Warming effects on biomass and composition of microbial communities and enzyme activities within soil aggregates in subtropical forest

Article *in* Biology and Fertility of Soils · December 2015

DOI: 10.1007/s00374-015-1081-5

7 authors, including:

Xiong Fang
Chinese Academy of Sciences
6 PUBLICATIONS **12** CITATIONS
SEE PROFILE

Guoyi Zhou
Chinese Academy of Sciences
230 PUBLICATIONS **4,459** CITATIONS
SEE PROFILE

Yuelin li
University of Bayreuth
18 PUBLICATIONS **171** CITATIONS
SEE PROFILE

Zhihong Xu
Griffith University
268 PUBLICATIONS **4,529** CITATIONS
SEE PROFILE

Biol Fertil Soils (2016) 52:353–365
DOI 10.1007/s00374-015-1081-5

ORIGINAL PAPER

Warming effects on biomass and composition of microbial communities and enzyme activities within soil aggregates in subtropical forest

Xiong Fang[1,2] · Guoyi Zhou[1] · Yuelin Li[1] · Shizhong Liu[1] · Guowei Chu[1] · Zhihong Xu[3] · Juxiu Liu[1]

Received: 12 August 2015 / Revised: 16 November 2015 / Accepted: 21 November 2015 / Published online: 1 December 2015

Abstract This study investigated the effects of warming (about 1 °C) on the biomass and composition of microbial communities and enzyme activities in soil macroaggregates and microaggregates. We fractionated the bulk soils from the control and warming treatments into large macroaggregates (>2000 μm), small macroaggregates (250–2000 μm) and microaggregates (<250 μm) using the optimal moist sieving approach. Warming did not significantly affect soil microbial biomass in all aggregate fractions, but significantly altered the soil microbial community composition in the large macroaggregates. The G^+:G^- ratio was significantly higher in the small macroaggregates and microaggregates than that in the large macroaggregates in warmed soils, while the stress ratio was significantly higher in the large and small macroaggregates than that in the microaggregates. Soil warming did not significantly affect β-glucosidase, cellobiohydrolase and *N*-acetylglucosaminidase activities, but significantly decreased acid phosphomonoesterase activity and increased oxidase activities. Our results suggest that soil microbial community composition in the large macroaggregates might be more sensitive to warming. The differential responses of soil microbial communities and enzyme activities in different aggregate fractions in the warmed soils may have important implications for C cycling in subtropical forest ecosystems.

Keywords Soil warming · Aggregate fractions · Microbial communities · Soil enzymes · Subtropical forest

Electronic supplementary material The online version of this article (doi:10.1007/s00374-015-1081-5) contains supplementary material, which is available to authorized users.

✉ Juxiu Liu
ljxiu@scbg.ac.cn

[1] Key Laboratory of Vegetation Restoration and Management of Degraded Ecosystems, South China Botanical Garden, Chinese Academy of Sciences, Xingke Road 723, Tianhe District, Guangzhou 510650, China

[2] University of Chinese Academy of Sciences, Beijing 100049, China

[3] Environmental Futures Research Institute and School of Natural Sciences, Griffith University, Nathan, Brisbane, QLD 4111, Australia

Introduction

Forest soil acts as a source of carbon (C) and plays an important role in the cycling of C in terrestrial ecosystems. Global warming is considered to promote the decomposition of soil organic C (SOC) and thereby to increase the C flux from soil to the atmosphere (Cox et al. 2000; Davidson and Janssens 2006). Nevertheless, SOC is decomposed by heterotrophic microorganisms which are one of the main drivers of the global C cycle (Schindlbacher et al. 2011; Zhou et al. 2012). Therefore, understanding how these microorganisms respond to increasing temperature is critical for predicting future atmospheric CO_2 concentrations.

Soil microorganisms are key components of below-ground ecosystems (Zhou et al. 2012; Streit et al. 2014). The biomass, composition and activities of such microorganisms are important in C cycling (Allison et al. 2010; Schindlbacher et al. 2011; Zhou et al. 2012). Microbial biomass may decrease in response to warming, especially when labile C pools are depleted (Yoshitake et al. 2015). And the decrease in available substrate may decrease the microbial impact on the C degradation (Davidson and Janssens 2006; Frey et al. 2008). However, it has been also observed that microbial biomass may remain constant or even increase, which would stimulate C degradation and the microbial mediated C feedbacks to climate warming (Belay-Tedla et al. 2009; Schindlbacher

et al. 2011; Wang et al. 2012). The response of microbial biomass and the relationship between microbial biomass and C pools due to warming remain under debate. Warming can greatly affect microbial community composition (Frey et al. 2008; Feng and Simpson 2009; Wang et al. 2012), and these changes are associated with altered microbial function. For example, the increase in fungal biomass compared to bacterial biomass in warmed soils (Castro et al. 2010) led to the changes in strategies to use C as a resource for microbial growth (Wang et al. 2012), which would stimulate the decomposition of recalcitrant C (Lipson et al. 2009; Wang et al. 2012). Soil enzymes, which are secreted by microorganisms, also play an important role in the degradation of soil organic matter and thus may provide a mechanistic link to alterations in C and nutrient utilization patterns (Nannipieri et al. 2012). Different microbial communities differ in the types of enzymes produced. Alterations in microbial community composition may affect different C pools degradation through secreting different extracellular enzymes (Sinsabaugh 2010). For instance, fungi mainly produce phenol oxidase and peroxidase that can degradate phenolic compounds which are indicative of more recalcitrant organic matter (Sinsabaugh 2010; Wang et al. 2012; Tian and Shi 2014).

Recently, substantial efforts have been made to investigate the effects of warming on biomass and composition of soil microbial communities and enzyme activities (Zhang et al. 2011; Long et al. 2012; Zhou et al. 2013; Weedon et al. 2014). Decrease in the relative abundance of Gram-positive (G^+) bacteria, Gram-negative (G^-) bacteria, total bacteria or fungi and enzyme activities; increase in G^+, bacteria or fungal richness and enzyme activities; or no significant changes in microbial community composition or enzyme activities were all observed in warmed soils (Allison and Treseder 2008; Frey et al. 2008; Feng and Simpson 2009; Rinnan et al. 2009; Castro et al. 2010; Schindlbacher et al. 2011; Zhang et al. 2011; Wang et al. 2012; Tian and Shi 2014; Weedon et al. 2014). Generally, the reported inconsistencies about the effects of warming on composition of soil microbial communities and enzyme activities may depend on various factors, such as the experimental warming approach, duration of warming treatment, substrate availability and ecosystem type (Karhu et al. 2010; Schindlbacher et al. 2011; Billings and Ballantyne 2013; Menichetti et al. 2015; Yoshitake et al. 2015). However, one of these factors which has received little attention is soil aggregate fraction, because (1) aggregate fraction can impact fluxes of water, oxygen, organic C and nutrient to microbial communities (Chenu et al. 2001; Six et al. 2004; Jastrow et al. 2007; He et al. 2008) and (2) aggregates of different sizes can provide different environments for microorganisms (Zhang et al. 2013). For example, microaggregates may provide relatively sequestered micro-habitats for microbial communities with low fauna predation, relatively stable moisture availability and steep diffusional gradients resulting in low nutrient and oxygen availability (Ranjard et al. 2000; Mummey and Stahl 2004; Jastrow et al. 2007; Ruamps et al. 2011). So inner-microaggregate communities may not be so significantly impacted by soil warming. Fungi were found to occupy macroaggregates because their hyphae could not penetrate small pores of soil microaggregates (Guggenberger et al. 1999; De Gryze et al. 2005). Much less information is available on microbial communities and enzyme activities within aggregates in response to soil warming. Therefore, the study of soil microbial communities and enzyme activities at an aggregate level may provide further insight into soil C cycling in response to global warming.

Cavaleri et al. (2015) have discussed the barriers and challenges in front of warming experiments in tropical forests and underlined that the tropics are indeed "a high priority region" for future climate change research. In the past decade, many experimental warming studies on soil microbial responses to warming have been conducted in temperate and alpine ecosystems. However, no field experiment has been conducted in tropical or subtropical forests of China, which are experiencing a significant increase in surface temperature (Zhou et al. 2011). Meanwhile, to date, warming effects on microbial communities and enzyme activities within soil aggregates have been poorly investigated. In this study, we conducted a translocation experiment from a high-elevation site to a low-elevation site, to study the effects of altitudinal transplant-induced ca. 1 °C soil temperature increase on biomass and composition of microbial communities and enzyme activities within soil aggregate fractions. We hypothesized that (1) the effects of warming on soil microbial communities and enzyme activities would differ among aggregate fractions, (2) soil microbial community composition and enzyme activities in the microaggregates might be less sensitive to soil warming.

