J Soils Sediments (2016) 16:405–416
DOI 10.1007/s11368-015-1200-9

SOILS, SEC 2 • GLOBAL CHANGE, ENVIRON RISK ASSESS, SUSTAINABLE LAND USE • RESEARCH ARTICLE

Stoichiometric variation of carbon, nitrogen, and phosphorus in soils and its implication for nutrient limitation in alpine ecosystem of Eastern Tibetan Plateau

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Received: 31 January 2015 / Accepted: 7 July 2015 / Published online: 12 August 2015

Abstract
Purpose The main objectives of this research are to decipher the stoichiometric characteristics of carbon (C), nitrogen (N), and phosphorus (P) in soils from the alpine ecosystem and to obtain information about nutrient limitation on plants and microbes.
Materials and methods The soils were sampled along an altitudinal gradient (2000 to 4300 m above sea level) from the eastern slope of Gongga Mountain in eastern Tibetan Plateau. In total of 102 soil samples in profiles and 27 soil microbial biomass (SMB) samples from five vegetation zones were collected to analyze the concentrations of C, N, and P as well as their ratios. The concentrations of C and N were measured using an automated C/N analyzer, total P was detected by inductively coupled plasma-atomic emission spectrometer, and the concentrations of microbial biomass C, N, and P were measured by the chloroform fumigation-extraction method. Soil P fractions were extracted by modified Hedley sequential extraction method.
Results and discussion The concentrations of C, N, and P in the soils and SMB varied spatially, whereas the variation of their ratios was constrained. The C:N:P ratios were 556:22:1 for the O horizon, 343:16:1 for the A horizon, 154:7:1 for the B horizon, and 63:3:1 for the C horizon, indicating a significant decrease with depth. The mean ratio in the SMB was 51:6.6:1. Microbial biomass C, N, and P were important components of soil nutrients, especially the microbial biomass P which accounted for 40.8 % of soil available P. The C:P and N:P were higher in the soils of broadleaf-coniferous and coniferous forests, whereas the ratios in the SMB were higher in the broadleaf forest. The ratios of C and N to available P in the soils decreased significantly with altitude.
Conclusions The local climate, vegetation succession, and soil development in the high mountain resulted in the soil nutrient cycling different from that in other terrestrial ecosystems. Among the different vegetation zones, the P-limitation of plants and microbial communities might be possible in the soils of lower land forests in the long term.

Keywords Alpine soils · C:N:P stoichiometry · Gongga Mountain · Nutrient limitation · Soil microbial biomass · Vegetation succession

1 Introduction

Whether a similar stoichiometric ratio of carbon (C), nitrogen (N), and phosphorus (P) exists across terrestrial ecosystems has been explored to understand their biogeochemical processes and nutrient limitation (Elser et al. 2000; McGroddy et al. 2004; Mooshammer et al. 2012; Beermann et al. 2015). Unlike marine ecosystems, terrestrial ecosystems are more complex due to the various conditions (e.g., topography, vegetation, human intervene, etc.), and hence, result in a large spatial heterogeneity of biogenic element distribution and their ratios. Aponte et al. (2010) concluded that the season, vegetation type, and soil depth were the main factors affecting the stoichiometry of C, N, and P in the soils of Mediterranean forests. Li et al. (2012) found that the variation of C:N:P ratios

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in the soils of subtropical China was closely related to landscape and land use. The research of Tian et al. (2010) showed that the variations of C:P and N:P in Chinese soils were attributed to different climatic characteristics, soil orders, soil depth, and weathering stages. In contrast, Cleveland and Liptzin (2007) reported a well-constrained C:N:P ratio in soil microbial biomass (SMB). Kirkby et al. (2011) obtained a constant stoichiometric ratio of C, N, and organic P in the stable portion of soil organic materials across a wide range of global soils. These researchers suggested that the stoichiometric ratio of C, N, and P in SMB may be homeostatic, whereas the ratio in soils exhibits a marked spatial heterogeneity in terrestrial ecosystems. To date, there has been limited research concerning the stoichiometry of C, N, and P in alpine ecosystems where the storage of nutrients is increasingly important with global warming and increasing N deposition. Mountain soils are an important pool of nutrients. Due to the hasher climatic conditions at high altitudes, the nutrients in mountain soils are facing high vulnerability, which not only modulates climatic warming through C dynamics in soils (Hagedorn et al. 2010) but also affects ecosystem functioning (e.g., nutrient limitation). Therefore, the research on biogeochemical cycling of C, N, and P in alpine soils is important for local and regional ecological stability.

The stoichiometry of C, N, and P is a powerful tool to decipher their coupling mechanisms and nutrient limitation in terrestrial ecosystems (Aponte et al. 2010; Kirkby et al. 2011; Ågren et al. 2012; Ostrowska and Porębska 2015). Many researchers have attempted to establish the critical value of foliar N:P to reveal nutrient limitation in terrestrial ecosystems (Koerselman and Meuleman 1996; Bennett and Adams 2001; Güsewell 2004), although some debates still exist in species-specific ecosystems (Drenovsky and Richards 2004; Kerkhoff et al. 2005; Craine et al. 2008). The research of potential nutrient limitation by the stoichiometry of C, N, and P in soils of alpine ecosystems is far from complete. The high mountains are characterized by large altitudinal gradients, which form special climate conditions and vegetation distribution. Climate, plant community, and soil development are regarded as major factors controlling soil nutrient availability by changing soil physiochemical properties and litter quality (Binkley et al. 2000; De Kovel et al. 2000; Chen et al. 2008). Furthermore, microbes, an important component in soils, regulate key processes of nutrient cycling through litterfall decomposition, mineralization, and immobilization (Ushio et al. 2010). On the other hand, the development and activity of microbes also respond to nutrient levels in soils as well as the environmental variation (Wan et al. 2015). Therefore, the stoichiometry of C, N, and P in soils and SMB from alpine ecosystems can improve our understanding of the nutrient availability and their potential limitation.

