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Editor: Denise Akob

Editor

Biogeosciences

Dear Dr. Akob,

Herewith, I would like to submit a revised version of our manuscript entitled '**Effects of temperature on the composition and diversity of bacterial communities in bamboo soils at different elevations**' (bg-2017-116), to be considered for publication in *Biogeosciences*.

We have thoroughly revised the manuscript according to your suggestions. We would like to gratefully acknowledge the insightful perspectives and suggestions that helped us in improving the manuscript. We hope we have adequately addressed them and that our manuscript is now ready for publication. Please find below our point-by-point responses.

Once again, we would like to thank you for your prompt management and constructive comments on our manuscript, and we are looking forward to hearing from you.

Kind regards,

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Responses to the Editor's comments

BG-2018-116-RC1

Comments: Currently, the discussion is highly microbial/soil focused and it would be nice to discuss the implications for plant interactions. Also, link back to management of the former plantations--how will changes in soil respiration/activity affect these legacy sites? or further management of them?

Response: We have added some paragraphs in the Discussion to link our findings to plant interactions and management practices:

“In d0 samples, which represent the original composition of the bamboo soils, the bacterial diversity was higher in the 1,800 m soils, followed by the 600 and 1,200 m soils. Communities with higher diversity are reportedly more resistant to environmental changes (Loreau and de Mazancourt 2013). In a study by Ren et al (2015) in rice paddies, the diverse soil communities were more resistant to elevated CO₂ and temperature than the less diverse foliar bacterial communities. The increasing concentration of recalcitrant C with increasing elevation (Wang et al., 2016) could be helpful in providing more carbon resources to the community at high elevation. Together, these findings indicate that bamboo soil bacterial communities with higher diversity could be more capable to maintain soil community and function when exposed to climatic changes and subjected to management at high elevation (1,800 m).” (p. 11, line15 to line 26)

“*Bradyrhizobium* of α -*Proteobacteria* at 1,800 m increased at all three incubation temperatures. This genus includes species capable of nitrogen fixation and may significantly contribute to soil function (Yarwood et al., 2009). The increase in their abundance might explain the elevated SON. Moreover, these bacteria are plant growth-promoting bacteria, stimulating plant growth by fixing N₂, increasing the availability of nutrients in the rhizosphere, positively influencing root growth and morphology, and promoting other beneficial plant-microbe symbioses (Vessey, 2003). Their response to incubation temperatures indicates their potential roles in bamboo growth and responses to application of fertilizers under climatic

change.” (p. 14, lines 2–11)

“Within β -*Proteobacteria*, the abundant genus *Burkholderia* is nutritionally versatile and is commonly found in rhizosphere soils. Their functional diversity, including nitrogen fixation and plant growth promotion (Coenye and Vandamme, 2003), could help maintain soil community stability.” (p. 14, lines 15–19)

“*Actinobacteria* are involved in the organic matter degradation. Under climatic changes, managements of bamboo forests need to consider the responses of *Actinobacteria* to temperature, especially that in N fertilizers, since the abundance of this phylum was positive affected by N fertilization treatments (Zhou et al., 2015).” (p. 14, lines 22 to line 26)

Comments: pg. 4, l. 9: correct spelling to oligotrophs

Response: The word has been corrected (p. 4, line 10).

Comments: pg. 4, l. 6-9: language is a bit awkward and pluralization is missing.

Response: This section has been rephrased as “Soil bacterial communities include different phylotypes that likely represent different functional groups, and their relative abundances are affected by carbon (C) availability. For example, some members of *Proteobacteria* are considered copiotrophs, and their relative abundances appear to be higher in C-rich environments.” (p. 4, lines 6–10).

Comments: pg. 6: please release the sequences prior to resubmission.

Response: The sequences (accession number: SRS1923345) have been released.

Comments: pg. 8, l. 4: define day/time notation, e.g., "d28", here to make it clear to the reader for the rest of the paper.

Response: We would like to kindly point out that we had defined this notation at first use in the previous revision. To further improve clarity, we have rephrased it as “(after day 28 [d28])” in the current manuscript (p. 8, line 2).

Comments: pg. 8, l. 17: OTU's formed is not the correct word choice. Please change to an based on an OTU cutoff of ≤ 0.03

Response: We have corrected this accordingly (p. 8, line 15).

Comments: pg. 13, l. 15: correct to "life strategies as an oligotroph or copiotroph."

Response: We have corrected this accordingly (p. 15, line 11).

Comments: Fig. 3: optional--change to a 3 part single column graph

Response: Fig. 3 was changed to a 3-part single-column graph.

