# Genotype and slope position control on the availability of soil soluble organic nitrogen in tea plantations

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Abstract Soluble organic nitrogen (SON) plays a vital role in ecosystem N cycling processes and is controlled by a number of biotic and abiotic factors. We compared soil SON availability, microbial biomass, protease and asparaginase activities and phospholipids fatty acid (PLFA) profiles at the 0-15 and 15-30 cm layers in 10 year old tea plantations of two genotypes—Oolong tea (Camellia sinensis (L.) O. Kuntze cv. Huangjingui) (designated as 'OT') and Green tea (C. sinensis (L.) O. Kuntze cv. Fuyun 6) (designated as 'GT')—established at different slope positions. Concentrations of soil SON measured by the 2 M KCl extraction under the OT plantation were greater than under the GT plantation, while concentrations of soil SON were greater in the middle slope (MS) and lower slope (LS) positions than in the upper slope (US) position. Trends in soil microbial biomass C and N and protease and asparaginase activities between the two genotypes and across the slope positions were similar to the SON pools. The fungal-to-bacterial ratios were higher in the US position than in the MS and LS positions and higher under the GT plantation than under the OT plantation. Results from this study support that the genotype and the slope position are key factors controlling the availability of soil SON in tea plantations and also imply the importance of plant traits (e.g. litter quantity and chemistry) and soil texture in determining overall soil N availability and transformation processes and microbial community composition at the landscape

Keywords Soil soluble organic nitrogen (SON) ·

Camellia sinensis (L.) O. Kuntze ·

Genotype · Slope position ·

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# Introduction

Soluble organic nitrogen (SON), along with inorganic N (i.e. NH<sub>4</sub><sup>+</sup>–N, NO<sub>3</sub><sup>-</sup>–N), has been considered to play a vital role in ecosystem processes and to be combined with inorganic N to form a more comprehensive index for soil N availability in terrestrial



ecosystems (e.g. Chen and Xu 2008). The SON also represents major inputs of N to surface water in forest watersheds and affects regional water quality (e.g. Qualls and Richardson 2003). Due to its significance in plant N nutrition and environmental quality, the availability, nature, dynamics and ecological function of soil SON have attracted much attention among soil scientists and ecologists (Murphy et al. 2000; Jones et al. 2004; Chen and Xu 2008). In the past decade, most studies have focused on the availability of SON pools in forest, grassland, vineyard and other arable soils (e.g. Murphy et al. 2000; Jones et al. 2004; Chen et al. 2005a, b; Christou et al. 2006); information on soil SON availability and associated microbial processes in tea orchards is scant. Soil SON is greatly affected by a number of biotic and abiotic factors, such as soil type, plant species, management regimes, land-use change, environmental conditions and analytical methods used (e.g. Kalbitz et al. 2000; Smolander and Kitunen 2002; Chen et al. 2005b; Ros et al. 2009). The variations in soil SON have been attributed to the organic matter inputs of different quantity and quality and the extent of disturbance (Burton et al. 2007; Chen and Xu 2008), and the production and transformation of soil SON is microbially mediated (e.g. Chen and Xu 2008).

Tea (Camellia sinensis L. O. Kuntze) is one of the most important beverage crops in the world. Green, Oolong and black teas are the most common types. Green tea is produced by drying and roasting and black tea after drying and fermentation, while Oolong tea is somewhere between green and black tea in oxidation. In recent years, massive tea plantations have been established in subtropical China to meet the demand from domestic and overseas markets. In Fujian province alone, the area of tea plantations reached 189,000 ha in 2008, consisting of 11.8% of total tea plantations in China. Most of the newly established tea plantations in Fujian province (ca. 50,000 ha established over the period 2003–2008) were in erosion-prone hill-country areas. A large amount of N fertilizer (including inorganic N and manure) had been applied in these intensively managed tea plantations in order to obtain both higher quality and output of tea. However, heavy N fertilization, topographical condition (i.e. steep slope) and high rainfall in this area may potentially lead to low N use efficiency and the enhanced transfer of N SON) from land to (particularly

watersheds due to soil erosion, surface runoff and leaching, thus affecting water quality.

Slope position is a key topographic factor influencing microclimate, species composition and ecosystem functions in many terrestrial ecosystems (McNab 1993; Barnes et al. 1998; Burke et al. 1999; Hook and Burke 2000; Sariyildiz et al. 2005; Xu and Wan 2008). It has been suggested that slope position affects soil particle distribution, soil temperature and moisture, and C and nutrient cycling processes in grasslands (e.g. Turner et al. 1997; Burke et al. 1999; Hook and Burke 2000; Xu and Wan 2008) and forestlands (e.g. Tateno and Takeda 2003; Sariyildiz et al. 2005). Effects of soil properties on plant growth and function and species composition have been well documented (e.g. Clark and Tilman 2008; van der Heijden et al. 2008; Xu et al. 2009). However, plant species can also feed back to soil C and nutrient cycling processes via nutrient use efficiency, quantity and quality of organic inputs, nutrient availability, preference of specific types of nutrients, specialized timing of nutrient uptake and soil-microbe interactions (e.g. Chapman et al. 2005; Carrera et al. 2009). It has been suggested that different genotypes of the same plant species influence substrate availability through inputs of leaf and root litters of different quantity and quality, and may control soil microbial community structure and associated nutrient cycling processes (Kasurinen et al. 2005; Madritch and Cardinale 2007; Schweitzer et al. 2008a, b). Different genotypes of tea cultivars vary with physiological processes and nutrient uptake and stocks (Fernández-Cáceres et al. 2001; De Costa et al. 2007; Kamau et al. 2008) and may respond differently to nutrient supply (Kamau et al. 2008). However, little is known about the effects of slope position and genotype on soil SON availability and the role of microbial processes in the transformation of SON in tea ecosystems. Improved understanding of the source, availability and dynamics of soil SON is critical for adopting appropriate management regimes to minimize ecosystem N loss, sustain tea plantations and reduce potential N pollution in the associated watersheds. The major objective of this study was to examine impacts of tea plantations of different genotypes (Oolong and Green teas) and topographic condition (slope position) on soil SON availability and the potential biological mechanisms involved. In this study, it was hypothesized that: (1)



different genotypes of tea cultivars affect the availability of soil SON directly through the inputs of root litters of varying quantity and quality (e.g. plant chemistry) and indirectly through influencing soil microbial community composition and activity and thus the availability of soil SON; and (2) different slope positions influence the soil texture and the movement of carbon and nutrients and thus availability of soil SON. To our knowledge, this work represents the first report on the soil SON availability and associated microbial processes in tea ecosystems.