Gongga Mountain (29° 20′–30° 20′ N, 101° 30′–102° 15′ E), with the peak of 7556 m a.s.l. (above sea level), is located at the eastern border of the Tibetan Plateau (Thomas 1999). The eastern slope of Mt. Gongga is characterized by high mountain-deep valley (1100–7556 m a.s.l.), a transitional seasonal climate and typical pristine forests, which offers an ideal area to investigate the stoichiometry of C, N, and P. Previous research found that the soil development on the eastern slope of Mt. Gongga was in the early stage (He and Tang 2008). In the present study, five vegetation zones along an altitudinal gradient (2000–4300 m a.s.l.) were selected on the eastern slope of Mt. Gongga, including broadleaf forest (BLF), broadleaf and coniferous forest (BCF), coniferous forest (CF), shrub, and meadow (Fig. 1). The objectives of this work are (1) to quantify the spatial distribution of C, N, and P concentrations as well as their ratios in the soils and SMB; (2) to identify the possible factors influencing the stoichiometry of C, N, and P in the soils; and (3) to provide evidence for nutrient limitation among different vegetation zones using the C:N:P stoichiometry.

2 Materials and methods

2.1 Study area

Soil sampling was conducted on the eastern slope of Mt. Gongga within the Hailuogou Glacier valley (Fig. 1), where the climate is typical temperate monsoon. The main components of parent materials are residual deposit and slope wash deriving from weathered Cenozoic feldspar granite and Permian quartz schist. There are many debris flow gullies and deposits developed by frequent debris flow (Zhang et al. 2006). The geographical features, meteorology, and vegetation distribution with altitude are summarized in Table 1.

2.2 Field sampling

After preliminary survey on topography, altitude gradients and vegetation, the sampling sites were selected to include all vegetation zones (Table 1). The soil sampling was conducted in September 2010 at nine altitudes: 2032, 2772, 2856, 3090, 3544, 3614, 3896, 4015, and 4221 m a.s.l. (Fig. 1). At each site, three soil profiles were hand-dug until the bedrock was reached. Four soil units were divided according to the primary aspects. The O horizon represents the soils with brown color and decomposition/semi-decomposition organic matter; the A horizon represents the soils with dark brown color and humus; the B horizon represents the soils with illuvial and/or eluvial materials; and the C horizon is the soil parent materials. Due to the heterogeneous development of the soils, the B horizon at the altitude of 2856 m a.s.l. and the O horizon at the altitude of 4221 m a.s.l. were absent. In total, 102 soil samples including 24 samples from the O horizon, 27 samples from the A

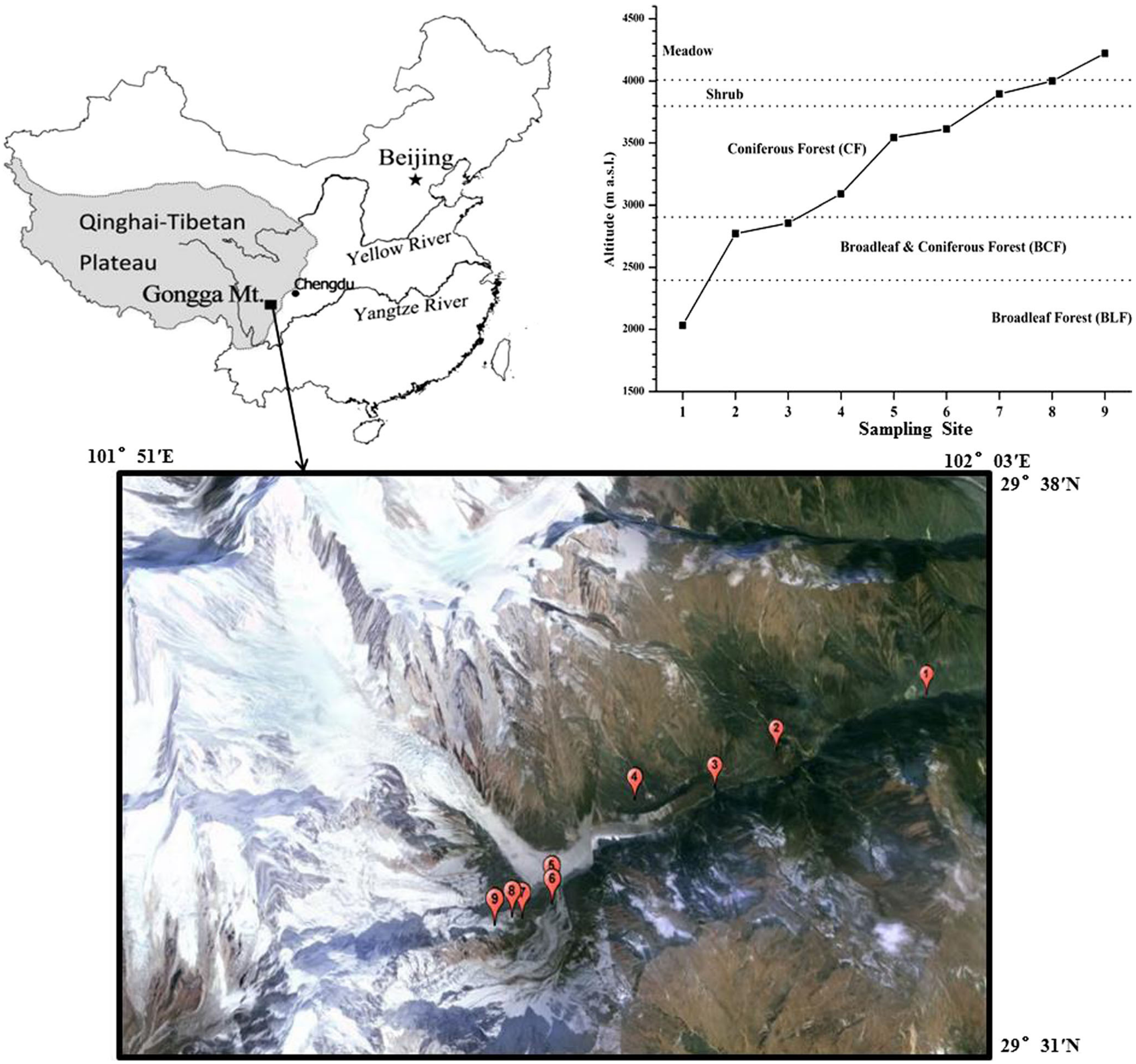

Fig. 1 The study area and the sampling sites on the eastern slope of Mt. Gongga

horizon, 24 samples from the B horizon, and 27 samples from the C horizon were collected.