Comments: Fig. 4: please check to see if this is readable for those that are color-blind. As is, the slight variations in darkness of the reds could be difficult to distinguish.

Response: In agreement with the editor's apt consideration, we have presented the data originally depicted in Fig. 4 in the newly added Table 1.

1 **Effects of temperature on the composition and diversity of bacterial**
2 **communities in bamboo soils at different elevations**

3

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5

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1 **Abstract.** Bamboo is an important resource distributed in mountain areas in Asia.
2 Little is known about the impact of temperature changes on bamboo soil bacterial
3 communities. In this study, responses of bacterial communities collected at 600,
4 1,200, and 1,800 m to different incubation temperatures (15°C, 20°C, and 35°C)
5 were examined using barcoded pyrosequencing and soil analyses. Soil respiration
6 was greater at higher elevation and incubation temperature. The bacterial diversity
7 decreased after 112 days of incubation at 35°C. Before incubation, *Acidobacteria*
8 and *Proteobacteria* were the most abundant phyla in all communities. The relative
9 abundance of *Acidobacteria* generally decreased after 112 days of incubation at the
10 three temperatures. α -*Proteobacteria* showed a similar trend, while γ -*Proteobacteria*
11 increased after incubation, except in samples from 1,800 m incubated at 35°C.
12 Non-metric multi-dimensional scaling analysis revealed structural variability under
13 different incubation times and temperatures. Principal component analysis indicated
14 that the bacterial structure in samples incubated at 35°C correlated with temperature
15 and soil respiration, while structures in samples incubated at 15°C and 20°C
16 correlated with time. These results suggest that a temperature rise could result in
17 increasing soil respiration and soluble carbon and nitrogen consumption, and
18 differentially influences bacterial diversity and structure at different elevations.

19

20 *Keywords:* temperature, bamboo, soil

1 **1 Introduction**

2

3 Temperature is one of the most important factors influencing soil organic matter
4 decomposition and microbial communities. For example, temperature significantly
5 affects the soil microbial phospholipid fatty acid composition associated with straw
6 decomposition at the early stage (Zhou et al., 2016). Bacterial abundance increases
7 in conditions of elevated temperature and CO₂ concentration (Castro et al., 2010).
8 The complex responses of bacterial composition and diversity of bamboo soils
9 across altitudinal gradients have been suggested to result from interactions with
10 multiple factors, including temperature (Lin et al., 2015).

11 In Taiwan, moso bamboo (*Phyllostachys pubescens*) is an important versatile
12 forest resource that is widely used for food, construction, and as a furniture material.
13 It is distributed from low to high mountain regions at approximately 1,800 m above
14 sea level (a.s.l.). Management practices for increasing bamboo production, including
15 regular removal of understory vegetation, tillage, and fertilizer application, can
16 increase the soil CO₂ efflux (Liu et al., 2011) and water-soluble organic N
17 concentration (Wu et al., 2010). However, these management practices can lower the
18 microbial functional diversity (Xu et al., 2008). Considering the effects of bamboo
19 plantations on soil properties and microbial communities, it is worth elucidating the
20 changes in bamboo soil bacterial communities under environmental changes.

21 Our previous study revealed that bamboo invasion could increase bacterial
22 diversity and alter the bacterial structure of adjacent cedar forest soils (Lin et al.,
23 2014). Soil bacterial diversity in bamboo plantations showed a hump-backed trend,
24 with less diversity at low and high elevations, and maximum diversity at middle
25 elevations, and community structure formed different clusters at different elevations
26 (Lin et al., 2015). Our parallel study showed that invasion of bamboo into adjacent

1 forest soils increased humification of soil organic matter (SOM) (Wang et al.,
2 2016b). In addition, changes in the SOM pool and the rate of humification with
3 elevation were primarily affected by changes in climatic conditions along the
4 elevation gradient in the bamboo plantations (Wang et al., 2016a). However, it is not
5 known whether bamboo soil bacterial groups respond to temperature changes.