# Materials and methods

Site description and sample collection

Two adjacent tea plantations, established at the Research Station of Tea Research Institute (27°13′S, 119°34′E), Fujian Academy of Agricultural Sciences, Fujian Province, China, were selected for this study. The mean annual rainfall and temperature at this site are 1646 mm and 19.3°C, respectively. The landform of the sites belongs to hilly mountainous terrain and the parent material is slope and residual deposits weathering from tuff. The soil type is a Typic Alliti-Udic Ferrosols (Soil Survey Staff 1999). The research sites were located on two adjacent slopes facing in the same direction (east) to the sun, with an average slope of 20°. The two slopes were developed into terrace land with the width of about 3-4 m. The area of each site measures 0.4 ha in which one was planted with Oolong tea (C. sinensis (L.) O. Kuntze cv. Huangjingui) (designated as 'OT') and the other was planted with Green tea (C. sinensis (L.) O. Kuntze cv. Fuyun 6) (designated as 'GT'). A buffer area of 50 m was kept between the OT and GT sites to avoid the edge effect. The splitplot design was adopted for this particular study, with two major plots (i.e. OT and GT plots) and three secondary plots (i.e. upper, middle and lower slope positions). Each of the secondary plots had three  $15 \times 10 \text{ m}^2$  replicate plots with an interval of 3 m between each plot as a buffer area. The OT cultivar Huangjingui was bred through the sole plant selection from Anxi tea plants at Anxi, Fujian, while the GT cultivar Fuyun 6 was a hybrid of Fuding Dabai x Yunnan Daye at Fuan, Fujian. These two cultivars, using inter-simple sequence repeat (ISSR) and target region amplification polymorphism (TRAP) techniques, have been found to be genetically different and belong to different genotypic groups (Guo et al. 2008). Both Fuyun 6 and Huangjingui are among the most widely grown tea cultivars in tropical and subtropical China due to their high yield, top quality and ease in management. Fuyun 6 buds earlier in spring and has larger and thicker leaves compared with Huangjingui.

Both OT and GT plantations were managed using conventional cultivation techniques. In brief, 4000 tea seedlings per hectare, with a spacing of  $1.3 \text{ m} \times 0.3 \text{ m}$ , were planted in March 1998 after application of base fertilizers at a rate of 4.5 t ha<sup>-1</sup> manure and 0.75 t ha<sup>-1</sup> calcium superphosphate. Tea plants were pruned three to four times during the first three years of the growing period, and plucking occurred three times per year in April, July and October once the coverage of tea canopy was higher than 60%. Three instances of inter-tillage with a depth of 10–15 cm, combined with three applications of additional fertilizer, were conducted in March, June and August each year; the application rate of additional fertilizer [inorganic complex fertilizer  $(N:P_2O_5:K_2O = 20\%:8\%:8\%)]$  were 3.0 t ha<sup>-1</sup> yr<sup>-1</sup>, spread over March (50%), June (20%) and August (30%). Furthermore, a deep-tillage with a depth of 20-25 cm was carried out in winter every 3 years.

Fifteen soil cores were randomly collected from each plot of these two tea plantations at two depths (0-15 and 15-30 cm) in May 2008, using a 7.5 cm diameter auger and bulked. All samples were transported on ice to the laboratory where field moist soils were well mixed and passed through a 2 mm sieve and stored at 4°C prior to analyses of soil SON, microbial biomass, enzyme activity and PLFA profiling. These analyses were carried out within a week after sampling. A subsample of each soil was taken and air-dried at room temperature for analysis of basic soil chemical and physical properties (e.g. pH, CEC, total C and N and texture, etc.). The samples of fine roots (<2 mm) were collected from these cores at the same time, washed with distilled water and ovendried at 60-70°C. The samples of leaf litters (the L layer) were also randomly collected from each plot and oven-dried at 60-70°C. The F and H layers were not visible due to rapid decomposition at high temperature and rainfall in this subtropical area. Root and leaf litter samples were finely ground for analysis



of total C and N and the solid state <sup>13</sup>C nuclear magnetic resonance (NMR) spectroscopy.

Analysis of soil properties and solid state <sup>13</sup>C NMR spectroscopy of leaf litter and roots

Total C (TC) and N (TN) of soils, leaf litters and roots were analyzed using an isotope ratio mass spectrometer with a Eurovector Elemental Analyser (Isoprime-EuroEA3000, Milan, Italy). Soil CEC, pH (soil:water 1:2.5) and particle size composition were measured using the methods described by Rayment and Higginson (1992).

Solid-state <sup>13</sup>C CPMAS NMR spectra of the tea leaf and root samples were obtained at a frequency of 100.6 MHz on a Varian Unity Inova 400 spectrometer (Varian Inc., CA). Samples were packed in a silicon nitride rotor (OD = 7 mm) and spun at 5 kHz at the magic angle. Single contact times of 2 ms were applied, with an acquisition time of 13.6 ms, and a recycle delay of 2.5 s. Approximately 9000 transients were collected for all samples and a Lorentzian line broadening function of 20 Hz was applied to all spectra. Chemical shift values were referenced internally to the di-O-alkyl peak which was adjusted to 105 ppm. The solid-state <sup>13</sup>C CPMAS NMR spectra were divided into the 4 common chemical shift regions: alkyl C (0-50 ppm), O-alkyl C (50-110 ppm), aromatic and olefinic C (110–160 ppm) and carboxyl C (160-210 ppm), and the relative intensity for each region was determined by integration using the Varian NMR software package (Version 6.1c, Varian Inc., CA). The A/O-A ratio, the ratio of alkyl C region intensity (0-50 ppm) to O-alkyl C region intensity (50-110 ppm), which has been used as an index of the extent of decomposition (Baldock and Preston 1995) or of substrate quality for microbes (Webster et al. 2000), was also calculated in this study as an indicator of the quality of soil organic C.

Analysis of soil soluble organic N and C

The KCl extracts were obtained by mixing 5 g (dry weight equivalent) of field moist soils with 50 ml of 2 M KCl, shaking on an end-to-end shaker for 1 h and filtering through a Whatman 42 paper followed by a 0.45  $\mu$ m filter membrane. Water and hot water extraction were also carried out for comparison with the KCl extraction using the methods described by

Chen et al. (2005a). Concentrations of  $\mathrm{NH_4}^+-\mathrm{N}$  and  $\mathrm{NO_3}^--\mathrm{N}$  in the extracts were measured using a LACHAT Quickchem Automated Ion Analyser (QuikChem Method 10-107-06-04-D for  $\mathrm{NH_4}^+-\mathrm{N}$  and QuikChem Method 12-107-04-1-B for  $\mathrm{NO_3}^--\mathrm{N}$ ). The  $\mathrm{NO_2}^--\mathrm{N}$  in the extracts was below the detection limit and therefore no values were reported. Soluble organic carbon (SOC) and total soluble N (TSN) in the extracts were analyzed by the high temperature catalytic oxidation method using SHIMADZU TOC analyzer (fitted with TN unit) as described by Chen et al. (2005a). The SON in the different extracts was calculated as the difference between TSN and the sum of  $\mathrm{NH_4}^+-\mathrm{N}$  and  $\mathrm{NO_3}^--\mathrm{N}$ .

Analysis of soil microbial biomass and enzyme activities

Soil microbial biomass C (MBC) and N (MBN) were measured by the chloroform fumigation-extraction method using an Ec factor of 2.64 (Vance et al. 1987) and an En factor of 2.22 (Brookes et al. 1985). Soluble organic C and total soluble N in the  $K_2SO_4$  extracts of the fumigated and unfumigated soil samples were determined by the high temperature catalytic oxidation method using SHIMADZU TOC analyzer (fitted with TN unit) (Chen et al. 2005a). MBC (MBN) = {soluble organic C(N) in the fumigated sample - soluble organic C(N) in the unfumigated sample}/Ec (En). The activities of soil protease and L-asparaginase were estimated using the methods by Ladd and Bulter (1972) and Frankenberger and Tabatabaim (1991), respectively.