The soil samples of the SMB were also collected in the five vegetation zones (at the same sites to the bulk soil samples, respectively), and the surface soils (0–15 cm) were used to analyze the concentrations of microbial biomass C, N, and P (MBC, MBN, and MBP). In total, nine sites and 27 soil samples (three replicates at each site) for the SMB analysis were obtained (Fig. 1).

Each soil sample was put into a polyethylene plastic bag and brought to the laboratory in an icebox. The soil samples were sieved to <2 mm to remove the large rocks and plant remains. The wet soil samples for the measurements of MBC, MBN, and MBP as well as soil moisture were immediately treated after the sieving. The other soil samples for physical and chemical analysis were stored at 4 °C, and then air-dried for analysis of the concentrations of soil organic carbon (SOC), N, and P.

2.3 Chemical analysis

The SOC and N concentrations were measured using a FlashEA1112 elemental analyzer linked to a Thermo Delta$^{\mathrm{Plus}}$ Advantage mass spectrometer after the soils were treated by 5 % HCl to remove carbonates. The standard reference material (GSS-11) was used during the

Table 1 Geographic features, meteorology, and vegetation distribution in the study area

Vegetation zone	Altitude (m a.s.l.)	Soil type	Soil pH [a]	Soil moisture (%) [b]	Dominant plants	Annual precipitation (mm)[c]	Annual temperature (°C)[c]
Broadleaf forest (BLF)	<2400	Yellow-brown soil	6.49 (6.20–6.95)	236	Lithocarpus *cleistocarpus* (Seem.) Rehd. Et Wils., Betula *insignis*	1020–1600	7.1–12.8
Broadleaf-coniferous forest (BCF)	2400–2900	Brown soil	4.66 (3.40–5.32)	340	Picea *brachytyla*, Betula *insignis*	1600–1870	4.5–7.1
Coniferous forest (CF)	2900–3800	Dark-brown soil	4.40 (3.30–5.99)	265	Abies *fabri*	1870–1930	0.9–4.5
Shrub	3800–4000	Meadow soil	4.62 (3.88–6.13)	274	Rhododendron *cephalanthum*, R. Phaeochrysum	–	–
Meadow	4000–4600	Meadow soil	4.94 (4.70–5.27)	85.7	Kobresia, Potentilla, Festucaovina	1000	−5.7–0.9

[a] Data were detected in situ. Values in the *brackets* are the ranges of pH, which represents the values of surface soils (0–15 cm)

[b] Soil moisture was measured by drying subsamples (surface soil) overnight at 105 °C. The soil moisture was calculated as the ratio of the weight of water to dry soil

[c] Data from Wu et al. (2013a) and Gao and Peng (1993)

measurement of SOC and N, and the standard deviations were <10 % of the certified value. The soils for analyzing total P concentration were digested with HNO_3-HF-$HClO_4$, and the P concentration was measured using an American Leeman Labs Profile inductively coupled plasma-atomic emission spectrometer. Standard solution SPEX^{TM} from the USA was used as the standard. Quality control was assured by the analysis of duplicate samples, blanks, and reference materials (GSD-9 and GSD-11, Chinese geological reference materials). The precision was good with variability in repeated analysis of samples and reference materials below 5 %. Recovery was 90±6 % (error expressed as 95 % confidence interval) for the reference materials.

The concentrations of MBC, MBN, and MBP were measured by the chloroform fumigation-extraction method (Brookes et al. 1985; Vance et al. 1987). For the measurements of MBC and MBN, 10 g of the moist soil samples were extracted using 40 mL of 0.5 M K_2SO_4 for 30 min by shaking at 300 rpm. Meanwhile, the other soil subsamples (10 g) were fumigated with chloroform for 24 h in a vacuum desiccator, followed by the same extraction procedure as the unfumigated samples. The concentrations of C and N in the extracts were measured by the C/N analyzer. The concentrations of MBC and MBN were estimated as the difference in K_2SO_4-extractable dissolved organic C and N between fumigated and unfumigated soils using the correction factors: K_C=0.45 for C and K_N=0.40 for N (Jonasson et al. 1996; Rinnan et al. 2008). For the measurement of MBP, 5 g of the moist soil samples were extracted by 100 mL of 0.025 N HCl and 0.03 N NH_4F for 30 min by shaking at 300 rpm. The other soil subsamples (5 g) were fumigated with chloroform for 24 h in a vacuum desiccator, followed by the same extraction procedure as the unfumigated samples. The concentration of MBP in the extracts was measured using the Molybdate colorimetric method. It was estimated as the difference of available P between the fumigated and unfumigated soils using a correction factor K_P=0.40 (Brookes et al. 1982).

The soil P fractions in the A horizon were detected by the modified Hedley sequential extraction method (Tiessen and Moir 1993). This method uses a sequence of increasingly strong reagents to successively remove more recalcitrant fractions of inorganic (Pi) and organic (Po) P. The available P is defined as the sum of resin-extractable Pi (resin-Pi), HCO_3^- extractable Pi (HCO_3^- Pi), and HCO_3^- extractable Po (HCO_3^- Po).

2.4 Statistical analysis

One-way ANOVA (Fisher test, $p<0.05$) was used to identify the significant differences in the mean concentrations of C, N, and P and their ratios at each soil horizon among different vegetation zones. In order to discuss the effects of vegetation types on the stoichiometry of C, N and P, the ANOVA was also applied to analyze the altitudinal differences of the stoichiometric ratios of C, N, and P in the soils and SMB. The results showed the same trend as the data compiled based on the form of vegetation zones. Thus, the variation of the stoichiometric ratios of C, N, and P in soils among different vegetation zones was presented in the text (Fig. 2). Linear fit was applied to establish the relationship between elements and their ratios with altitude (or different vegetation zones). All statistical analysis in this study was performed using the software package Origin 8.0 for Windows.