Materials and methods

Study site

This study was conducted at the Dinghushan Biosphere Reserve (23° 09′ N-23° 11′ N, 112° 30′ E-112° 33′ E, DBR), with an area of 1155 ha. DBR is located in the middle of Guangdong Province in southern China and is characterized by a typical subtropical monsoon climate. Mean annual temperature is ca. 21 °C, ranging from mean coldest in January (12.6 °C) and hottest in July (28.0 °C). Mean annual precipitation is ca. 1700 mm, and nearly 80 % of the rain falls in the wet season (April–September) and 20 % in the dry season (October–March). Major vegetation types include pine forest (PF), mixed pine and broadleaved forest (MF), and monsoon evergreen broadleaved forest (BF). The PF, MF and BF belong to different natural successional stages, from the pioneer community PF to the regional climax vegetation BF. Soils are

oxisols (lateritic red earths) formed from sandstone approximately 30 to 70 cm in depth.

Translocation experiment design

A high-elevation site (300 m.a.s.l., with a slope <10 %) and a low-elevation site (30 m.a.s.l., with a slope <10 %) in the DBR were selected to conduct the translocation experiment. In March 2012, three open top chambers (OTCs) were constructed at the high-elevation site (as control group) and another three OTCs were constructed at the low-elevation site (as warming group). The two transplant sites were located in open area where they were exposed to full light and rain. Each chamber had an edge length of 3 m, with a 0.8-m deep below-ground section. The below-ground section was surrounded by concrete brick wall bonding with ceramic tiles to prevent the lateral or vertical movement of water or element from the surrounding soils. There was a hole at the top and another one at the bottom of the wall. The holes (inner diameter: 2 cm) were connected to a stainless steel water collection boxes to collect surface and ground water samples, respectively. Both holes were capped by a 2-mm plastic net to prevent losses other than those of leachates.

In April 2012, three different layers of soils (0–20, 20–40 and 40–70 cm) which were collected from the MF with approximately 300 m altitude were homogenized separately and then transported into the chambers correspondingly. This resulted in 7.2 m^3 ($3\times3\times0.8=7.2$ m^3) soil in each chamber. Then six species of seedlings were specifically selected for this study due to their common occurrence and distribution range in the MF. All seedlings (1-year-old) were also collected from the MF with approximately 300 m altitude. They included *Schima superba* Gardn. et Champ., *Syzygium rehderianum* Merr. et Perry, *Machilus breviflora* (Hance) hemsl., *Pinus massoniana* Lambert, *Castanopsis hystrix* Hook.f. & Thomson ex A. DC and *Ardisia lindleyana* D. Dietr. In early May 2012, when seedlings of all species were collected, 6 individuals per species were transplanted into each of the OTCs randomly. Thus there were 36 seedlings located in each chamber. The trees grow very well and fast. One tree per species was harvested at the end of 2014, to avoid crowding in the chambers.

Microclimate monitoring

Soil profile temperatures (at 5, 20 and 40 cm) were recorded in each chamber using Campbell 109 constantan-copper thermocouples. Volumetric water content was measured from the soil surface to a depth of 5 cm using Campbell CS616 water content reflectometer probes. Data were recorded every hour using Campbell Scientific (Logan, UT, USA) CR1000 data loggers since May 2013.

Soil sampling and aggregate fractionation

Soil samples from each chamber were collected from a depth of 0–10 cm in late June of 2014. Each soil sample, pooled from five soil cores (inner diameter: 5 cm), was stored in a hard plastic container and immediately transported to the laboratory. To minimize effects on composition and activity of microbial communities, soil aggregates were isolated by the optimal moist sieving approach according to Dorodnikov et al. (2009a, b). All soil samples were air-dried to optimal moisture (water content ca. 10–15 %) that would allow limited mechanical stress to induce maximum brittle failure along natural planes of weakness, and then they were gently sieved through an 8-mm sieve. The recovered soil samples (500 g) were transferred to a nest of sieves (2000 and 250 μm) and shaken 100 min^{-1} for 2 min. All visible roots and stones were removed and the aggregates >2000 μm (large macroaggregates) were collected. The same procedure was carried out for the material retained on the 250 μm sieve, isolating aggregate size class of 250–2000 μm (small macroaggregates). The remaining material passed through the 250 μm sieve was identified as aggregate class <250 μm (microaggregates). Then each soil sample was divided into two parts. One part was stored at 4 °C and analysed for soil water content, soil microbial biomass C (SMBC), phospholipid fatty acid (PLFA) and soil enzyme activities. The other part was air-dried and analysed for chemical properties.

Soil chemical analyses

Soil pH was measured using a soil:water ratio at 1:5. Soil water content was determined from mass loss after drying at 105 °C for 24 h. SOC was determined by the Walkley-Black's wet digestion method (Nelson and Sommers 1982). Soil total N (TN) was measured using the micro-Kjedahl method (Jackson 1964). Total P (TP) concentration was measured photometrically after samples were digested with HNO_3. SMBC was determined by subjecting fresh soil samples to the chloroform fumigation-extraction method (Martens 1995). And the unfumigated samples were used to estimate background dissolved organic C (DOC) and N (DON) values.

PLFA analyses

PLFA analysis was determined according to Bossio and Scow (1998). Briefly, PLFAs were extracted from 8 g of dry soil, fractionated and analyzed. Peak areas were converted to nanomole per gram of dry soil using internal standards (19:0 nonadecanoic methylester). The soil microbial community composition was investigated on the basis of specific PLFAs for different microbial groups (Schindlbacher et al. 2011; Jiang et al. 2013). PLFAs for Gram-negative (G^-) bacterial biomass were the cyclic 17:0cy and 19:0cy as well as

16:1ω7c and 18:1ω7c. Gram-positive (G^+) bacterial biomass was identified by the PLFAs: 15:0a, 15:0i, 16:0i, 17:0a and 17:0i. We calculated the sum of G^+, G^- bacterial biomass and 14:0, 15:0, 16:0 and 18:0 as indicators of bacterial biomass. The methylic, mid-chain-branched saturated PLFA 10Me16:0, 10Me17:0 and 10Me18:0 were used as indicators for actinomycetes. Fungal biomass was calculated by summing 18:1ω9c, 18:2ω6c and 18:3ω3c PLFA biomarkers. Total PLFA was calculated by summing all determined PLFAs. The bacterial to fungal biomass ratio (B:F ratio) was calculated by dividing the sum of all bacterial PLFA markers by the sum of all fungal PLFA markers (Streit et al. 2014). We used the ratio of the sum concentration of cyclopropyl fatty acids (cy17:0 and cy19:0) divided the sum of the respective mono-unsaturated precursor concentration (16:1ω7 and 18:1ω7) as an indicator of microbial stress, since G-bacteria are forming more stable cyclopropyl fatty acids in their membrane under stress conditions, such as water limitations or high temperature (Streit et al. 2014).

Soil enzyme analysis

Activities of soil enzymes involved in C, N and P cycling were measured. These enzymes included four hydrolytic enzymes: β-glucosidase (BG), cellobiohydrolase (CBH), *N*-acetylglucosaminidase (NAG), and acid phosphomonoesterase (AP) and two oxidases: phenol oxidase (PhOx) and peroxidase (Perox). The measurement of BG, CBH, NAG and AP activities was performed by the release of *p*-nitrophenol (PNP) after cleavage of the enzyme-specific synthetic substrate according to Tabatabai (1994). The specific substrates were bought from Sigma, St. Louis, USA. For BG activity, 1 g of soil was mixed with 4 mL of modified universal buffer at pH 6.0, and 1 mL 0.025 M *p*-nitrophenyl-b-D-glucopyranoside. The mixture was then incubated for 1 h at 37 °C. Reactions were stopped by adding 0.5 M $CaCl_2$ and 0.1 M trihydroxymethyl aminomethane which was buffered to pH 12.0. Controls were performed with the substrate being added after the reactions were stopped. The mixture was filtered through Whatman filter paper and the reaction product was measured colorimetrically at 400 nm (Tabatabai 1994) with a UV–VIS spectrophotometer (UV-1700, Shimadzu). The procedures for the assays of CBH, NAG and AP activities were the same as for BG except using *p*-nitrophenyl-b-D-Cellobioside, *p*-nitrophenyl-*N*-acetyl-b-D-glucosaminidine and *p*-nitrophenylphosphate as the substrate and buffering pH of reaction systems to 5.0, 5.5 (Wang et al. 2015) and 6.5 (Tabatabai 1994), respectively. For PhOx activity, 1 g of soil was mixed with 4.5 mL of modified universal buffer at pH 5.0 and 4.5 mL 0.01 M L-3,4-dihydroxy phenylalanine (L-DOPA). The mixture was then rapidly mixed and then incubated for 1 h at 25 °C. Then, that is immediately centrifuged at 12,000×*g* at 5 °C for 5 min to terminate the reaction. The products were filtered through Whatman filter paper and measured at 450 nm (Iyyemperumal and Shi 2008). The assay of Perox activity was the same as PhOx except adding 1 mL 0.3 % H_2O_2 to the mixture before incubation (Iyyemperumal and Shi 2008). The specific activities of the enzymes were calculated by dividing enzyme activities by the SMBC (Trasar-Cepeda et al. 2007).