Analysis of phospholipid fatty acid and microbial community composition in soil

Soil phospholipid fatty acid (PLFA) was extracted and measured using the modified methods described by Frostegärd et al. (1993) and Kourtev et al. (2002). In brief, 10 g (dry weight equivalent) of field moist soil was extracted by 20 ml of 0.2 M KOH–methanol in a hot water bath for 1 h at 37°C, and then 3 ml of 1.0 M acetic acid was added to the extract to neutralize pH and 10 ml of n-hexane to dissolve PLFA into organic phase. After separating the extracts into two phases at 2000 rpm for 15 min, the n-hexane phase of the extracts was transferred into a conical beaker and dried under a stream of  $N_2$ 



gas. The PLFA in the conical beaker was fully dissolved with 1 ml of 1:1 (v/v) n-hexane and methyltert-butyl ether for 3-5 min, and then transferred into a GC bottle. The resulting PLFA and mixed guide sample of fatty acid methyl ester were parallel analyzed on an Agilent 6890 N gas chromatograph under the following chromatographic condition: column temperature was increased by two-stage program, beginning at 170°C, increasing at 5°C min<sup>-1</sup> to 260°C and then increasing at 40°C min<sup>-1</sup> to the final temperature of 310°C which was kept constant for 90 s. The temperature of vaporizing chamber and detector were 250 and 300°C, respectively. The carrier and makeup gases  $(2 \text{ ml min}^{-1})$ hvdrogen and nitrogen (30 ml min<sup>-1</sup>), respectively. The column inlet pressure was 10.00 psi. The sampling volume was 1  $\mu$ l with split ratio of 100:1. The amounts of individual PLFA were expressed as mol% of total PLFA. The amount of bacteria in soils was estimated from the sum of percentages of the following PLFAs: i15:0, a15:0, 15:0 3OH, i16:0, a16:0, 16:1 2OH, 16:1ω5c, 10Me 16:0,  $16:1\omega$ 7c, i17:0, a17:0, 10Me 17:0,  $18:1\omega$ 7c and cy19:0 $\omega$ 8c (Frostegärd et al. 1993; Frostegärd and Bääth 1996; Klamer and Baath 1998; Fritze et al. 2000; Wilkinson et al. 2002; Bääth and Anderson 2003; Marhan et al. 2007). The PLFAs i15:0, a15:0, i16:0, i17:0, a17:0, 10Me 16:0 and 10Me 17:0 represent Gram positive bacteria, while  $16:1\omega 5c$ ,  $16:1\omega 7c$ ,  $18:1\omega 7c$  and  $cy 19:0\omega 8c$  represent Gram negative bacteria (O'Leary and Wilkinson, 1988; Frostegärd et al. 1993; Frostegärd and Bääth 1996). The sum of percentages of  $18:2\omega6,9$ ,  $18:1\omega9c$ and  $18:3\omega6c$  (6, 9, 12) was considered to represent the percentage of fungi (Frostegärd and Bääth 1996; Zelles 1997; Karlinski et al. 2007; Marhan et al. 2007). The percentages of actinomycetes were estimated from the percentages of 10Me 18:0 (Frostegärd and Bääth 1996; Klamer and Baath 1998).

# Statistical analysis

Analysis of split plot design with two factors (main factor, genotype of cultivars; secondary factor, slope position) was performed on basic soil properties, SON pools, microbial biomass, enzyme activities, and PLFA profiling data using Statistica Version 6.1 (Statsoft, Inc.). Least significant difference (LSD, P < 0.05) was used to separate the means when

differences were significant. Pearson linear correlations between SON pools, soil microbial biomass C and N, total soil N and enzyme activity were also conducted in Statistica Version 6.1 (Statsoft, Inc.). Data (mol%) on the PLFA profile were log-transformed [log (n + 1)] and were subject to principal component analysis (PCA) using Statistica Version 6.1 (Statsoft, Inc.).

#### Results

Properties of soil and leaf litters and roots in tea plantations of different genotypes at different slope positions

Soil sand and silt contents in the US and MS positions were higher than or equivalent to those in the LS position in both depths (0–15 and 15–30 cm), while the clay contents were greater in the LS position than in the US and MS positions (Table 1). This indicated that the soil was derived from slope and residual deposits and the finer particles moved down the slope during the soil forming processes. There were no significant differences in the particle size composition in the soils under the OT and GT plantations (Table 1), further indicating the soils had developed from the same parent materials. Soil pH values (1:2.5 soil: $H_2O$ ) were similar in the soils (4.2–4.3) under the OT plantation across the slope positions at both depths, while the pH values were lower in the MS position (3.4) than the US and LS positions (3.9–4.3) under the GT plantation (Table 1). Soil pH values were significantly higher under the OT plantation than under the GT plantation (Table 1). Soil moisture contents were higher in the LS position than the US and MS positions at both depths except for the 15-30 cm layer under the GT plantation (Table 1). Soil moisture contents were markedly lower under the OT plantation than under the GT plantation at both depths (Table 1). Soil CEC was higher in the MS and LS positions than in the US position, while soil CEC was higher under the OT plantation than under the GT plantation at both depths (Table 1). Soil TC and TN contents were higher in the MS and LS positions than in the US position at both depths except for TN in the 15-30 cm layer under the OT plantation (Table 1). This indicated that soil organic matter tended to accumulate at the lower position of slope in



Table 1 Selected soil properties under adjacent Oolong tea and Green tea plantations in subtropical China

| Soil property                | Oolong tea | a     |        |       | Green tea |        |        |       |  |
|------------------------------|------------|-------|--------|-------|-----------|--------|--------|-------|--|
|                              | US         | MS    | LS     | Mean  | US        | MS     | LS     | Mean  |  |
| 0–15 cm                      |            |       |        |       |           |        |        |       |  |
| Sand (%)                     | 12.9a      | 13.7a | 10.8b  | 12.5a | 10.4b     | 12.5a  | 10.1b  | 11.0a |  |
| Silt (%)                     | 40.5a      | 42.1a | 41.0a  | 41.2a | 46.0a     | 40.7b  | 41.2ab | 42.6a |  |
| Clay (%)                     | 46.6ab     | 44.2b | 48.2a  | 46.4a | 43.6b     | 46.9ab | 48.7a  | 46.4a |  |
| pН                           | 4.3a       | 4.2a  | 4.3a   | 4.2a  | 3.9b      | 3.4c   | 4.3a   | 3.8b  |  |
| Soil moisture (%)            | 6.2c       | 7.4b  | 12.5a  | 8.7b  | 15.0b     | 16.0ab | 17.3a  | 16.1a |  |
| CEC (cmol kg <sup>-1</sup> ) | 5.6b       | 6.4a  | 6.0ab  | 6.0a  | 4.1b      | 6.0a   | 6.1a   | 5.4b  |  |
| $TC (g kg^{-1})$             | 10.7b      | 12.8a | 11.9a  | 11.8a | 7.4b      | 12.2a  | 12.6a  | 10.7b |  |
| $TN (g kg^{-1})$             | 0.57b      | 0.62a | 0.59ab | 0.59a | 0.47c     | 0.57b  | 0.62a  | 0.55a |  |
| C:N ratio                    | 18.7b      | 20.6a | 19.9ab | 19.8a | 15.7b     | 21.3a  | 20.3a  | 19.1a |  |
| 15-30 cm                     |            |       |        |       |           |        |        |       |  |
| Sand (%)                     | 13.1a      | 13.9a | 10.7b  | 12.5a | 11.7a     | 11.2a  | 10.9a  | 11.2a |  |
| Silt (%)                     | 40.1a      | 41.0a | 40.1a  | 40.4a | 43.6a     | 41.1ab | 40.4b  | 41.7a |  |
| Clay (%)                     | 46.8ab     | 45.1b | 49.3a  | 47.1a | 44.8b     | 47.7ab | 48.7a  | 47.1a |  |
| pН                           | 4.2a       | 4.2a  | 4.3a   | 4.3a  | 4.0a      | 3.4b   | 4.2a   | 3.9b  |  |
| Soil moisture (%)            | 8.9b       | 7.8b  | 11.0a  | 9.2b  | 17.6a     | 17.0a  | 17.2a  | 17.3a |  |
| CEC (cmol kg <sup>-1</sup> ) | 4.6a       | 5.1a  | 4.9a   | 4.9a  | 3.6b      | 4.7a   | 4.2ab  | 4.2b  |  |
| $TC (g kg^{-1})$             | 9.3b       | 10.6a | 9.9ab  | 9.9a  | 5.8b      | 8.9a   | 8.6a   | 7.8b  |  |
| $TN (g kg^{-1})$             | 0.48ab     | 0.55a | 0.47b  | 0.51a | 0.37b     | 0.44ab | 049a   | 0.43a |  |
| C:N ratio                    | 19.2a      | 19.2a | 20.9a  | 19.8a | 15.9b     | 20.5a  | 17.7ab | 18.0b |  |