3 Results

3.1 The concentrations and ratios of SOC, N, and P in soils

As shown in Table 2, the concentrations of SOC and N in the soil profiles decreased significantly with depth, whereas the variation of P concentrations was little. Among the vegetation

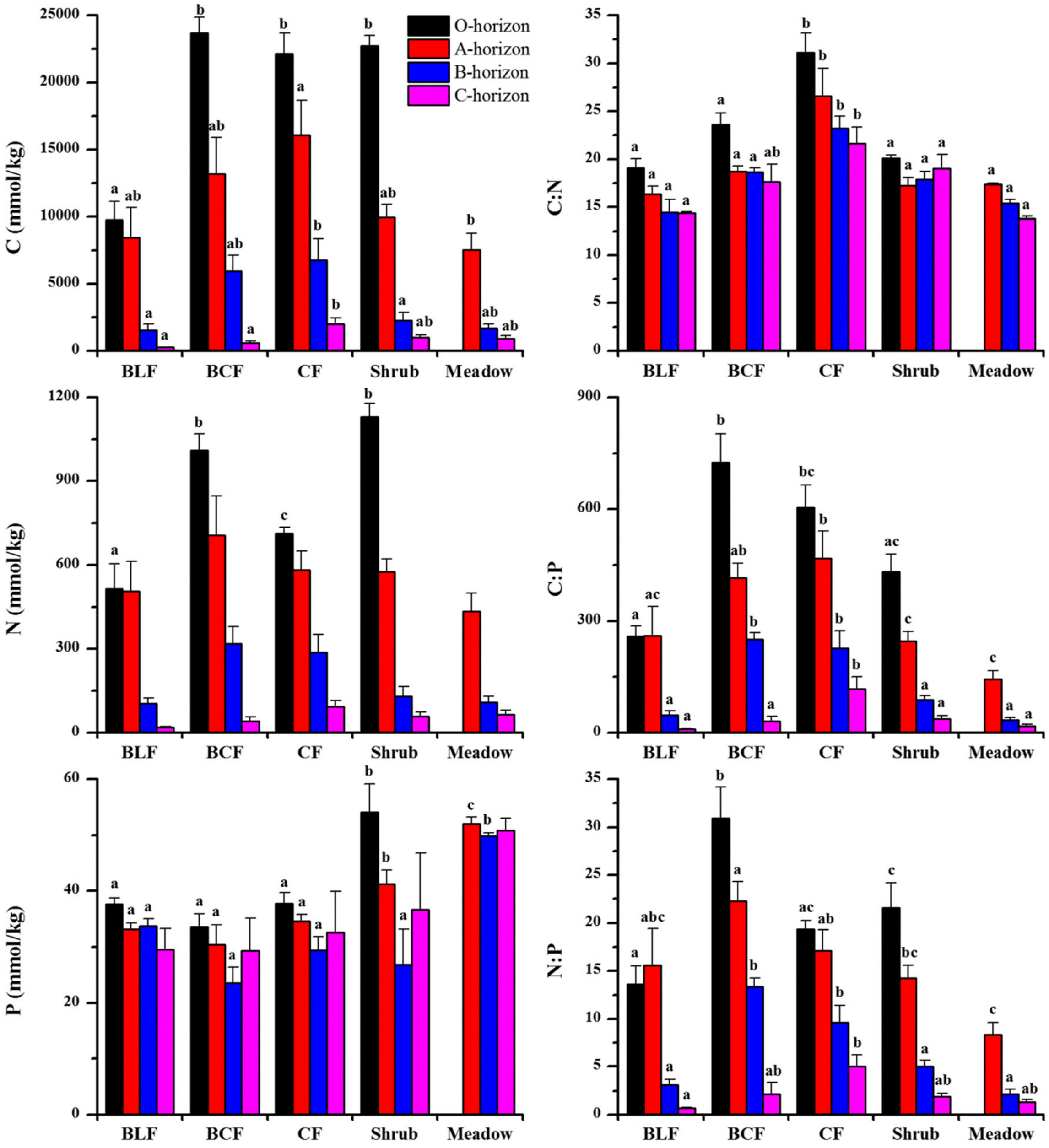

Fig. 2 The concentrations and atomic ratios of SOC, N, and P in the soils. The different letters represent the significant difference of the means at each soil horizon among the vegetation zones ($p<0.05$), whereas the same letter (or no letter) indicates insignificant difference. The error bars represent the standard error

zones, the concentrations of SOC at each soil horizon were generally higher in the CF zone, followed by the BCF, shrub, and BLF zones, and the lowest concentration was observed in the meadow zone (Fig. 2). Except in the O horizon, the N concentrations were not significantly different among the vegetation zones. The concentrations of P varied insignificantly in the C horizon indicating a uniform background of P in the soils of the eastern slope of Mt. Gongga. However, the P concentrations in other horizons were markedly higher in the soils of shrub and meadow zones than of other zones.

The atomic ratios of C:N, C:P, and N:P varied in a constrained magnitude compared with their concentrations (Table 2). These ratios were significantly higher in the O horizon than in other horizons, respectively, and the ratios of C:P and N:P showed a marked decreasing trend with depth. Specifically, the C:N:P ratio was 556:22:1 for the O horizon, 343:16:1 for the A horizon, 154:7:1 for the B horizon, and 63:3:1 for the C horizon, with the mean of 279:12:1 in the soils. Among the vegetation zones, the C:N at each soil horizon was notably higher in the CF zone (Fig. 2). The ratios of C:P and N:P were significantly higher in the BCF and CF zones followed by the shrub and BLF zones, and the lowest was observed in the meadow zone.