Data analysis

Data were transformed to meet the assumptions of normality and homogeneity of variances when necessary. A repeated-measures general linear model was used to evaluate the effects of the translocation treatments on soil temperature and moisture. Two-way ANOVA was used to test the effects of warming, soil aggregate sizes and their interactions on all response variables. Individual treatment means within each soil aggregate sizes were compared with Duncan's Multiple Range test. Principal component analyses (PCA) were conducted on the PLFA contents and on the enzyme activities to investigate the differences in soil microbial community composition and functions. Correlation among microbial parameters, soil enzyme activities and soil characteristics was tested by Pearson correlation coefficient. All statistical analyses were performed by the SAS software (Statistical Analysis System, version 9.2, SAS Institute, Inc.) and statistical significance was determined at $P<0.05$.

Results

Soil temperature and moisture

Translocation treatment led to soil temperature increased during the studied period (Fig. 1). The daily average temperature at 10 cm in the warmed chambers was 0.93 °C higher ($P<0.01$) than in the ambient chambers (from 24 May 2013 to 23 May 2014). During the same period, soil daily maxima and minima were on average 1.15 and 0.67 °C higher ($P<0.01$) in the warmed chambers, respectively. Soil moisture was also significantly affected by transplant treatments (Fig. 1; $P<0.01$). The soil moisture was 19.9 % in the warming chambers while it was 24.8 % in the control chambers.

Aggregate distribution and soil properties

The weight distribution among the aggregate size classes of the bulk soil was as follows: large macroaggregates contributed 55–58 %, small macroaggregates 35–36 % and microaggregates 6–7 % of the weight of bulk soil (Table 1). Soil warming did not affect the size distribution of aggregates.

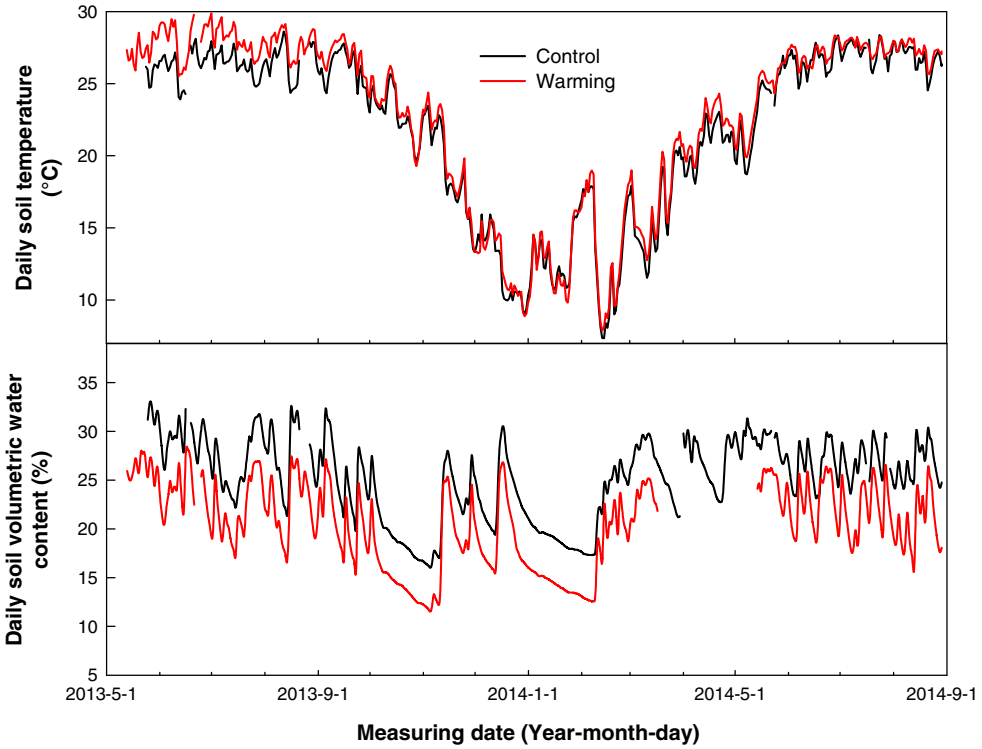

Fig. 1 Daily soil temperature and volumetric water content at 5 cm soil depth in the warm chambers and the controls (from May 2013 to September 2014)

Warming significantly decreased ($P<0.05$) soil water content, SOC, TN, TP, SMBC and DOC contents, but increased soil pH, regardless of aggregate fractions (Table 1). Considering aggregate size level, warming only significantly decreased ($P<0.05$) SOC in the small macroaggregates and in the microaggregates and TP in the microaggregates. Both SOC and TN were significantly increased ($P<0.05$) as aggregate size decreased (Table 1). Soil aggregate fraction did not affect soil water content in the control chambers, but soil water content significantly decreased in the microaggregates in the warming chambers.

Biomass and composition of soil microbial communities

Soil warming did not significantly affect the concentrations of total PLFA, and of biomarkers related to bacteria, G^+ bacteria, G^- bacteria, fungi and actinomycetes (Table 2, Fig. 2) as well as the B:F ratio, regardless of aggregate fractions. But, G^-

Table 1 Effects of experimental warming on fraction amount (% of whole soil), soil water content, pH, soil organic C, total N, total P, soil microbial C and dissolved organic C in different soil aggregate fractions

Aggregate/ treatment		Fraction amount (%)	SWC (%)	pH	SOC (g kg^{-1})	TN (g kg^{-1})	TP (g kg^{-1})	SMBC (mg kg^{-1})	DOC (mg kg^{-1})
LA	Control	58.5 ± 2.6^{A}	19.7±1.7	3.52±0.03	15.3 ± 2.3^{B}	1.11 ± 0.20^{B}	0.15±0.01	242.2±99.5	331.0±15.4
	Warming	55.2 ± 6.2^{A}	17.6 ± 0.3^{A}	3.59±0.05	14.±1.6	0.91±0.07	0.14 ± 0.00^{AB}	219.5±29.9	297.2±16.2
SA	Control	35.6 ± 3.0^{B}	20.3±1.2	3.58±0.05	21.0 ± 3.0^{aAB}	1.19 ± 0.08^{AB}	0.16±0.01	379.0±55.0	310.0±24.0
	Warming	36.1 ± 5.0^{B}	17.1 ± 0.7^{A}	3.63±0.09	15.9 ± 2.8^{b}	1.12±0.16	0.15 ± 0.00^{A}	231.1±79.4	296.3±31.3
IA	Control	6.0 ± 0.7^{C}	18.4±2.9	3.56±0.05	26.8 ± 4.8^{aA}	1.59 ± 0.32^{A}	0.16 ± 0.01^{a}	349.6±51.0	329.3±48.4
	Warming	7.3 ± 1.2^{C}	14.0 ± 1.8^{B}	3.63±0.07	17.2 ± 1.0^{b}	1.09±0.08	0.14 ± 0.00^{bB}	261.4±30.0	271.3±39.8
Analysis of variance (P values)									
Warming (W)		0.964	*0.003*	*0.043*	*0.020*	*0.024*	*<0.001*	*0.021*	*0.023*
Aggregate (A)		*<0.001*	0.142	0.425	*0.019*	*0.037*	0.536	0.203	0.781
W×A		*<0.001*	*0.008*	0.333	*0.001*	*0.002*	*0.003*	*0.040*	0.250

Values are the means±SD ($n=3$). Different superscript letters indicate significant differences among aggregate size classes in the same warming treatment (uppercase letters) and warming treatments in the same class of aggregate size (lowercase letters) at $P<0.05$

LA large macroaggregates (>2000 μm), *SA* small macroaggregates (250–2000 μm), *IA* microaggregates (<250 μm)

Table 2 Effects of experimental warming, aggregate fraction and their interactions on content of phospholipids acid biomarkers, ratios of biomarkers and soil enzyme activities. The probability values are shown in the table