both OT and GT plantations due to the leaching and runoff of organic matter from the upper to the lower positions of slope. Soil TC and TN contents were higher under the OT plantation than under the GT plantation (Table 1). Soil C:N ratio was higher in the MS and LS positions than in the US position at both depths except for no differences observed in the C:N ratio at the 15–30 cm layer across the slopes under the OT plantation (Table 1). There was no significant difference in the soil C:N ratio in the 0–15 cm depth between the OT and GT plantations, but the C:N ratio was greater in the 15–30 cm soil under the OT plantation than under the GT plantation (Table 1).

The C content in the leaf litter of OT (41.3%) was slightly higher than that of GT (39.8%), but the N content was markedly lower in the leaf litter of OT (1.85%) than that of GT (3.15%) (Table 2). The

similar trends were found in roots between the OT and GT plantations. The C:N ratios were higher in both leaf litters and roots of OT (22.3-60.1) than those of GT (12.6–34.7) (Table 2). Results from <sup>13</sup>C CPMAS NMR spectroscopy showed that the composition of C functional groups was not greatly different in leaf litters between the OT and GT plantations (Table 2). However, the proportions of relatively recalcitrant C groups (e.g. Alkyl C, aromatic and olefinic and carboxyl C) in the root samples of the OT plantation were lower than those of the GT plantation, while the proportion of the labile C group (e.g. O-alkyl C) was greater in the root samples of the OT plantation than those of the GT plantation. The A/O-A ratio was lower in both leaf litters and roots of the OT plantation than those of the GT plantation (Table 2).



**Table 2** Composition of carbon (C) functional groups in leaf litters and roots of adjacent Oolong tea and Green tea plantations as characterized by <sup>13</sup>C CPMAS NMR spectroscopy

| Tea<br>plantation | C (%) | N (%) | C:N ratio | Alkyl C (%) | O-Alkyl C (%) | Aromatic and olefinic C (%) | Carboxyl C (%) | A/O-A<br>ratio |
|-------------------|-------|-------|-----------|-------------|---------------|-----------------------------|----------------|----------------|
| Leaf litter       |       |       |           |             |               |                             |                |                |
| Oolong tea        | 41.3  | 1.85  | 22.3      | 29.5        | 44.7          | 15.3                        | 10.5           | 0.660          |
| Green tea         | 39.8  | 3.15  | 12.6      | 30.8        | 43.4          | 15.6                        | 10.2           | 0.710          |
| Root              |       |       |           |             |               |                             |                |                |
| Oolong tea        | 47.8  | 0.80  | 60.1      | 12.9        | 64.7          | 15.5                        | 6.9            | 0.199          |
| Green tea         | 47.9  | 1.38  | 34.7      | 15.6        | 59.6          | 17.0                        | 7.7            | 0.261          |

Data in the column are values of the composite samples. A/O-A ratio is the ratio of alkyl C region intensity (0–50 ppm) to O-alkyl C region intensity (50–110 ppm) and used as an index of the extent of decomposition

Soil SON concentrations under tea plantations of different genotypes at different slope positions

Concentrations of soil SON in KCl extracts (SON<sub>KCl</sub>) ranged from 27.3 to 54.1 mg kg<sup>-1</sup>, comprising 33.5– 86.5% of total soluble N and 4.5-15.0% of total soil N (Table 3). Concentrations of SON<sub>KCl</sub> and SOC<sub>KCl</sub> were significantly higher in the MS and LS positions than in the US position at both depths (0-15 and 15-30 cm) under both the OT and GT plantations, and decreased with soil depth (Table 3). Concentrations of soil SON<sub>KCl</sub> and SOC<sub>KCl</sub> were higher under the OT plantation than under the GT plantation. The SON:SIN ratio [a ratio of SON to soluble inorganic N (the sum of  $NH_4^+$ -N and  $NO_3^-$ -N)] was not consistent among different slope positions at both soil depths under both OT and GT plantations. However, this ratio was significantly higher at both depths under the OT plantation than under the GT plantation (Table 3). The C:N<sub>KCl</sub> ratios were generally lower in the MS and LS positions than in the US position, while significantly higher at both depths under the OT plantation than under the GT plantation.

The NH<sub>4</sub><sup>+</sup>–N is the predominant form among the inorganic N pools except for the MS position under the GT plantation (Table 3). Concentrations of NH<sub>4</sub><sup>+</sup>–N and NO<sub>3</sub><sup>-</sup>–N in KCl extracts were not significantly different among the slope positions except for the MS position at both depths (Table 3). On the other hand, concentrations of NH<sub>4</sub><sup>+</sup>–N and NO<sub>3</sub><sup>-</sup>–N in KCl extracts were generally higher under the GT plantation than under the OT plantation (Table 3).

Both water and hot water extracted less amounts of soluble organic N and C than the KCl extraction,

ranging from 6.5 to 14.7 mg kg $^{-1}$  (39.6%-67.6% of total soluble N and 1.1%-4.3% of total soil N) in water extracts and from 9.6 to 30.9 mg kg $^{-1}$  (40.1–74.6% of total soluble N and 1.4–9.4% of total soil N) in hot water extracts. The patterns in the concentrations of water and hot water extractable organic N and C, NH<sub>4</sub> $^+$ –N and NO<sub>3</sub> $^-$ –N as well as the SON:SIN ratio and SOC:SON ratios across different slope positions and the tea plantations were similar to those for the KCl extractable pools (Data not shown).