Table 2 The concentrations of C, N, P, and Av_P (unit; mmol/kg), and atomic ratios of C:N, C:P, N:P, C:Av_P, N:Av_P, and C:N:P in the soils and SMB

		C	N	P	C:N	C:P	N:P	Av_P	C:Av_P	N:Av_P	C:N:P
Soil											
O	Range	7920–30,500	422–1270	26.3–68.2	18.0–39.4	217–981	11.6–36.5				
n=24	SE	1220	48.0	1.9	1.5	44.7	1.5				
	Mean	*21,000*^{a}	*825*^{a}	*39.6*^{a}	*26.0*^{a}	*556*^{a}	*21.5*^{a}				556:22:1
A	Range	3560–23,500	223–1136	21.4–54.1	13.6–38.2	91.6–738	5.7–29.0	2.5–15.9	716–3770	35.9–209	
n=27	SE	1190	42.7	1.7	1.2	34.3	1.2	0.9	213	11.4	
	Mean	*12,100*^{b}	*583*^{b}	*37.0*^{ab}	*20.4*^{b}	*343*^{b}	*16.4*^{b}	*7.6*	*1870*	*91.6*	343:16:1
B	Range	992–15,400	47.0–639	9.2–50.9	13.0–29.8	20.7–484	1.2–18.6				
n=24	SE	831	33.2	2.2	0.9	26.0	1.0				
	Mean	*4450*^{c}	*213*^{c}	*31.0*^{b}	*19.6*^{b}	*154*^{c}	*7.4*^{c}				154:7:1
C	Range	250–4080	14.3–247	8.0–76.9	10.6–35.1	4.3–278	0.2–10.9				
n=27	SE	207	10.4	3.7	0.9	15.6	0.6				
	Mean	*1250*^{d}	*66.2*^{d}	*34.5*^{ab}	*18.7*^{b}	*63.0*^{d}	*3.0*^{d}				63:3:1
SMB											
n=27	Range	27.6–166	4.3–31.7	0.7–9.4	2.2–28.5	7.4–194	1.0–25.7				
	SE	9.7	1.5	0.4	1.4	10.0	1.5				
	Mean	*95.8*	*11.5*	*3.1*	*10.2*	*51.0*	*6.6*				51:6.6:1

Av_P available P. Different letters represent the significant difference of the mean values at each soil horizon ($p<0.05$), whereas the same letters indicate insignificant difference

In the A horizon, the available P (Av_P) concentrations varied between 2.5 and 15.9 mmol/kg with the mean of 7.6 mmol/kg (Table 2). The atomic ratios of C:Av_P and N:Av_P in the A horizon were 1870 and 91.6 on average, respectively. Spatially, both ratios of C:Av_P and N:Av_P in the A horizon showed a marked decreasing trend with altitude (or from lower-land to higher-land vegetation zones) (Fig. 3).

3.2 The concentrations and ratios of C, N, and P in the SMB

The concentrations of MBC, MBN, and MBP were much lower than those in the soils (Table 2). Among the vegetation zones, the concentrations of MBC were significantly higher in the BLF, BCF, and CF zones than in the shrub and meadow zones (Fig. 4). The MBN concentrations did not show marked difference among the vegetation zones, whereas they decreased gradually with altitude. The MBP concentrations increased from the BLF to the shrub, but decreased considerably in the meadow zone.

The atomic ratio of C:N:P in the SMB was 51:6.6:1 on average (Table 2). The relatively high C:N in the SMB was observed in the BCF and CF zones, although the difference was not significant among the vegetation zones (Fig. 4). The similar variation of C:P and N:P was observed in the SMB among the vegetation zones, and these ratios were significantly higher in the BLF zone.

4 Discussion

4.1 Possible factors affecting the stoichiometry of C, N, and P in the soils

Many researchers have reported the stoichiometry of C, N, and P in different terrestrial ecosystems (Table 3). Compared with these results, our analysis showed much higher C:P in the O and A horizons, but comparable N:P with global forest soils and grassland soils. For the ratios in the SMB, a little lower C:P was observed on the eastern slope of Mt. Gongga, whereas the N:P was similar to those of Chinese and global soils. The difference of C:N:P stoichiometry between the alpine and other terrestrial ecosystems is mainly attributed to the complex conditions in alpine ecosystems, which are currently experiencing strong climatic warming, more precipitation and anthropogenic impacts (Hagedorn et al. 2010). Soil formation, changes of vegetation cover and atmospheric N deposition during last decades have intensive impact on nutrient biogeochemical process in mountain soils even within a short distance.

4.1.1 Climate

Climate plays an important role in soil development and consequently nutrient cycling and availability (Oleksyn et al. 2003; Dijkstra et al. 2012). An increase in temperature can enhance N availability to plants with increased microbial activity in soils (Melillo et al. 2002). In the alpine ecosystem, the