Parameters	Warming (W)	Aggregate (A)	W×A
Total PLFAs	0.415	<0.001	0.001
Bacteria	0.361	<0.001	0.001
G^+ bacteria	0.42	<0.001	<0.001
G^- bacteria	0.245	<0.001	<0.001
Fungi	0.662	<0.001	0.003
Actinomycetes	0.345	<0.001	0.002
B:F ratio	0.133	0.525	0.557
G^+:G^- ratio	0.087	0.03	0.001
Stress ratio	0.025	0.042	0.001
Acid phosphatase activity	0.004	0.073	<0.001
β glucosidase activity	0.153	0.594	0.715
Cellobiohydrolase activity	0.266	0.19	0.463
N-acetyl-glucosaminidase activity	0.286	0.522	0.808
Phenol oxidase activity	<0.001	0.45	0.004
Peroxidase activity	0.008	0.102	0.011

G^+ bacteria Gram-positive bacteria, *G^- bacteria* Gram-negative bacteria, *B:F ratio* the ratio of bacterial biomass to fungal biomass, *G^+ :G^- ratio* the ratio of Gram-positive bacterial biomass to Gram-negative bacterial biomass

bacteria was marginal, significantly lower in the small macroaggregates (P=0.06) and microaggregates (P=0.07) in the warming chambers with reference to the control treatment. The ratio of G^+ bacteria to G^- bacteria (G^+:G^- ratio) was significantly higher (P<0.05) in the small macroaggregates and microaggregates in the warming chambers than that of the respective aggregates of the control chambers. Compared to the control chambers, warming significantly increased (P<0.05) stress ratio in the large macroaggregates and small macroaggregates.

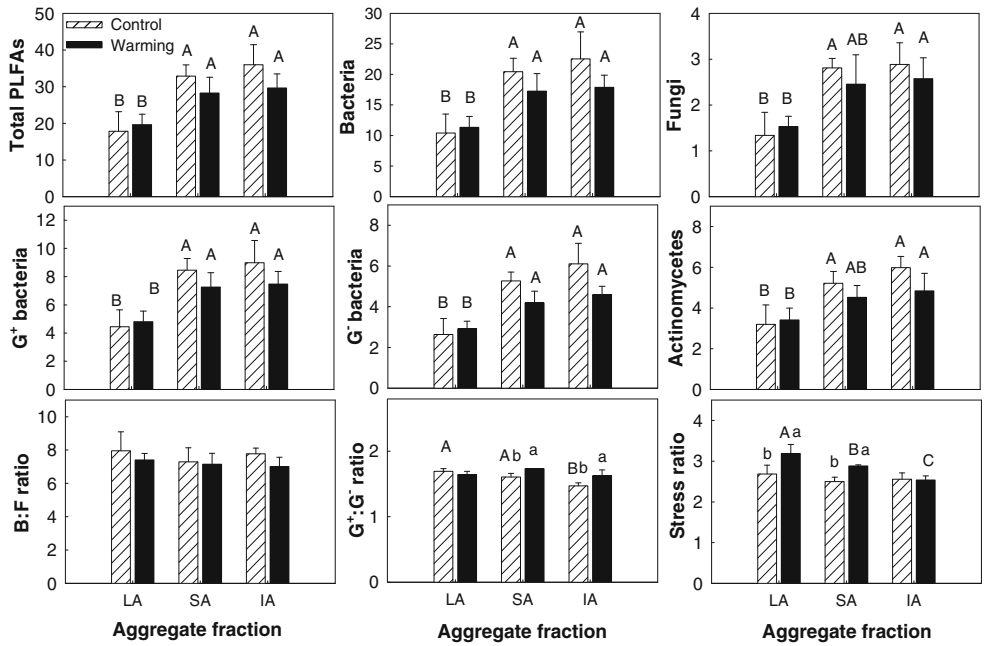

Fig. 2 The concentration of phospholipids acid biomarkers (nmol g^{-1} dry soil) and ratios of biomarkers were affected by experimental warming in three aggregate fractions. LA is large macroaggregates; SA is small macroaggregates and IA is microaggregates. G^+ bacteria is Gram-positive bacteria; G^- bacteria is Gram-negative bacteria; B:F ratio is the ratio of bacterial biomass to fungal biomass. G^+:G^- ratio is the ratio of Gram-positive bacterial biomass to Gram-negative bacterial biomass; Stress ratio is the ratio of cyclopropyl PLFA (cy17:0 and cy19:0) to precursor (16:1ω7 and 18:1ω7). Different superscript letters indicate significant differences among aggregate size classes in the same warming treatment (uppercase letters) and warming treatments in the same class of aggregate size (lowercase letters) at P<0.05. Values are means±SD (n=3)

Soil aggregate fractions exerted significant influences on all microbial biomasses, which increased as aggregate size decreased (Table 2 and Fig. 2). The total PLFA and concentrations of biomarkers of bacteria, G^+ bacteria, G^- bacteria, fungi and actinomycete were all significantly lower ($P<0.001$) in the large macroaggregates compared with those in the small macroaggregates and microaggregates. In the control chambers, stress ratio did not change among aggregates, but it was significantly decreased ($P<0.05$) as the aggregate size decreased in the warming chambers. In contrast, the B:F and G^+:G^- ratios were not significantly changed among aggregate fractions.

Individual PLFA concentrations were respectively subjected to PCA according to warming and aggregate size. The first principal component (PC1) accounted for 75.31 % and the second component (PC2) for 10.48 % of the variation in the dataset (Fig. 3a). The large macroaggregates was clearly separated from the small macroaggregates and microaggregates on the PC1 axis ($P<0.05$). Meanwhile, warming only significantly ($P<0.05$) altered soil microbial community composition in the large macroaggregates.

Soil enzyme activities

In general, we found no significant effects of warming on the activities of BG, CBH and NAG, whereas there was a significant decrease ($P<0.01$) in AP activity in all three aggregate fractions (Table 2 and Fig. 4). In contrast, PhOx and Perox activities increased ($P<0.01$) in the warming chambers compared to the control chambers. Considering aggregate size level, warming only increased PhOx activity in the large macroaggregates and small macroaggregates and increased Perox activity in the small macroaggregates. Overall, aggregate fraction did not significantly affect soil enzyme activities, but enzyme activities tended to increase as aggregate size decreased (Fig. 4).

Soil warming did not affect the ratio of soil hydrolase activities to MBC, but significantly increased the ratio of soil oxidase activities to SMBC (Table 3). Soil aggregate fraction did not affect all six specific enzyme activities. Individual enzyme activities were respectively subjected to PCA according to warming and aggregate size. We found that warming also significantly ($P<0.05$) altered soil enzyme activities the large macroaggregates (Fig. 3b).

Correlations between soil chemical and biological characteristics

The abundance of total PLFA, and half of biomarkers related to bacteria, G^+ bacteria, G^- bacteria and actinomycete was significantly and positively correlated with most hydrolytic enzyme activities in the large macroaggregates and small macroaggregates (Tables 4 and 5). The abundance of most microbial groups was positively correlated ($P<0.05$) with SOC in the large macroaggregates and small macroaggregates, and positively correlated ($P<0.05$) with TP in the small macroaggregates and microaggregates (Tables 5 and 6). The stress ratio was negatively correlated ($P<0.05$) with SOC and water content in the large macroaggregates and small macroaggregates (Tables 4 and 5). The abundance of most microbial groups did not correlate with soil moisture in all three aggregate fractions, but most hydrolase activities were positively correlated ($P<0.05$) with soil moisture in the large and small macroaggregate (Table S1).

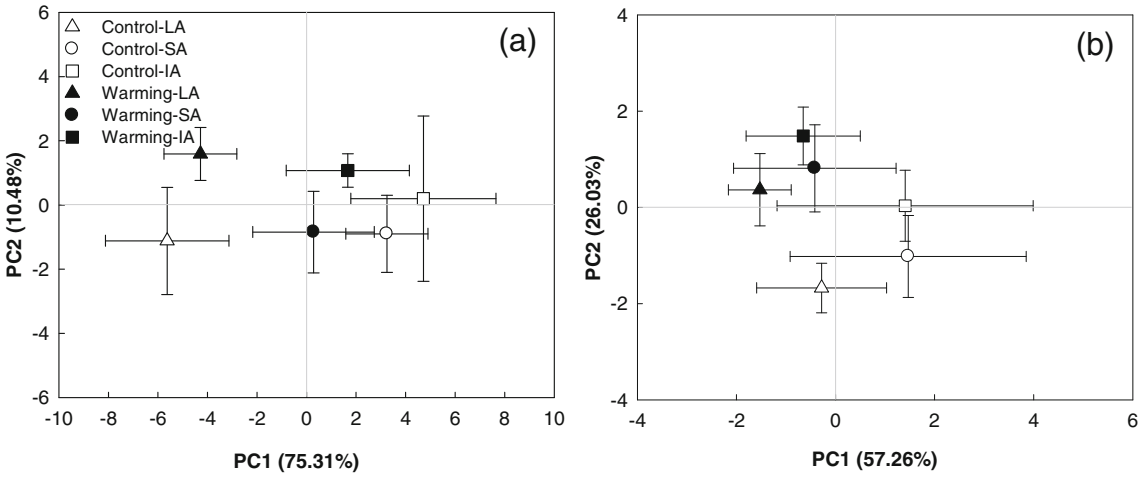

Fig. 3 Soil microbial communities (**a**) and enzyme activities (**b**) pattern in soil samples from three aggregate fractions. PCA scores from aggregate fractions were separated along PC1, while PCA scores from warming treatment were separated along PC2 ($n=3$). LA is large macroaggregates; SA is small macroaggregates; IA is microaggregates