Soil microbial biomass and enzyme activities under tea plantations of different genotypes at different slope positions

Concentrations of soil MBC and MBN were higher in the MS and LS positions than in the US position at both depths under both OT and GT plantations, and also higher under the OT plantation than under GT plantation at both depths (Fig. 1). This trend is generally consistent with those of concentrations of soil SON and SOC measured as above in various soil extracts. The trend in the microbial C:N ratio was not consistent across different slope positions, while there was no significant difference in the microbial C:N ratio between the OT and GT plantations (Fig. 1). Soil protease activities were generally higher in the MS and LS positions than in the US position at both depths under both tea plantations (Fig. 2). Soil asparaginase activities showed a similar trend across different slope positions to that for the soil protease activity. Soil protease activities were generally higher in the 0-15 and 15-30 cm layers under the OT plantation than under the GT plantation, respectively, while there was no significant difference in soil



Table 3 Soil soluble inorganic N  $(NH_4^+-N)$  and  $NO_3^--N)$  and organic N  $(SON_{KCl})$  in 2 M KCl extracts under adjacent Oolong tea and Green tea plantations in subtropical China

| Soil soluble N and C                        | Oolong te | a      |        |        | Green tea |        |         |        |  |
|---|-----------|--------|--------|--------|-----------|--------|---------|--------|--|
|   | US        | MS     | LS     | Mean   | US        | MS     | LS      | Mean   |  |
| 0–15 cm                                     |           |        |        |        |           |        |         |        |  |
| $NO_3^-$ -N (mg kg <sup>-1</sup> )          | 0.3a      | 1.1a   | 0.0a   | 0.5b   | 1.3b      | 23.6a  | 0.0b    | 8.3a   |  |
| $NH_4^+ - N \ (mg \ kg^{-1})$               | 10.6a     | 10.6a  | 13.1a  | 11.4b  | 12.0b     | 23.5a  | 15.5b   | 17.0a  |  |
| SON <sub>KCl</sub> a (mg kg <sup>-1</sup> ) | 41.7b     | 52.8a  | 52.6a  | 49.0a  | 30.5b     | 54.1a  | 49.4a   | 44.7a  |  |
| SON:SIN ratiob                              | 4.53a     | 4.16a  | 4.09a  | 4.26a  | 2.68a     | 1.12b  | 3.18a   | 2.23b  |  |
| SOC <sub>KCl</sub> c (mg kg <sup>-1</sup> ) | 460.6b    | 551.1a | 548.1a | 519.9a | 272.6c    | 478.6a | 369.6b  | 373.6b |  |
| C:N <sub>KCl</sub> ratio <sup>d</sup>       | 11.1a     | 10.4a  | 10.4a  | 10.6a  | 8.9a      | 8.8a   | 7.5b    | 8.4b   |  |
| 15-30 cm                                    |           |        |        |        |           |        |         |        |  |
| $NO_3^ N \text{ (mg kg}^{-1}\text{)}$       | 0.5a      | 2.0a   | 0.0a   | 0.8b   | 3.5b      | 13.4a  | 1.2b    | 6.0a   |  |
| $NH_4^+ - N \ (mg \ kg^{-1})$               | 13.0a     | 12.9a  | 10.4a  | 12.1a  | 11.8a     | 14.2a  | 14.4a   | 13.5a  |  |
| SON <sub>KCl</sub> a (mg kg <sup>-1</sup> ) | 36.0b     | 48.2a  | 47.5a  | 43.9a  | 27.3b     | 41.8a  | 37.7a   | 35.6b  |  |
| SON:SIN ratiob                              | 4.20b     | 3.62b  | 4.58a  | 4.13a  | 1.78a     | 0.75b  | 2.81a   | 1.78b  |  |
| $SOC_{KCl}^{c} (mg \ kg^{-1})$              | 442.5b    | 522.4a | 509.8a | 491.6a | 239.3b    | 323.2a | 268.2ab | 276.9b |  |
| C:N <sub>KCl</sub> ratio <sup>d</sup>       | 12.3a     | 10.8b  | 10.7b  | 11.2a  | 8.8a      | 7.7ab  | 7.1b    | 7.8b   |  |

asparaginase activity between the OT and GT plantations. Both soil protease and asparaginase activities decreased with soil depth.

Soil PLFA profile and microbial community composition under tea plantations of different genotypes at different slope positions

Across different slope positions and genotypes of tea cultivars, 17 types of PLFAs were detected in soils (Table 4). The PLFAs 16:00, 18:1ω9c, i15:0, i16:0, i17:0, 18:00 and cy19:0ω8c were predominant accounting for 71.3–73.0% of total PLFA regardless of slope position and genotype of tea cultivars (Data not shown). Among these predominant PLFAs, the PLFAs 16:0 and 18:1ω9c consisted of 23.4–32.8% and 8.7–13.7% of total soil PLFA, respectively. In the 0–15 cm soil, significant differences were observed in the relative abundance of some PLFAs (e.g. 14:0, 16:0, a16:0, 10Me 16:0 and 18:1ω9c) across the slope

positions under the OT plantations, while more PLFAs (e.g. i15:0, a15:0, 16:0, i16:0, a16:0, 16:1 2OH, i17:0, a17:0,  $18:1\omega 9c$ ,  $18:3\omega 6c$  and  $cy 19:0\omega 8c$ ) were significantly different across the slope positions under the GT plantation (Table 4). On the other hand, the variations in the PLFA composition were small between the genotypes of tea cultivars compared with those among the slope positions (Table 4). The relative abundance of the PLFAs 16:0 and  $16:1\omega7c$ were significantly greater in the 0-15 cm soil under the OT plantation than under the GT plantation, while  $18:3\omega$ 6c was lower in the soil at the same depth under the OT plantation than under the GT plantation (Table 4). The trend in the variation of PLFA composition across slope positions and genotypes of tea cultivars in the 15-30 cm depth were similar to that in the 0–15 cm depth (data not shown).

Results from the PCA of PLFA patterns showed that soil samples at the 0–15 cm depth under the OT plantation concentrated in the middle of the plot,



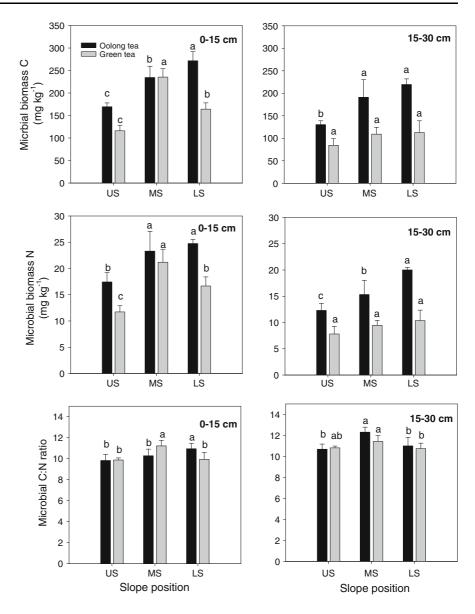
<sup>&</sup>lt;sup>a</sup> SON<sub>KCl</sub>, soluble organic N in 2 M KCl extracts

<sup>&</sup>lt;sup>b</sup> The ratio of SON to SIN (soluble inorganic N, sum of NO<sub>3</sub><sup>-</sup>-N and NH<sub>4</sub><sup>+</sup>-N)

<sup>&</sup>lt;sup>c</sup> SOC<sub>KCl</sub>, soluble organic carbon in 2 M KCl extracts

<sup>&</sup>lt;sup>d</sup> C:N<sub>KCl</sub> ratio, the ratio of SOC to SON in 2 M KCl extracts

Fig. 1 Soil microbial biomass C and N at different slope positions (US upper slope, MS middle slope, LS lower slope) under adjacent Oolong tea and Green tea plantations in subtropical China. Error bars indicate the standard error of the mean (n = 3). Lower case letter indicate statistically significant differences among the slope positions under each tea cultivar