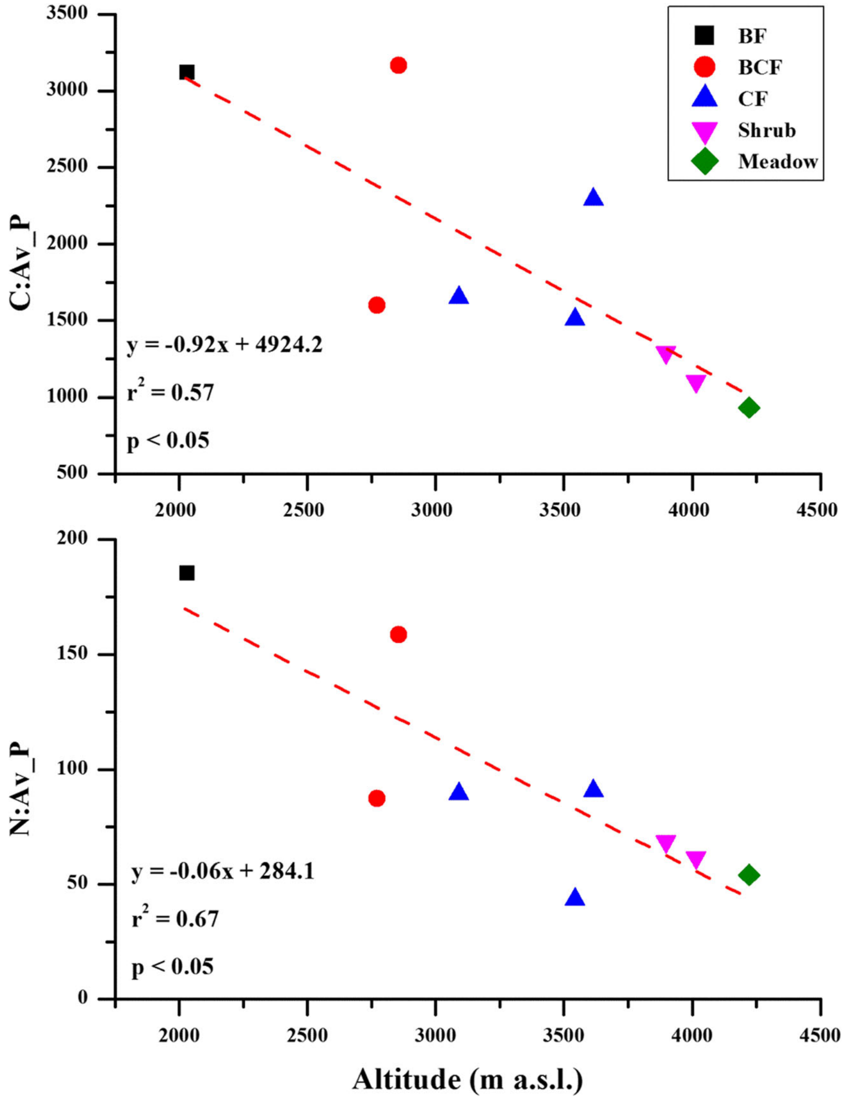

Fig. 3 The ratios of C and N to available P (C:Av_P and N:Av_P) with altitude in the soils

temperature markedly decreased with increasing altitude (Table 1). Correspondingly, the concentrations of MBC and MBN were higher in the forests of the lower altitude, indicating the sensitivity of microbes to temperature and then their effect on nutrient cycling. However, the concentrations of C, N, and P in the soils of Mt. Gongga were not significantly higher in the lower land forests. This could be attributed to the influence of precipitation as well as the soil moisture. The higher precipitation and soil moisture can increase soil weathering, and then lead to rapid release of P from parent materials.[1] Under the high precipitation and soil moisture (Table 1), the higher ratios of C:P and N:P were observed in the soils of the BCF and CF zones (Fig. 2). On the one hand, the higher precipitation increases organic matter production, and thus leads to higher organic matter content (high SOC and N concentrations) in the soils. On the other hand, the high P leaching or low P concentrations in decomposers may exist under the higher precipitation and humid conditions (Manzoni et al. 2010). In addition, the higher soil moisture can increase the accessibility of nutrients, enhance microbial growth, and consequently, induce N and P immobilization in microbial biomass and plants (Nielsen et al. 2009; Aponte et al. 2010).

4.1.2 Plant species

Plants regulate the soil stoichiometry through nutrient uptake, litterfall inputs, and root exudates. Plants can control their homeostatic stoichiometry under the stable environment but not in the variable environment (Sardans et al. 2012). Meanwhile, the physiological strategies of plants to optimize use of potentially limiting nutrients, such as nutrient resorption, leaf turnover rate, and nutrient investment in biomass, can induce

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[1] Zhou J (2014) Weathering, pedogenesis and changes of soil phosphorus speciation of Hailuogou Glacier foreland chronosequence. (unpublished dissertation)

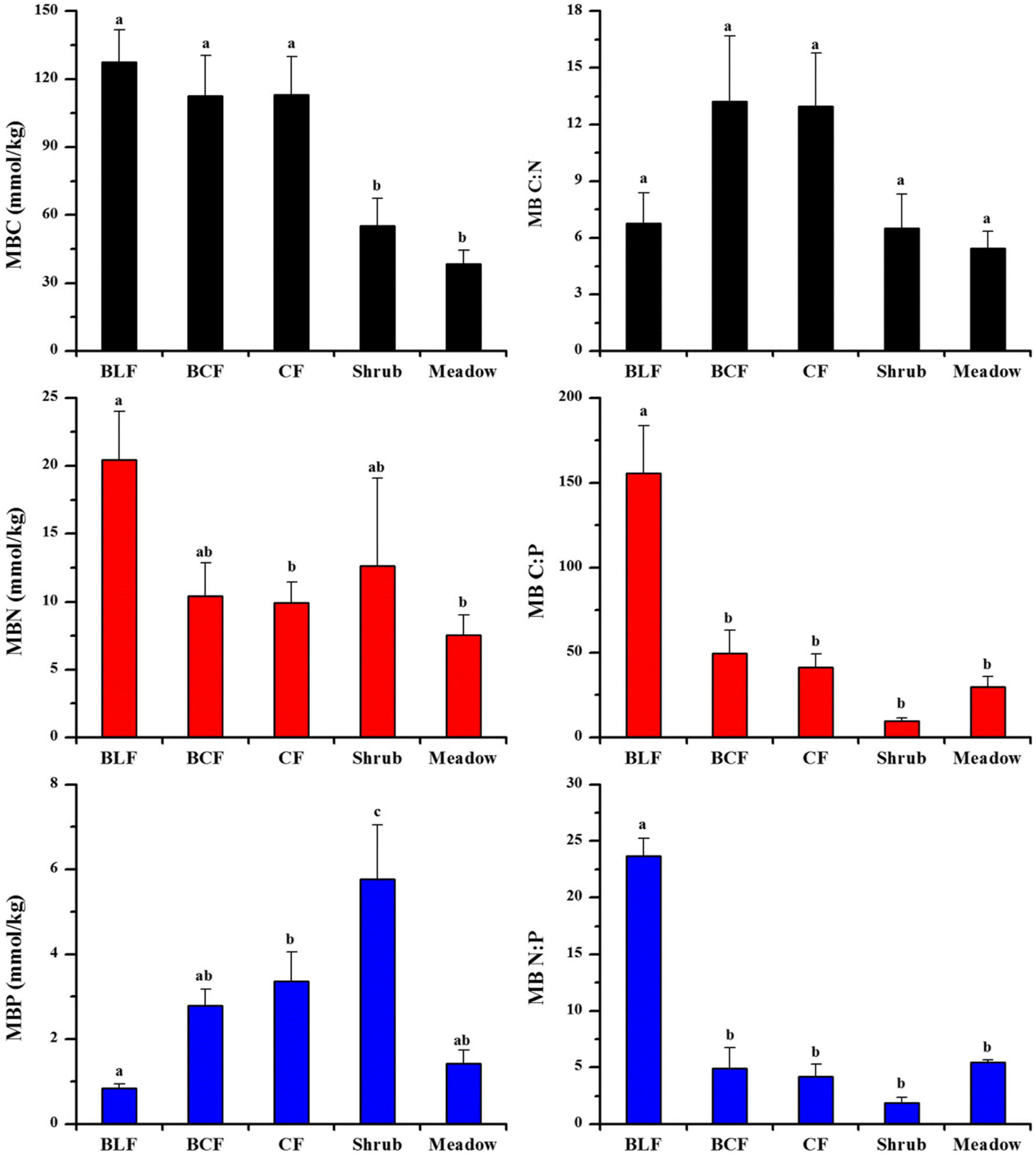

Fig. 4 The concentrations and atomic ratios of C, N, and P in the SMB. The different letters represent the significant difference of the means at each soil horizon among the vegetation zones ($p<0.05$), whereas the same letter indicates insignificant difference. The error bars represent the standard error