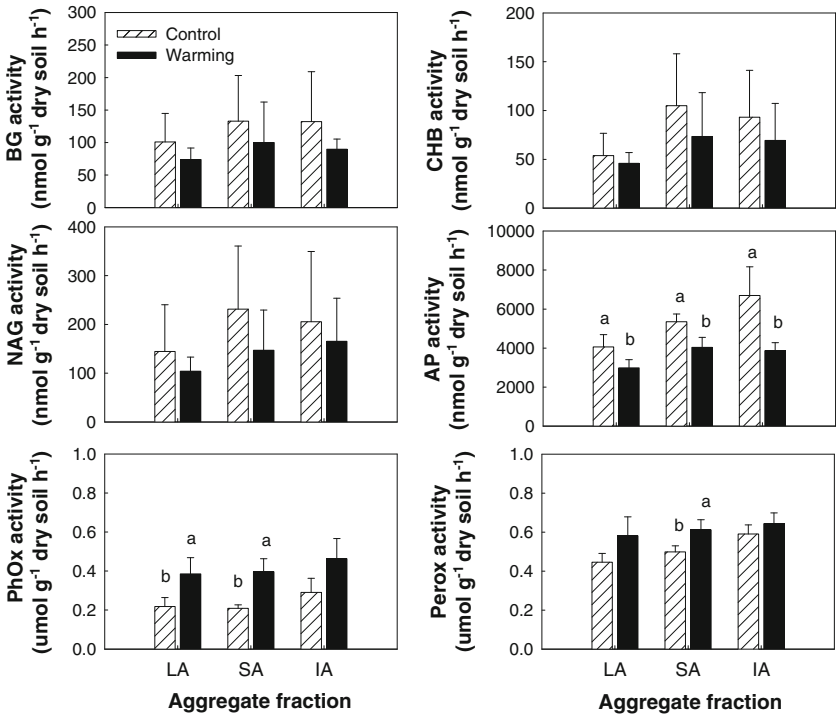

Fig. 4 Soil enzyme activities were affected by experimental warming in three aggregate fractions. LA is large macroaggregates; SA is small macroaggregates and IA is microaggregates. AP is acid phosphomonoesterase activity; BG is β-glucosidase activity; CBH is cellobiohydrolase activity; NAG is *N*-acetylglucosaminidase activity; PhOx is phenol oxidase activity; Perox is peroxidase activity. Different superscript letters indicate significant differences among aggregate size classes in the same warming treatment (uppercase letters) and warming treatments in the same class of aggregate size (lowercase letters) at $P<0.05$. Values are means±SD ($n=3$)

Discussion

Soil microbial communities in soil aggregates under warming

Some earlier researchers have reported that microbial biomass is greater in macroaggregates than in microaggregates (Chotte et al. 1998; Guggenberger et al. 1999). Contrary to these earlier reports, our study showed that the soil microbial biomass increased as soil aggregate size decreased when optimal moist sieving approach was used to separate different aggregate fractions (Table 2 and Fig. 2). These contrasting results may reflect the differences in aggregate separation by wet or dry sieving method. Indeed, it has been reported that the soil microbial biomass can increase as soil aggregate size decrease (Dorodnikov et al. 2009a, b; Jiang et al. 2013; Wang et al. 2015), when aggregates are prepared by the method of optimal moist sieving. A substantial portion of soil microbes is thought to live on or near the aggregate surface (Oades 1984), and the optimal moist sieving approach is less

Table 3 Effects of experimental warming on specific enzyme activities (ratios of enzyme activities to the soil microbial biomass C) in different soil aggregate fractions

Aggregate/treatment		AP:SMBC	BG:SMBC	CBH:SMBC	NAG:SMBC	PhOx:SMBC	Perox:SMBC
LA	Control	18.4±6.7	0.42±0.04	0.22±0.00	0.56±0.13	0.0010±0.0002^{b}	0.002±0.001
	Warming	13.8±3.3	0.34±0.11	0.21±0.07	0.48±0.17	0.0018±0.0004^{a}	0.003±0.000
SA	Control	14.4±2.8	0.35±0.18	0.28±0.14	0.61±0.52	0.0006±0.0001^{a}	0.001±0.000
	Warming	19.2±7.8	0.42±0.19	0.33±0.16	0.67±0.32	0.0020±0.0008^{b}	0.003±0.001
IA	Control	19.2±3.3	0.38±0.20	0.27±0.12	0.59±0.37	0.0008±0.0001^{b}	0.002±0.000
	Warming	14.9±2.0	0.34±0.03	0.26±0.13	0.63±0.30	0.0018±0.0004^{a}	0.002±0.000
Analysis of variance (*P* values)							
Warming (W)		0.564	0.809	0.796	0.960	*<0.001*	*0.004*
Aggregate (A)		0.945	0.949	0.387	0.786	0.976	0.825
W×A		0.555	0.963	0.843	0.988	*0.024*	*0.082*

Values are the means±SD ($n=3$). Different superscript letters indicate significant differences between warming treatments in the same class of aggregate size (lowercase letters) at $P<0.05$

AP acid phosphomonoesterase activity, *BG* β-glucosidase activity, *CBH* cellobiohydrolase activity, *NAG N*-acetylglucosaminidase activity, *PhOx* phenol oxidase activity, *Perox* peroxidase activity, *SMBC* soil microbial biomass C, *LA* large macroaggregates (>2000 μm), *SA* small macroaggregates (250–2000 μm), *IA* microaggregates (<250 μm)

Table 4 Pearson correlation between microbial variables, soil enzyme activities and soil organic C, total N, total P and water content in the large macroaggregates (n=6)

Variable	Total PLFAs	Bacteria	G^+ bacteria	G^- bacteria	Fungi	Actinomycetes	G^+:G^- ratio	Stress ratio
AP	0.259	0.365	0.309	0.264	0.285	0.217	0.315	−0.713
BG	0.738	0.781*	0.761*	0.740	0.680	0.733	−0.011	−0.496
CBH	0.856**	0.871**	0.872 **	0.848**	0.771*	0.864**	−0.037	−0.295
NAG	0.809*	0.830**	0.817**	0.809*	0.760*	0.804*	−0.130	−0.453
PhOx	0.647	0.571	0.625	0.629	0.641	0.604	−0.280	0.779*
Perox	0.029	−0.160	−0.117	−0.046	0.104	−0.040	−0.555	0.371
SOC	0.825**	0.895**	0.863**	0.837**	0.807*	0.748*	−0.033	−0.826**
TN	0.412	0.509	0.439	0.440	0.446	0.343	−0.071	−0.776*
TP	−0.461	−0.469	−0.426	−0.495	−0.660	−0.281	0.680	−0.040
Water content	0.379	0.437	0.396	0.390	0.329	0.400	0.013	−0.806*

G^+ bacteria Gram-positive bacteria, *G^- bacteria* Gram-negative bacteria, *B:F ratio* the ratio of bacterial biomass to fungal biomass, *G^+:G^- ratio* the ratio of Gram-positive bacterial biomass to Gram-negative bacterial biomass, *Stress ratio* the ratio of cyclopropyl PLFA (cy17:0 and cy19:0) to precursor (16:1ω7 and 18:1ω7), *AP* acid phosphomonoesterase activity, *BG* β-glucosidase activity, *CBH* cellobiohydrolase activity, *NAG* *N*-acetylglucosaminidase activity, *PhOx* phenol oxidase activity, *Perox* peroxidase activity, *SOC* soil organic C, *TN* total N, *TP* total P

*P<0.1, **P<0.05

destructive than the wet-sieving method (Kristiansen et al. 2006) and more appropriate for soil microbiological studies (Dorodnikov et al. 2009b). The increase of microbial biomass as soil aggregate size decreased can depend on the presence of small pore sizes in microaggregates (Chenu et al. 2001; Jastrow et al. 2007), which may protect microorganisms from predation by protozoa or from desiccation (Zhang et al. 2013). Meanwhile, smaller aggregate sizes have higher specific surface to which microbial cells can attach (Amato and Ladd 1992; Van Gestel et al. 1996).