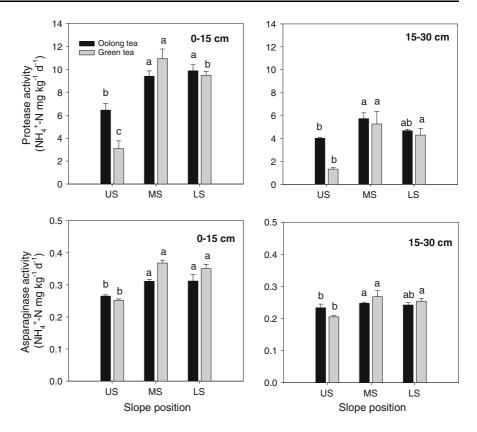


while soil samples under the GT plantation were scattered around the plot (Fig. 3a). This may indicate the samples in the OT plot were more consistent and possibly more influenced by the plant genotype compared to the GT plot. The PLFA patterns in the 0–15 cm soils were not distinct among slope positions under the OT plantation, while samples at the upper slope were well separated from the others at the 0–15 cm depth under the GT plantation (Fig. 3a), indicating that samples in the GT plot were predominantly affected by edaphic factors. The PLFA patterns in the 15–30 cm soils were clearly separated between the OT and GT plots, and more affected by

the genotypic factor than the slope position (Fig. 3c), and within each genotype, the samples at the lower slope position could generally separated from those at middle and upper slopes (Fig. 3c). The sum of PC1 and PC2 accounted for 47.3% of the variation in the PLFA composition for the 0–15 cm layer. The PLFAs i15:0, i16:0,  $16:1\omega$ 5c,  $18:2\omega$ 6,9c, i17:0,  $18:3\omega$ 6c and cy19:0 $\omega$ 8c were responsible for the separation of samples along the PC1 (total contributions to the PC1: 72.2%) at the 0–15 cm depth, while a15:0, a16:0,  $16:1\omega$ 5c,  $18:3\omega$ 6c, 16:0 and cy19:0 $\omega$ 8c along the PC2 at this depth (total contributions to the PC2: 81.4%) (Fig. 3b). In the 15–30 cm soil, samples



Fig. 2 Activities of soil proteases and asparaginases at different slope positions (US upper slope, MS middle slope, LS lower slope) under adjacent Oolong tea and Green tea plantations in subtropical China. Error bars indicate the standard error of the mean (n = 3). Lower case letter indicate statistically significant differences among the slope positions under each tea cultivar



from different genotypes of tea cultivars were better separated from each other along the PC1 and PC2, compared with those in the 0–15 cm soil (Fig. 3c). However, samples collected from different slope positions under both tea plantations were not clearly separated (Fig. 3c). The PLFAs i15:0, a15:0, i16:0, a16:0,  $18:2\omega6,9c$ , i17:0 and cy19:0 $\omega8c$  were most important in separating the 15–30 cm soils of different genotypes (total contributions to the PC1: 81.3%), while a15:0, 16:0, 18:0, a17:0 and  $18:3\omega6c$  made the most important contributions to the separation of the 15–30 cm soil samples of different genotypes along the PC2 (total contributions to the PC2: 74.8%) (Fig. 3d).

The soil microbial community composition in both 0–15 and 15–30 cm layers under the OT and GT plantations was similar, with bacteria being the dominant group followed by fungus (Table 5). Actinomycetes (represented by the PLFA 10Me 18:0) were not detected in any samples, probably due to strong acidity in these soils. There were no significant differences in the abundance of bacteria at both depths across the slope positions and the genotypes of tea cultivars. The relative abundance of Gram

positive bacteria was greater at the 0–15 cm depth in the MS than in the US and LS under the GT plantation, while Gram negative bacteria were greater in the LS than in the US and MS at the 15–30 cm depth under the GT plantation (Table 5). There were no significant differences in Gram positive and Gram negative bacteria across slope positions at both depths under the OT plantation. The relative abundance of fungus was consistently greater in the US than in the MS and LS at both depths under both tea plantations and greater in the soils under the GT plantation than under the OT plantation (Table 5). The fungal-to-bacterial ratio was also higher in the US than in the MS and LS and higher in soils under the GT plantation than under the OT plantation (Table 5).

## Discussion

Effects of tea genotype on soil SON

Concentrations of soil SON in tea plantations from this study (27.3–54.1 mg kg<sup>-1</sup> in KCl extracts, 6.5–14.6 mg kg<sup>-1</sup> in water extracts and 9.6–



**Table 4** Soil PLFA profile in the 0–15 cm layer under adjacent Oolong tea and Green tea plantations in subtropical China

| PLFA fraction | Oolong te | ea (mol%) |       |       | Green tea (mol%) |       |        |       |  |
|---------------|-----------|-----------|-------|-------|------------------|-------|--------|-------|--|
|               | US        | MS        | LS    | Mean  | US               | MS    | LS     | Mean  |  |
| 14:0          | 1.7b      | 1.6b      | 2.3a  | 1.9a  | 1.4a             | 1.2a  | 1.8a   | 1.5a  |  |
| i15:0         | 6.6a      | 6.3a      | 6.5a  | 6.5a  | 5.0b             | 7.2a  | 6.9a   | 6.4a  |  |
| a15:0         | 3.3a      | 3.7a      | 3.6a  | 3.5a  | 4.9a             | 3.5b  | 3.2b   | 3.9a  |  |
| 16:0          | 26.4c     | 32.8a     | 29.7b | 29.7a | 23.4b            | 26.7a | 27.5a  | 25.9b |  |
| i16:0         | 6.0a      | 7.4a      | 7.5a  | 7.0a  | 5.5b             | 8.8a  | 6.3b   | 6.9a  |  |
| a16:0         | 2.3a      | 1.0b      | 1.0b  | 1.4a  | 2.3a             | 0.6b  | 0.0b   | 1.0a  |  |
| 16:1ω5c       | 1.0a      | 1.6a      | 1.4a  | 1.3a  | 2.4a             | 1.9a  | 2.9a   | 2.4a  |  |
| 16:1ω7c       | 1.5a      | 1.5a      | 2.1a  | 1.7a  | 0.0a             | 0.0a  | 0.4a   | 0.1b  |  |
| 10Me 16:0     | 4.7a      | 4.8a      | 2.9b  | 4.2a  | 5.0a             | 3.9a  | 4.5a   | 4.5a  |  |
| 16:1 2OH      | 4.7a      | 2.2a      | 4.1a  | 3.7a  | 4.8a             | 1.4b  | 3.3ab  | 3.2a  |  |
| i17:0         | 6.2a      | 5.7a      | 6.2a  | 6.0a  | 4.b2             | 8.4a  | 5.9b   | 6.2a  |  |
| a17:0         | 2.7a      | 2.6a      | 2.8a  | 2.7a  | 3.3a             | 2.2b  | 2.5b   | 2.7a  |  |
| 18:0          | 5.3a      | 5.4a      | 6.4a  | 5.7a  | 6.2a             | 6.6a  | 6.3a   | 6.4a  |  |
| 18:1ω9c       | 12.7a     | 9.7b      | 8.7b  | 10.4a | 14.6a            | 12.8b | 13.2ab | 13.6a |  |
| 18:2ω6,9c     | 3.5a      | 3.2a      | 3.4a  | 3.4a  | 4.7a             | 3.4a  | 3.3a   | 3.8a  |  |
| 18:3ω6c       | 3.5a      | 3.2a      | 3.2a  | 3.3b  | 5.7a             | 3.5b  | 3.4b   | 4.2a  |  |
| cy19:0ω8c     | 7.7a      | 7.2a      | 8.1a  | 7.7a  | 6.5b             | 7.8a  | 8.4a   | 7.6a  |  |

 $30.9 \text{ mg kg}^{-1}$  in hot water extracts) were comparable to those reported for agricultural and forest soils in literature (see reviews by Murphy et al. 2000; Chen and Xu 2008). Concentrations of soil SON in water, hot water and KCl extracts were significantly related to each other (Fig. 4a,  $R^2 = 0.535-0.584$ , P < 0.01) with an order:  $SON_{KCl} > SON_{hw} > SON_w$ . This indicated the different extraction efficiencies, and these SON pools might overlap to some extent, which was consistent with our previous study in forest plantations (Xing et al. 2010).