various changes of C, N, and P in soils (McGroddy et al. 2004). Thus, the difference of plant communities may change nutrient distribution in soils. On the eastern slope of Mt. Gongga, five different vegetation zones have formed along the large altitude gradients (Fig. 1). The stoichiometric variation of C, N, and P in the soils, especially for the significantly lower P in the BLF, BCF, and CF zones (Fig. 2), evidenced the "pumping" effects of plants. The large amounts of biomass and production have been observed in the lower land forests of Mt. Gongga, especially in the BCF zone (Luo et al. 2000), confirming that the plants indeed modify the nutrient distribution in the soils through the biomass production. Furthermore, Shen et al. (2004) reported that the richness of plant species on the eastern slope of Mt. Gongga decreased with the increasing altitude. The diversity of plant species in the lower land forests could increase the competition of organisms for P, which was evident from the relatively higher ratios of C:P and N:P in the soils.

Another effect of plants on the C:N:P stoichiometry in soils is related to the quality and quantity of litterfall inputs from different plant species. The inputs of litterfall can modify community structure and microbial biomass (Yeates and Saggar 1998; Ehlers et al. 2010) and then the decomposition processes (Rutigliano et al. 2004; Aponte et al. 2010). The quantity of

Table 3 Comparison of the C:N:P ratios in the soils and SMB with other reports

	Soil	SMB	Study sites	Refs
1	556:22:1	–	O horizon	This study
	343:16:1	51:6.6:1	A horizon	
	154:7:1	–	B horizon	
	63:3:1	–	C horizon	
	279:12:1	–	Mean in all soils	
2	186:13:1	60:7:1	Global soils (0–10 cm)	Cleveland and Liptzin (2007)
	212:15:1	74:9:1	Forest soils	
3	287:17:1	42:6:1	Global soils	Xu et al. (2013)
4	60:5:1	–	Chinese soils	Tian et al. (2010)
	134:9:1	–	Surface soils (0–10 cm)	
5	219:18:1	36:5:1	Grassland soils	Griffiths et al. (2012)
6	–	78:9:1	Mediterranean oak forest soils	Aponte et al. (2010)
7	80:7.9:1	70:6:1	Soils in Subtropical China (0–20 cm)	Li et al. (2012)

litterfall can change the contents of soil organic matter and total soil N, and subsequently, influence the microbial nutrient level (Kara et al. 2008; Rinnan et al. 2008). The deciduous trees provide nutrient richer litterfall to microbial growth. Luo et al. (2003) found much more litterfall inputs in the BLF zones (3810 kg/hm^2/year) than in the CF zones (2810 kg/hm^2/year), and they also observed higher N and P concentrations returned from litterfall in the BLF zones than in the CF zones. Combined with the higher temperature at the lower altitude, the higher litterfall inputs are conducive to microbial decomposition processes through high energy supply. Our data supported this assertion. Compared with the CF zone, the C:P and N:P ratios in the BLF zone were higher in the SMB but lower in the soils (Figs. 2 and 4), which suggested a high transformation rate of P by microbes.

The higher ratios of C:P and N:P in the soils of BCF and CF zones are also related to the root exudes including acid phosphatases, low-molecular-weight organic acid, and proton (Wu et al. 2013b), which can acidify the soils and then modify the soil nutrient solubility. The coniferous trees have been found to secret much more acid exudes than broadleaf trees (Raulund-Rasmussen and Vejre 1995). In the CF zone, the soil pH is markedly lower than that in other zones (Table 1), indicating that the soil acidification by root exudes would increase soil weathering and P solubility. These exudes have been reported to improve nutrient mobility and availability in soils, and then increase their uptake by organisms (Wang et al. 2008; Carvalhais et al. 2011; Richardson et al. 2011).

4.1.3 Soil development

Soil development (e.g., substrate age and weathering intensity) can change nutrient availability and then the C:N:P ratios in soils (Frizano et al. 2002; Reich and Oleksyn 2004). Tian et al. (2010) found that soil C:N ratios increased significantly with soil weathering intensity, and the stronger the soil weathering the higher C:P and N:P ratios. Although there is a similar parent material in the soils on the eastern slope of Mt. Gongga, the soil development is not consistent along the large altitudinal gradients, which was well observed by the soil physicochemical properties in profiles (Table 2). The strongest weathering of the soils should occur in the forests of lower altitude due to the abundant precipitation and the complex plant communities (Table 1), and correspondingly, we observed a notably higher ratios of C:P and N:P in the BCF and CF zones (Fig. 2). Meanwhile, the soil physicochemical properties (e.g., soil organic matter, pH, and soil texture) on the eastern slope of Mt. Gongga have been largely changed with the soil development (Tables 1 and 2). This not only modified indirectly the stoichiometry of C, N, and P in the soils, but also affected microbial development and then the nutrient mineralization (Hassink 1994). However, compared with the BCF and CF zones, the much lower C:P and N:P ratios were observed in the BLF zone. This was not an issue of the P weathering due to the same concentrations in the soils of the three zones (Fig. 2). On the one hand, the faster remineralization of SOC and N to CO_2 and N_2, respectively, could happen in the BLF zone than in the BCF and CF zones under the better climate conditions, and thus, removed their pools in the soils. On the other hand, the C and N in the soils may be taken up faster by the broadleaf trees in the BLF.