Similar to many previous findings (Rinnan et al. 2008; Schindlbacher et al. 2011; Streit et al. 2014), experimental warming (only about 1 °C) had no or negative impact on soil microbial community biomass, as indicated by phospholipid acid biomarkers. Finlay et al. (1997) suggested that the composition of microbial communities often change when environmental factors vary with high amplitudes or frequencies. In our study, an increase of annual average soil temperature by 0.93 °C might not be enough to induce great changes in soil microbial community biomass. Although soil moisture is a major factor controlling the biomass and activity of microorganisms, the abundance of all microbial groups had no positive correlations with soil water content. Probably soil moisture might not have declined to the threshold to significantly affect soil microbial biomass. While, to some extent, decreased soil moisture in the warmed soils probably reduced

Table 5 Pearson correlation between microbial variables, soil enzyme activities and soil organic C, total N, total P and water content in the small macroaggregates (n=6)

Variable	Total PLFAs	Bacteria	G^+ bacteria	G^- bacteria	Fungi	Actinomycetes	G^+:G^- ratio	Stress ratio
AP	0.824**	0.731	0.816**	0.940***	0.782*	0.726	−0.847*	−0.939***
BG	0.839**	0.828**	0.842**	0.760*	0.642	0.863**	−0.162	−0.446
CBH	0.913**	0.940***	0.904**	0.843**	0.768*	0.875**	−0.249	−0.545
NAG	0.803*	0.851**	0.807*	0.750	0.618	0.774*	−0.208	−0.523
PhOx	−0.437	−0.210	−0.456	−0.634	−0.223	−0.481	0.835**	0.823**
Perox	−0.172	0.006	−0.205	−0.388	0.099	−0.214	0.708	0.702
SOC	0.899**	0.801*	0.903**	0.952***	0.705	0.887**	−0.632	−0.833**
TN	0.191	0.265	0.220	0.255	−0.057	0.148	−0.195	−0.407
TP	0.903**	0.833**	0.921***	0.940***	0.592	0.902**	−0.545	−0.820**
Water content	0.618	0.466	0.640	0.791*	0.349	0.633	−0.813	−0.939***

G^+ bacteria Gram-positive bacteria, *G^- bacteria* Gram-negative bacteria, *B:F ratio* the ratio of bacterial biomass to fungal biomass, *G^+:G^- ratio* the ratio of Gram-positive bacterial biomass to Gram-negative bacterial biomass, *Stress ratio* the ratio of cyclopropyl PLFA (cy17:0 and cy19:0) to precursor (16:1ω7 and 18:1ω7), *AP* acid phosphomonoesterase activity, *BG* β-glucosidase activity, *CBH* cellobiohydrolase activity, *NAG* *N*-acetylglucosaminidase activity, *PhOx* phenol oxidase activity, *Perox* peroxidase activity, *SOC* soil organic C, *TN* total N, *TP* total P

*P<0.1, **P<0.05, ***P<0.01

Table 6 Pearson correlation between microbial variables, soil enzyme activities and soil organic C, total N, total P and water content in the microaggregates (n=6)

Variable	Total PLFAs	Bacteria	G^+ bacteria	G^- bacteria	Fungi	Actinomycetes	G^+:G^- ratio	Stress ratio
AP	0.747	0.696	0.663	0.846**	0.586	0.799*	−0.791*	0.195
BG	0.709	0.748	0.665	0.763*	0.666	0.593	−0.496	0.043
CBH	0.067	0.703	0.592	0.683	0.778*	0.575	−0.472	0.205
NAG	0.637	0.681	0.573	0.655	0.720	0.527	−0.418	0.116
PhOx	−0.499	−0.516	−0.547	−0.631	−0.159	−0.403	−0.465	0.443
Perox	−0.776*	−0.858**	−0.741	−0.833**	−0.839	−0.585	−0.571	−0.030
SOC	0.719	0.673	0.693	0.831**	0.537	0.770*	−0.809*	0.191
TN	0.717	0.618	0.622	0.782*	−0.546	0.842**	−0.700	0.217
TP	0.817**	0.803**	0.781*	0.892**	0.568	0.849**	−0.638	−0.119
Water content	0.565	0.526	0.520	0.688	0.290	0.604	−0.016	−0.664

G^+ bacteria Gram-positive bacteria, *G^- bacteria* Gram-negative bacteria, *B:F ratio* the ratio of bacterial biomass to fungal biomass, *G^+:G^- ratio* the ratio of Gram-positive bacterial biomass to Gram-negative bacterial biomass, *Stress ratio* the ratio of cyclopropyl PLFA (cy17:0 and cy19:0) to precursor (16:1ω7 and 18:1ω7), *AP* acid phosphomonoesterase activity, *BG* β-glucosidase activity, *CBH* cellobiohydrolase activity, *NAG* *N*-acetylglucosaminidase activity, *PhOx* phenol oxidase activity, *Perox* peroxidase activity, *SOC* soil organic C, *TN* total N, *TP* total P

*P<0.1, **P<0.05

the diffusion of soluble substrates, and thus affected microbial biomass (Bastida et al. 2006; Van Meeteren et al. 2008; Huseo et al. 2012). Changing availabilities of C, N and water can feedback to microbial resource requirements, which in turn may influence the biomass and composition of microbial community (Billings and Ballantyne 2013; Koyama et al. 2013; Stark et al. 2014; Yoshitake et al. 2015). Significant increased stress ratio and positive correlations between the stress ratio and soil C, N and water content in the large macroaggregates under warmed soils, suggest that water, C and N availability are limiting factors for microbial growth in these aggregates of the warmed soils (Schindlbacher et al. 2011). Microbial biomass may also be limited by the availability of P (Wardle 1992). Cleveland et al. (2002) reported that in a Ferralsol under a tropical rainforest microbial C degradation was strongly constrained by P availability. Microbial P limitation has been observed when C and N are applied in high quantities to tropical parkland soils (Gnankambary et al. 2008). The abundance of most microbial groups was positively correlated with TP in the small macroaggregates and microaggregates and this may indicate that P availability may be one of the limiting factors for microbial growth in the aggregates of warmed soils.

Significant or no significant changes in microbial community composition have been reported under warming in different experiments (Frey et al. 2008; Feng and Simpson 2009; Rinnan et al. 2009; Schindlbacher et al. 2011; Weedon et al. 2014). These discrepancies can be explained by the experimental warming approach, duration of warming treatment, and substrate availability (Karhu et al. 2010; Schindlbacher et al. 2011; Billings and Ballantyne 2013). In our study, PCA analysis indicated that warming only significantly altered soil microbial community composition in the large macroaggregates, and the effect was mainly due to alterations in the community composition of G^- bacteria. This indicates that the microenvironment in the large macroaggregates is easier to be changed by external environmental factors than those of the small macroaggregates and microaggregates. Microaggregate interior regions may be characterized by lower predation and more stable moisture availability for microorganisms than the large macroaggregates (Ranjard et al. 2000; Chenu et al. 2001; Ruamps et al. 2011). It could also be because the microbial communities in the large aggregate fraction are more sensitive to changes in temperature. Meanwhile, the results that significant increased stress ratio and positive correlations between the stress ratio and SOC, TN, and water contents of the large macroaggregates under warmed soils indicate that changes in the availability of water and substrate may be important in changing microbial community composition of the large macroaggregates (Feng and Simpson 2009; Billings and Ballantyne 2013; Yoshitake et al. 2015). Because the interactive effects between microbial resource requirements and substrate or water availabilities is a complex process (Davidson and Janssens 2006; Billings and Ballantyne 2013), understanding the warming effects on changes in microbial community composition of different aggregate fractions, requires additional research. These results suggest that microbial community composition might be more sensitive to experimental warming in larger than smaller soil aggregate fractions.

Soil enzymes in soil aggregates under warming

Soil enzyme activities were not significantly affected by aggregate fractions (Fig. 4). Wang et al. (2015) found relatively higher BG and AP activities in microaggregates

than small macroaggregates, but NAG activity was not affected by aggregate fractions. Grandy et al. (2008) showed that the activities of PhOx, Perox, BG and NAG increased as aggregate size decreased. Probably enzymes are mainly secreted by soil microorganisms to mineralize organic C, N and P from soil organic matter (Waring et al. 2014). The distribution of enzyme activities among aggregate fractions could be partially explained by the distribution of microbial biomass among aggregate fraction (Bell et al. 2010; Zhou et al. 2013).