It has been reported that soil SON concentrations varied with tree species (e.g. Smolander and Kitunen 2002; Chen et al. 2005b). For example, concentrations of SON in soils under birch (*Betula pendula* Roth.) and Norway spruce (*Picea abies* L.) were higher than under Scots pine (*Pinus sylvestris* L.) (Smolander and Kitunen 2002). However, little is known about the effects of genotypic difference within a species on soil SON availability. Results from this study have shown that concentrations of soil SON and SOC, SOC:SON ratios and SON:SIN ratios

in KCl extracts were significantly higher under the OT plantation than under the GT plantation (Tables 3), indicating significant effects of genotype of tea cultivars. It has been suggested that the majority of SON may be derived from dissolution and decomposition of litters and organic matter in soils, root secretion and debris in forest ecosystems (e.g. Kalbitz et al. 2000; Qualls and Richardson 2003; Zhong and Makeschin 2003). The variation in soil SON pool size among forest species may be attributed to differences in quantity and quality of organic inputs and associated microbial transformation processes, although the factors controlling the size of soil SON pools in forest ecosystems are poorly understood (Smolander and Kitunen 2002; Burton et al. 2007). Compared with forest ecosystems, much lower amounts of leaf litter are annually produced in tea ecosystems due to regular tea plucking. Therefore, root exudation and turnover may play a predominant role in the SON production. Unfortunately, root quantity and turnover under different tea genotypes were not measured in this study, but soil total C and



Fig. 3 a Scores plot of PCA showing the separation of different tea cultivars (OT Oolong tea, GT Green tea) and slope position (US upper slope, MS middle slope, LS lower slope) along principal components (PC) 1 and 2 in the 0-15 cm depth; b loading values of the individual PLFA for PC1 and PC2 in the 0-15 cm depth; c scores plot of PCA showing the separation of different tea cultivars and slope position along principal components (PC) 1 and 2 in the 15-30 cm depth; and d loading values of the individual PLFA for PC1 and PC2 in the 15-30 cm depth

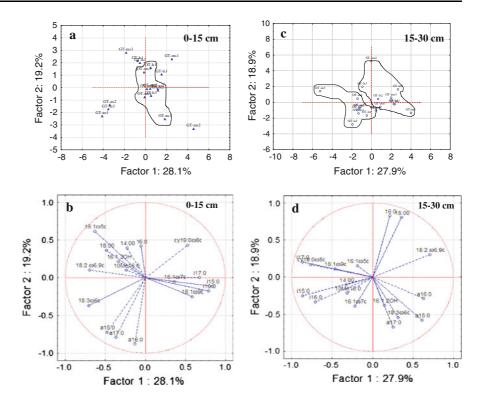


Table 5 Mole percentages (%) of soil microbes under adjacent Oolong tea and Green tea plantations in subtropical China

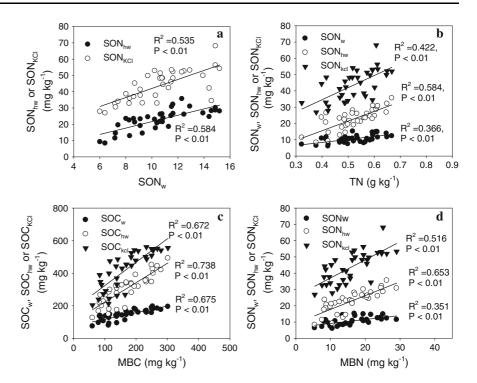
| Microbial community       | Oolong t | ea    |       |       | Green tea |       |       |       |
|---------------------------|----------|-------|-------|-------|-----------|-------|-------|-------|
|                           | US       | MS    | LS    | Mean  | US        | MS    | LS    | Mean  |
| 0–15 cm                   |          |       |       |       |           |       |       |       |
| Bacteria                  | 46.7a    | 45.0a | 46.2a | 46.0a | 43.9a     | 45.7a | 45.3a | 45.0a |
| Gram positive             | 29.5a    | 30.5a | 29.5a | 29.9a | 27.9b     | 34.0a | 29.3b | 30.6a |
| Gram negative             | 10.2a    | 10.3a | 11.6a | 10.7a | 8.9b      | 9.7b  | 11.7a | 10.1a |
| Fungus                    | 19.7a    | 16.1b | 15.4b | 17.0b | 25.1a     | 19.7b | 20.0b | 21.6a |
| Fungal-to-bacterial ratio | 0.42a    | 0.36b | 0.33b | 0.37b | 0.57a     | 0.43b | 0.44b | 0.48a |
| 15-30 cm                  |          |       |       |       |           |       |       |       |
| Bacteria                  | 47.8a    | 47.2a | 47.7a | 47.6a | 44.2a     | 45.6a | 46.0a | 45.3a |
| Gram positive             | 34.9a    | 31.5a | 31.1a | 32.5a | 31.5a     | 30.0a | 31.7a | 31.0a |
| Gram negative             | 9.1a     | 8.3a  | 9.6a  | 9.0a  | 6.1b      | 8.3b  | 11.1a | 8.5a  |
| Fungus                    | 21.2a    | 15.8b | 15.6b | 17.5b | 27.5a     | 18.7b | 18.7b | 21.6a |
| Fungal-to-bacterial ratio | 0.44a    | 0.33b | 0.33b | 0.37b | 0.62a     | 0.41b | 0.41b | 0.48a |

N in both 0–15 cm and 15–30 cm layers were higher under the OT plantation than under the GT plantation (Table 1), probably indicating greater organic matter

inputs from the root system under the former than the latter. Moreover, significant positive relationships between soil total N and SON pools (Fig. 4b)



Fig. 4 Relationships among soil soluble organic N in water ( $SON_w$ ), hot water ( $SON_{hw}$ ) and KCl ( $SON_{KCl}$ ) extracts (a) and their relationships with soil total N (TN) (b) and microbial biomass C (MBC) (c) and N (MBN) (d) under adjacent Oolong tea and Green tea plantations in subtropical China



indicated that higher soil TN contents may contribute to greater SON pools.

