapes and ecosystems. The growing recognition of the inter-relationship between biological and cultural diversity suggests that effective conservation policy should focus on ways to promote both (Stepp *et al*. 2005). The incorporation of both diverse ecosystems and indigenous management in conservation frameworks could result in policies that are more aligned towards biodiversity conservation and sustainable livelihoods while allowing communities to maintain their cultural identity through agrarian practices.

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