Soil enzyme activities are affected by soil temperature and moisture (Sardans et al. 2008; Menichetti et al. 2015), microbial biomass (Bell et al. 2010; Zhou et al. 2013), pH and nutrient availability (Stark et al. 2014). In line with changes in the composition of soil microbial communities, we found that soil warming also had no or negative effects on hydrolase activities (Fig. 4) and the ratios of soil hydrolase activities to SMBC. These results confirm that most hydrolase activities are not sensitive to small increases in soil temperature (Allison and Treseder 2008; Bell et al. 2010; Jing et al. 2014; Weedon et al. 2014). The abundance of most microbial groups was positively correlated with most hydrolase activities in the large macroaggregates and small macroaggregates; these enzyme activities were also related to the changes in soil microbial biomass (Bell et al. 2010; Zhou et al. 2013). Microbes may down-regulate enzyme production, especially substrate or other resources such as water becoming limiting (Nannipieri et al. 2012; Stark et al. 2014). The fact that AP activity was significantly and positively correlated with soil moisture in all three aggregates suggests that decreased soil moisture in the warmed soils may decrease AP activity (Sardans et al. 2008).

To date, most reports on the effects of warming on soil enzyme activity refer to hydrolase activities, but little is known about the temperature sensitivity of oxidase activities (Trasar-Cepeda et al. 2007; Jing et al. 2014). Oxidase activities are generally inhibited by low temperatures, low oxygen availability, and low pH (Freeman et al. 2001; Sinsabaugh 2010). Jing et al. (2014) reported that the activity of PhOx increased by increasing incubation temperature. In this study, we also found that warming significantly increased PhOx and Perox activities (Fig. 4) and the ratios of soil oxidase activities to SMBC (Table 3). Increased pH value in the warmed soils in our study may be one of the possible explanations for this result (Sinsabaugh 2010). Higher temperature and lower soil moisture might lead to higher oxygen availability, and thus may increase oxidase activities in the warmed soils (Freeman et al. 2001). In turn, increased soil oxidases activities in the warmed soils might partially explain the decreased SOC content by the experimental warming (Sinsabaugh 2010). Consistent with the changes in microbial community composition, soil warming also shifted soil enzyme activities of large macroaggregates (Fig. 3b). This change suggest that microbial function might be also more sensitive to experimental warming in the larger than the smaller soil aggregate fractions.

Conclusions

We found that warming (about 1 °C) had no effects on soil microbial biomass and hydrolase activities, but significantly increased oxidases activities. In line with the hypothesis, our results suggest that the composition and functions of microbial community may be more sensitive to experimental warming in the larger than in the smaller soil aggregate fractions. Nevertheless, additional research is needed to clear the mechanisms of these results and to determine whether these findings can be extrapolated to longer time and larger spatial scales.

Acknowledgments This study was funded by the National Natural Science Foundation of China (Grant Nos.31370530, 31570482 and 41430529).

References

Allison SD, Treseder KK (2008) Warming and drying suppress microbial activity and carbon cycling in boreal forest soils. Glob Chang Biol 14:2898–2909

Allison SD, Wallenstein MD, Bradford MA (2010) Soil-carbon response to warming dependent on microbial physiology. Nat Geosci 3:336–340

Amato M, Ladd JN (1992) Decomposition of ^{14}C-1abelled glucose and legume material in soils: properties influencing the accumulation of organic residue C and microbial biomass C. Soil Biol Biochem 24:455–464

Bastida F, Moreno JL, Hernández T, García C (2006) Microbiological activity in a soil 15 years after its devegetation. Soil Biol Biochem 38:2503–2507

Belay-Tedla A, Zhou XH, Su B, Wan SQ, Luo YQ (2009) Labile, recalcitrant, and microbial carbon and nitrogen pools of a tallgrass prairie soil in the US Great Plains subjected to experimental warming and clipping. Soil Biol Biochem 41:110–116

Bell TH, Klironomos JN, Henry HAL (2010) Season responses of extracellular enzyme activity and microbial biomass to warming and nitrogen addition. Soil Sci Soc Am J 74:820–828

Billings SA, Ballantyne F (2013) How interactions between microbial resource demands, soil organic matter stoichiometry, and substrate reactivity determine the direction and magnitude of soil respiratory responses to warming. Glob Chang Biol 19:90–102

Bossio DA, Scow KM (1998) Impacts of carbon and flooding on soil microbial communities: phospholipid fatty acid profiles and substrate utilization patterns. Microb Ecol 35:265–278

Castro HF, Classen AT, Austin EE, Norby RJ, Schadt CW (2010) Soil microbial community responses to multiple experimental climate change drivers. Appl Environ Microbiol 76:999–1007

Cavaleri MA, Reed SC, Smith WK, Wood TE (2015) Urgent need for warming experiments in tropical forests. Glob Chang Biol 21:2111–2121

Chenu C, Hassink J, Bloem J (2001) Short-term changes in the spatial distribution of microorganisms in soil aggregates as affected by glucose addition. Biol Fertil Soils 34:349–356

Chotte JL, Ladd JN, Amato M (1998) Sites of microbial assimilation, and turnover of soluble and particulate ^{14}C-labelled substrates decomposing in a clay soil. Soil Biol Biochem 30:205–218

Cleveland CC, Townsend AR, Schmidt SK (2002) Phosphorus limitation of microbial processes in moist tropical forests: evidence from short-term laboratory incubations and field studies. Ecosystems 5:680–691

Cox PM, Betts RA, Jones CD, Spall SA, Totterdell IJ (2000) Acceleration of global warming due to carbon-cycle feedbacks in a coupled climate model. Nature 408:184–187

Davidson EA, Janssens IA (2006) Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature 440:165–173

De Gryze S, Six J, Brits C, Merckx R (2005) A quantification of short-term macroaggregate dynamics: influences of wheat residue input and texture. Soil Biol Biochem 37:55–66

Dorodnikov M, Blagodatskaya E, Blagodatsky S, Fangmeier A, Kuzyakov Y (2009a) Stimulation of r-vs. K-selected microorganisms by elevated atmospheric CO_2 depends on soil aggregate size. FEMS Microbiol Ecol 69:43–52

Dorodnikov M, Blagodatskaya E, Blagodatsky S, Marhan S, Fangmeier A, Kuzyakov Y (2009b) Stimulation of microbial extracellular enzyme activities by elevated CO_2 depends on soil aggregate size. Glob Chang Biol 15:1603–1614

Feng XJ, Simpson MJ (2009) Temperature and substrate controls on microbial phospholipid fatty acid composition during incubation of grassland soils contrasting in organic matter quality. Soil Biol Biochem 41:804–812

Finlay BJ, Maberly SC, Cooper JI (1997) Microbial diversity and ecosystem function. Oikos 80:209–213

Freeman C, Ostle N, Kang H (2001) An enzymic 'latch' on a global carbon store—a shortage of oxygen locks up carbon in peatlands by restraining a single enzyme. Nature 409:149

Frey SD, Drijber R, Smith H, Melillo J (2008) Microbial biomass, functional capacity, and community structure after 12 years of soil warming. Soil Biol Biochem 40:2904–2907

Gnankambary Z, Ilstedt U, Nyberg G, Hien V, Malmer A (2008) Nitrogen and phosphorus limitation of soil microbial respiration in two tropical agroforestry parklands in the south-Sudanese zone of Burkina Faso: the effects of tree canopy and fertilization. Soil Biol Biochem 40:350–359

Grandy AS, Sinsabaugh RL, Neff JC, Stursova M, Zak DR (2008) Nitrogen deposition effects on soil organic matter chemistry are linked to variation in enzymes, ecosystems and size fractions. Biogeochemistry 91:37–49

Guggenberger G, Frey SD, Six J, Paustian K, Elliot ET (1999) Bacterial and fungal cell-wall residues in conventional and no-tillage agroecosystems. Soil Sci Soc Am J 63:1188–1198

He Y, Xu ZH, Chen CR, Burton J, Ma Q, Ge Y, Xu JM (2008) Light fraction and macroaggregate associated organic matters as early indicators for management-induced changes of soil in adjacent native and plantation forests of subtropical Australia. Geoderma 147:116–125

Huseo S, Garcia C, Hernandez T (2012) Severe drought conditions modify the microbial community structure, size and activity in amended and unamended soils. Soil Biol Biochem 50:167–173

Iyyemperumal K, Shi W (2008) Soil enzyme activities in two forage systems following application of different rates of swine lagoon effluent or ammonium nitrate. Appl Soil Ecol 38:128–136

Jackson ML (1964) Soil chemical analysis. Prentice-Hall Inc, Englewood Cliffs, pp 86–92

Jastrow JD, Amonette JE, Bailey VL (2007) Mechanisms controlling soil carbon turnover and their potential application for enhancing carbon sequestration. Clim Chang 80:5–23