Results from <sup>13</sup>C NMR spectroscopy in this study have found lower proportions of relatively recalcitrant C groups (e.g. Alkyl C, aromatic and olefinic and carboxyl C) and a greater proportion of the labile C group (O-alkyl C) in the root samples of the OT plantation than those of the GT plantation (Table 2), indicating that root litters of OT may be more easily decomposable to produce SON than those of GT. On the other hand, the C:N ratio has traditionally been used to indicate the N mineralization rate of organic residues (producing NH<sub>4</sub><sup>+</sup>-N) (e.g. Thomas and Asakawa 1993). Root and leaf litters of the OT plantation contained similar amounts of C but less N with a higher C:N ratio, compared with those of the GT plantation (Table 2), probably indicating lower mineralization potential to produce NH<sub>4</sub><sup>+</sup>-N under the OT plantation than under the GT plantation. This was also consistent with lower levels of inorganic N (both NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N) found in soils under the OT plantation than under the GT plantation (Table 3). In addition, different plant chemistry between the genotypes may have affected soil Ncycling processes. It has been suggested that high concentration of polyphenols shift the dominant pathway of N cycling from mineral to organic form to minimize potential N losses from the ecosystem (e.g. Northup and Dahlgren 1998; Schweitzer et al. 2008b). Polyphenols consist of 20–40% of dry matter in young tea plants (dry weight base) and are considered to be very important to the flavor of tea (Balentine et al. 1997), while information on the role of polyphenols in N cycling in the tea ecosystem is scant. The contents of extractable polyphenols in Oolong (Huangjingui) and Green (Fuyun 6) tea plants were not determined in this study, but in general, the Oolong tea plant has a relatively higher content of extractable ployphenols compared with the Green tea plant (Balentine et al. 1997) although the manufactured Green tea has higher contents of polyphenols compared with Oolong and black teas (Lin et al. 2003). The higher SON contents and SON:SIN ratios in soil under the OT plantation compared with the GT plantation (Table 3) have implied that organic N cycling may have been dominant in the OT plantation. The polyphenol may play a key role in maintaining the organic N cycling in soils under the OT plantation. Further study is warranted to look into the evidence for relationships among the tea plant



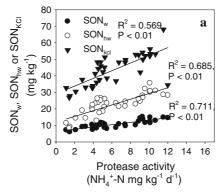
chemistry (e.g. polyphenols) and N form and dynamics in the tea ecosystem.

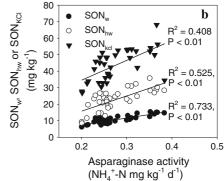
The SON can be directly produced by microbial turnover and indirectly through microbial generation of extra-cellular enzymes (Neff et al. 2003), and also can be mineralized by enzymes and microbes to release NH<sub>4</sub><sup>+</sup>. On the other hand, soil inorganic N (e.g. NH<sub>4</sub><sup>+</sup>) can be immobilized by microbes and the microbial N may thus enter SON pools as amino acids and other organic N by microbial death or damage (e.g. Deluca et al. 1992). Hence, soil microbe community and enzyme activity were considered to be another major factor controlling SON pools in soils (e.g. Kalbitz et al. 2000). Microbial biomass C and N were highly correlated with SON pools in various extracts (Fig. 4c, d), indicating active involvement of microbial processes in controlling the availability of soil SON. The PCA of PLFA fractions showed distinct microbial community compositions of soils under different tea genotypes (Fig. 3). The factor scores of PC1 and PC2 were highly correlated with SON pools  $(R^2 = 0.222 -$ 0.351, P < 0.05, data not shown), further indicating a linkage between SON and microbial community composition. Soil fungal-to-bacterial ratios were higher under the GT plantation than under the OT plantation (Table 5), indicating the shift in microbial community composition as a result of plantation of different tea genotypes. Bacteria mainly decompose relatively simple or low molecular weight organic compounds while fungi mostly decompose more complex or high molecular weight organic substances (e.g. Attiwill and Adams 1993). The increased fungal-to-bacterial ratio in soil under the GT plantation is consistent with the higher proportion of recalcitrant C groups found in root litters under the GT plantation compared with the OT plantation (see above), and is at least partially responsible for the differences in soil SON pools between tea genotypes. Protease and asparaginase are involved in the hydrolysis of complex organic N into peptides and then amino acids in soils. Results from this study showed that concentrations of soil SON were positively related to the activities of protease and asparaginase regardless of extraction methods ( $R^2 = 0.408-0.733$ , P < 0.01) (Fig. 5a, b). Higher protease in soils under the OT plantation than under the GT plantation might have contributed to greater concentrations of soil SON under the former than under the latter (Fig. 2).

# Effects of slope position on soil SON

Effects of slope position on microclimate, soils, plant species composition, community development and site productivity have been well documented (e.g. Barnes et al. 1998). Topographic position and soil texture can affect the landscape-scale variation of C and N (Hook and Burke 2000). Upper slope surfaces are exposed to intense solar radiation, strong wind, and soil movement and are drier than lower slopes (McNab 1993), while the lower slope tends to be sheltered from strong winds and subject to accumulation of organic matter and be moister than upper slope (Sariyildiz et al. 2005). In this study, soil total C and N, moisture and clay contents were higher in the MS and LS positions than in the US position (Table 1), which is consistent with other studies (Hook and Burke 2000; Tateno and Takeda 2003; Sariyildiz et al. 2005). These trends are likely related to the enhanced movement of soil and associated C and nutrients downwards the slope through the leaching and surface runoff due to the high rainfall and steep slope (ca. 20°) at the experiment site in this study.

Fig. 5 Relationships between soil soluble organic N in water (SONw), hot water (SON $_{hw}$ ) and KCl (SON $_{KCl}$ ) extracts and soil protease (a) and asparaginase (b) activities under adjacent Oolong tea and Green tea plantations in subtropical China







Burke et al. (1999) reported that total soil C and N, coarse and fine particulate organic matter C and N, and potentially mineralizable C were significantly affected by topography, with higher levels in the lower slope position than the middle slope position. Hook and Burke (2000) suggested that most lowland plots were enriched in silt, clay, C and N relative to adjacent upland plots. Sariyildiz et al. (2005) also reported that the decomposition rate of forest litters were higher in the lower position than the middle and upper slope. In this study, concentrations of SON and SOC were significantly higher in the MS and LS positions than in the US position regardless of genotypes and extraction methods (Tables 3, 4 and 5). This is in accordance with higher clay, moisture and total C and N contents in the MS and LS positions than those in the US position (Table 1). It has been suggested that soil texture is a key proximal control over biogeochemical processes (Hook and Burke 2000). Soil clay content may be largely responsible for observed topographic differences in soil total C and N, SON and SOC in this study. In addition, soil MBC and MBN and protease and asparaginase activities were greater in the MS and LS positions than the US position, which also contributed to the greater amount of SON and SOC in the MS and LS positions than in the US position. From the PCA of PLFA patterns, in addition to the influence by the genotypic factor, the slope position was also an important factor in determining the pattern in microbial community composition, particularly within each genotype (Fig. 3a, c). Moreover, the shift in microbial community composition with higher fungal-to-bacterial ratios in the US position than in the MS and LS positions regardless of genotypes and depths might also contribute to higher contents of soil SON and SOC in the MS and LS positions than in the US position in this study (see above). This, considering the significant relationships between soil texture and C, N and microbial parameters, may imply the significant role of soil texture in determining microbial community composition at the landscape level.

# Conclusions

It has been clearly demonstrated that the genotype and the slope position are key factors controlling the availability of soil SON in tea plantations. Concentrations of soil SON were generally greater under the OT plantation than under the GT plantation, while concentrations of soil SON were greater in the MS and LS positions than in the US position. Organic matter inputs (mainly root litters) of different quantity and chemistry (e.g. polyphenols, N content) under different genotypes of tea cultivars were largely responsible for the differences in the SON availability. The variation in soil SON availability at different slope positions may be attributed to different physical and chemical environments (clay content, moisture and soil total C and N, etc.) resulting from the downward movement of soil and associated C and nutrients along the slope through the leaching and surface runoff due to the high rainfall and steep slope. Soil microbial biomass, microbial community composition and organic N-related enzyme activities (protease and asparaginase) played a vital role in determining SON availability under different genotypes and at different slope positions. This work confirms that genotypic and topographic factors control the soil N cycling at the landscape level. Further studies should focus on how the quantity of root and leaf litters and plant chemistry affect the SON production, what key functional groups of soil microbial community are involved in the SON transformation and the chemical nature of SON as affected by the genotype and the slope position.

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