4.1.4 Microbial effect

Compared with the concentrations of C, N, and P in the A horizon, the concentrations of MBC, MBN, and MBP accounted for the corresponding concentrations in the soils of 0.8 %, 2.0 %, and 8.4 %, respectively, which was similar to the mean estimates of 1.2 %, 2.6 %, and 8.0 % globally (Xu et al. 2013). Meanwhile, the proportion of MBP to the available P in the A horizon was 40.8 %. This suggested that soil microbial biomass was an important pool of P in the soils, and microorganisms were actively involved in the P biogeochemical cycling as well as the P supply for plants (Turner et al. 2013; Mooshammer et al. 2014). The decrease of microbial population can release available P for plant growth, whereas its increase will be associated with strong competition for this grow-limiting resource (Lipson et al. 1999; Schmidt et al. 2007). The ratio of C:Po is often used to estimate the mineralization potential of organic P, and the mineralization occurs at C:Po below 200, whereas the immobilization process dominates when it is up to 300 (ChacÓn et al. 2005). This ratio varied between 92.5 and 804 with the mean of 460 in the A horizon, which indicated that the uptake and immobilization of P by microbes dominated in the soils. Compared with other vegetation zones, the relatively lower C:Po ratios were observed in the lower land forests (BLF and BCF), which suggested that the organic P mineralization was more favored in these zones under the strong microbial activity discussed above. Additionally, we also analyzed the soil microbial C:N:P ratios in May 2010 (beginning of growing season) with the mean of 48:9:1 (unpublished data), which was distinctly different from the mean of 51:6.6:1 in September 2010 (late growing season). This discrepancy confirmed that microbes did compete with plants for soil nutrients in the growing season.

4.2 Implication for nutrient limitation

Researchers have proposed the critical N:P ratios to indicate N- or P-limitation for terrestrial ecosystems using different plant tissues. For example, the critical N:P ratio in plant leaf has been applied to indicate N-limitation (N:P<14) and P-limitation (N:P>16) (Aerts and Chapin Iii 1999; Reich and Oleksyn 2004), and Güsewell (2004) suggested N:P<10 and>20 to correspond to N- and P-limited biomass production, respectively, by short-term fertilization experiment. On the eastern slope of Mt. Gongga, the foliar N:P of dominant plants in different vegetation zones decreased prominently with altitude, and the relatively high values were observed in the low altitude (N:P> 17.0 and>20 on 2362 m and 1750 m a.s.l., respectively, unpublished data). According to the definition above, the P-limitation is possible in the lower land forests. However, the foliar N:P ratio is not simply a function of soil availability, and the variation of climate, tree species and growth rates, soil order, and others can change the foliar chemistry and then the ratio (Güsewell 2004; Townsend et al. 2007; Elser et al. 2010). Soils, especially the surface soils, have the most active organism-environment interaction and are highly involved in the biogeochemical cycling of nutrients in terrestrial ecosystems (Tian et al. 2010; Izquierdo et al. 2013). Therefore, the nutrient limitation of ecosystems should be roundly investigated by the C:N:P ratios in soils and SMB.

On the eastern slope of Mt. Gongga, the C:N was significantly higher in the soils of the CF zone (C:N>20) despite the soil horizon, and the ratios of C:P and N:P were much higher in the BCF and CF zones (Fig. 2). Meanwhile, the ratios of C:Av_P and N:Av_P in the A horizon (Table 2), lower than those in Chinese soils (Tian et al. 2010), decreased notably with altitude (Fig. 4). These evidences suggested that with the pedogenesis P becomes ecologically scarce. In the Mediterranean oak forest soils, Aponte et al. (2010) found the higher proportion of N (C:N=7.6) and the lower fraction of P (C:P= 93.8) indicating the P-limitation in the soils. Izquierdo et al. (2013) reported that the N:P of mineral soils increased significantly during pedogenesis and pointed to P as the primary limiting nutrient in the forest ecosystem. Furthermore, tree species are responsible for the variation of nutrient stoichiometry in soils (Wan et al. 2015). Huang et al. (2013) found that the P limitation increased during forest succession on the Dinghu Mountain, southeastern China. However, our results suggested the possible P limitation in the soils of BCF and CF zones. This difference was attributed to the complex conditions in the alpine ecosystem. On the one hand, the climatic conditions are markedly different between the southeastern and southwestern China. As mentioned above, the abundant precipitation was observed around 3000 m a.s.l. on the eastern slope of Mt. Gongga corresponding to the BCF and CF zones (Table 1), which modulated the nutrient availability and microbial biomass. On the other hand, the differences of plant species and diversity can change nutrient-use efficiency and competition for nutrients. Compared with the Dinghu Mountain (pine forests, mixed pine and broadleaf forests, and monsoon evergreen broadleaf forests), the vegetation composition is much more complicated on the Mt. Gongga (Fig. 1).

The C:N:P ratios in the SMB showed different patterns from those in the soils, which were related to the microbial homeostasis (Mooshammer et al. 2014). The direct evidence was that there was no significant difference of the C:P and N:P ratios among the vegetation zones except the BLF zone (Fig. 4). Compared with the results of Cleveland and Liptzin (2007) (mean microbial biomass N:P ratio of 6.9), our results showed a similar N:P ratio of 6.6 in the SMB. This seemed to indicate little nutrient limitation to microorganisms on the eastern slope of Mt. Gongga. However, the obviously higher ratios of C:P and N:P in the SMB of the BLF zone suggested the potential P-limitation for plants.

5 Conclusions

The concentrations of C, N, and P in the soils and SMB exhibited spatial heterogeneity on the eastern slope of Mt. Gongga, whereas the variations of their ratios were constrained. The soil stoichiometric ratios of C, N, and P on the eastern slope of Mt. Gongga were different from those in other regions of the world. The local climate, vegetation variation, and soil development played a key role in the soil nutrient stoichiometry. Microbial biomass C, N, and P were important components of soil nutrients, especially the microbial biomass P content which accounted for 40.8 % of the soil available P. The ratios of C and N to the available P also increased with the decreasing altitude. According to the C:N:P stoichiometry, the P-limitation of plants was possible in the soils of broadleaf-coniferous and coniferous forests, whereas the limitation of microbial biomass existed in the soils of broadleaf forests. Our data would provide supplementary information for the stoichiometry of C, N, and P in global terrestrial ecosystems.

Acknowledgments This work is supported by National Natural Science Foundation of China (41402313 and 41272200) and Chinese Academy of Sciences (CAS "Light of West China" Program and SDS-135-1201-04).

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