Jiang YJ, Sun B, Jin C, Wang F (2013) Soil aggregate stratification of nematodes and microbial communities affects the metabolic quotient in an acid soil. Soil Biol Biochem 60:1–9

Jing X, Wang YH, Chung HG, Mi ZR, Wang SP, Zeng H, He JS (2014) No temperature acclimation of soil extracellular enzymes to experimental warming in an alpine grassland ecosystem on the Tibetan Plateau. Biogeochemistry 117:39–54

Karhu K, Fritze H, Tuomi M, Vanhala P, Spetz P, Kitunen V, Liski J (2010) Temperature sensitivity of organic matter decomposition in two boreal forest soil profiles. Soil Biol Biochem 42:72–82

Koyama A, Wallenstein MD, Simpson RT, Moore JC (2013) Carbon-degrading enzyme activities stimulated by increased nutrient availability in arctic tundra soils. PLoS One 8, e77212

Kristiansen SM, Schjnning P, Thomsen IK, Olesen JE, Kristensen K, Christensen BT (2006) Similarity of differently sized macroaggregates in arable soils of different texture. Geoderma 137:147–154

Lipson DA, Monson RK, Schmidt SK, Weintraub MN (2009) The trade-off between growth rate and yield in microbial communities and the consequences for under-snow soil respiration in a high elevation coniferous forest. Biogeochemistry 95:23–35

Long XA, Chen CR, Xu ZH, Linder S, He JZ (2012) Abundance and community structure of ammonia oxidizing bacteria and archaea in a Sweden boreal forest soil under 19-year fertilization and 12-year warming. J Soil Sediments 12:1124–1133

Martens R (1995) Current methods for measuring microbial biomass C in soil: potentials and limitations. Biol Fertil Soils 19:87–99

Menichetti L, Ortigoza ALR, Garcia N, Giagnoni L, Nannipieri P, Renella G (2015) Thermal sensitivity of enzyme activity in tropical soils assessed by the Q(10) and equilibrium model. Biol Fertil Soils 51:299–310

Mummey DL, Stahl PD (2004) Analysis of soil whole-and inner-microaggregate bacterial communities. Microb Ecol 48:41–50

Nannipieri P, Giagnoni L, Renella G, Puglisi E, Ceccanti B, Masciandaro G, Fornasier F, Moscatelli MC, Marinari S (2012) Soil enzymology: classical and molecular approaches. Biol Fertil Soils 48:743–762

Nelson DW, Sommers LE (1982) Carbon and organic matter. In: Page, Mille AL, Keeney RH (eds) Methods of soil analysis part 2: chemical and microbiological properties. American Society of Agronomy, Madison, pp 561–579

Oades JM (1984) Soil organic matter and structural stability: mechanisms and implications for management. Plant Soil 76:319–337

Ranjard L, Poly F, Combrisson J, Richaume A, Gourbiere F, Thioulouse J, Nazaret S (2000) Heterogeneous cell density and genetic structure of bacterial pools associated with various soil microenvironments as determined by enumeration and DNA fingerprinting approach (RISA). Microb Ecol 39:263–272

Rinnan R, Michelsen A, Jonasson S (2008) Effects of litter addition and warming on soil carbon, nutrient pools and microbial communities in a subarctic heath ecosystem. Appl Soil Ecol 39:271–281

Rinnan R, Stark S, Tolvanen A (2009) Responses of vegetation and soil microbial communities to warming and simulated herbivory in a subarctic heath. J Ecol 97:788–800

Ruamps LS, Nunan N, Chenu C (2011) Microbial biogeography at the soil pore scale. Soil Biol Biochem 43:280–286

Sardans J, Peñuelas J, Estiarte M (2008) Changes in soil enzymes related to C and N cycle and in soil C and N content under prolonged warming and drought in a Mediterranean shrubland. Appl Soil Ecol 39:223–235

Schindlbacher A, Rodler A, Kuffner M, Kitzler B, Sessitsch A, Zechmeister-Boltenstern S (2011) Experimental warming effects on the microbial community of a temperate mountain forest soil. Soil Biol Biochem 43:1417–1425

Sinsabaugh RL (2010) Phenol oxidase, peroxidase and organic matter dynamics of soil. Soil Biol Biochem 42:391–404

Six J, Bossuyt H, Degryze S, Denef K (2004) A history of research on the link between (micro) aggregates, soil biota, and soil organic matter dynamics. Soil Tillage Res 79:7–31

Stark S, Mannisto MK, Eskelinen A (2014) Nutrient availability and pH jointly constrain microbial extracellular enzyme activities in nutrient-poor tundra soils. Plant Soil 383:373–385

Streit K, Hagedorn F, Hiltbrunner D, Portmann M, Saurer M, Buchmann N, Wild B, Richter A, Wipf S, Siegwolf RTW (2014) Soil warming alters microbial substrate use in alpine soils. Glob Chang Biol 20: 1327–1338

Tabatabai MA (1994) Soil enzymes. In: Mickelson SH, Bifham JM (eds) Methods of Soil Analysis. Part 2: Microbiological and Biochemical Properties. Soil Science Society of America, Inc, Madison, pp 775–833

Tian L, Shi W (2014) Soil peroxidase regulates organic matter decomposition through improving the accessibility of reducing sugars and amino acids. Biol Fertil Soils 50:785–794

Trasar-Cepeda C, Gil-Sotres F, Leiros MC (2007) Thermodynamic parameters of enzymes in grassland soils from Galicia, NW Spain. Soil Biol Biochem 39:311–319

Van Gestel M, Merckx R, Vlassak K (1996) Spatial distribution of microbial biomass in microaggregates of a silty–loam soil and the relation with the resistance of microorganisms to soil drying. Soil Biol Biochem 28:503–510

Van Meeteren MJM, Tietema A, Van Loon EE, Verstraten JM (2008) Microbial dynamics and litter decomposition under a changed climate in a Dutch heathland. Appl Soil Ecol 38:119–127

Wang H, He ZL, Lu ZM, Zhou JZ, Van Nostrand JD, Xu XH, Zhang ZJ (2012) Genetic linkage of soil carbon pools and microbial functions in subtropical freshwater wetlands in response to experimental warming. Appl Environ Microb 78:7652–7661

Wang RZ, Dorodnikov M, Yang S, Zhang YY, Filley TR, Turco RF, Zhang YG, Xu ZW, Li H, Jiang Y (2015) Responses of enzymatic activities within soil aggregates to 9-year nitrogen and water addition in a semi-arid grassland. Soil Biol Biochem 81:159–167

Wardle DA (1992) A comparative assessment of factors which influence microbial biomass carbon and nitrogen levels in soil. Biol Rev 67: 321–358

Waring BG, Weintraub SR, Sinsabaugh RL (2014) Ecoenzymatic stoichiometry of microbial nutrient acquisition in tropical soils. Biogeochemistry 117:101–113

Weedon JT, Aerts R, Kowalchuk GA, Van Bodegom PM (2014) No effects of experimental warming but contrasting seasonal patterns for soil peptidase and glycosidase enzymes in a sub-arctic peat bog. Biogeochemistry 117:55–66

Yoshitake S, Tabei N, Mizuno Y, Yoshida H, Sekine Y, Tatsumura M, Koizumi H (2015) Soil microbial response to experimental warming in cool temperate semi-natural grassland in Japan. Ecol Res 30:235–245

Zhang NL, Xia JY, Yu XJ, Ma KP, Wan SQ (2011) Soil microbial community changes and their linkages with ecosystem carbon exchange under asymmetrically diurnal warming. Soil Biol Biochem 43: 2053–2059

Zhang SX, Li Q, Lü Y, Zhang XP, Liang WJ (2013) Contributions of soil biota to C sequestration varied with aggregate fractions under different tillage systems. Soil Biol Biochem 62:147–156

Zhou GY, Wei XH, Wu YP, Liu SZ, Huang YH, Yan J, Zhang D, Zhang DQ, Liu JX, Meng Z (2011) Quantifying the hydrological responses to climate change in an intact forested small watershed in Southern China. Glob Chang Biol 17:3736–3746

Zhou JZ, Xue K, Xie JP, Deng Y, Wu LY, Cheng XL, Fei SF, Deng SP, He ZL, Nostrand JDV, Luo YQ (2012) Microbial mediation of carbon-cycle feedbacks to climate warming. Nat Clim Chang 2:106–110

Zhou XQ, Chen CR, Wang YF, Xu ZH, Han HY, Li LH, Wan SQ (2013) Warming and increased precipitation have differential effects on soil extracellular enzyme activities in a temperate grassland. Sci Total Environ 444:552–558