6 Soil bacterial communities include different phylotypes that likely represent
7 different functional groups, and their relative abundances are affected by carbon (C)
8 availability. For example, some members of *Proteobacteria* are considered
9 copiotrophs, and their relative abundances appear to be higher in C-rich
10 environments. In contrast, oligotrophs (e.g., *Acidobacteria*) can live in stressful
11 environmental conditions (Fierer et al., 2007). However, little is known about how
12 these two groups respond to the environmental temperature changes. Here, we
13 hypothesized that the temperature changes would alter the structure and diversity of
14 soil bacterial communities at different elevations, and that bacterial taxa, including
15 copiotrophic and oligotrophic groups, would have distinct responses to altered
16 nutrient availability caused by temperature changes. To test these hypotheses, soil
17 communities sampled at bamboo plantations at three elevations were incubated at
18 different temperatures and investigated by using the barcoded pyrosequencing
19 technique. The objectives of this study were to elucidate (1) changes in soil organic
20 carbon, nitrogen, and respiration at elevation gradients and at different incubation
21 temperatures, (2) differences in bacterial structure and diversity under different
22 incubation temperatures and periods, and (3) changes in the abundances of different
23 phylogenetic groups at different incubation temperatures.

24

25 **2 Methods**

26

1 **2.1 Site description and soil sampling**

2

3 This study was conducted in Mt. Da-an, a subtropical mountain area in Nantou
4 County, central Taiwan (23°42' N, 120°41' E). The soil samples were collected from
5 moso bamboo plantations at 600, 1,200, and 1,800 m a.s.l. along a county road. The
6 three sampling sites were all dominated by moso bamboo with few understory plants.
7 Based on weather station records and the temperature–elevation correlation, the
8 annual mean air temperature was estimated as 20.3°C at 600 m, 17.2°C at 1,200 m,
9 and 14.1°C at 1,800 m with a decrease of 0.52°C per 100 m elevation gain (Wang et
10 al., 2016a). At each elevation, three 25 × 25 m plots were established along transect
11 lines in March 2015. Within each plot, three subsamples were collected with a soil
12 auger 8 cm in diameter and 10 cm deep and pooled. Visible detritus, such as roots
13 and litter, were manually removed prior to passing the soil through a 2-mm sieve.
14 Soil samples collected at each elevation were combined and homogenized for
15 further incubation and analysis. The sieved soils were stored at 4°C before
16 incubation experiments.

17

18 **2.2 Incubation experiment and soil analysis**

19

20 Three replicates (25 g each) from each elevation were incubated at 15°C, 20°C, or
21 35°C for 112 days. The temperatures of 15°C and 20°C were selected based on the
22 mean annual temperature, while 35°C was selected to simulate the summer
23 condition. During the entire incubation period, the soil moisture was maintained at
24 60% of the water-holding capacity. At various incubation times, soil samples were
25 taken from the same container. Soil respiration (CO₂-C) was measured as described
26 (Huang et al., 2014). Soluble organic carbon (SOC) and nitrogen (SON) were

1 extracted from the soil samples after different incubation periods with 2 M KCl, and
2 measured with the Fisons NA1500 elemental analyzer (ThermoQuest Italia, Milan,
3 Italy) as described (Huang et al., 2014).

4

5 **2.3 Barcoded pyrosequencing of the 16S rRNA genes**

6

7 Soil community DNA was extracted using the PowerSoil[®] Soil DNA Isolation kit
8 (MoBio Industries, Carlsbad, CA, USA) in accordance with the manufacturer's
9 instructions. The V1 to V2 regions of the bacterial 16S rRNA gene were amplified
10 using 27F and 338R primers (Lane, 1991). Polymerase chain reactions (PCR) were
11 performed as described previously (Lin et al., 2015). Secondary PCR (using 3 cycles
12 instead of 20) was carried out to barcode the DNA in each sample. The unique and
13 error-correcting bar codes facilitated sorting of sequences from a single
14 pyrosequencing run (Hamady et al., 2008). The barcoded PCR products were
15 purified on a column filter using a PCR clean-up system (Viogene Biotek Corp.,
16 New Taipei City, Taiwan). The qualities and concentrations of the purified barcoded
17 PCR products were determined using a NanoDrop spectrophotometer (Thermo
18 Fisher Scientific, Waltham, MA, USA). Amplicon pyrosequencing was performed
19 by Mission Biotech (Taipei, Taiwan) using the 454/Roche GS-FLX Titanium
20 Instrument (Roche, Branchburg, NJ, USA). All sequences have been submitted to
21 the Short Read Archives under accession number SRS1923345.

22

23 **2.4 Sequence analyses**

24

25 The pyrosequences were processed through the RDP pyrosequencing pipeline
26 (<http://pyro.cme.msu.edu>; RDP Release 11.5; release date: 2016.09.30). The

1 sequences were assigned to the samples by recognition of the bar code from a tag file,
2 followed by trimming of bar codes, primers, and linkers. The pyrosequences were
3 filtered, and sequences that did not contain Ns, were more than 200 bp in length, and
4 possessed quality scores >25 were selected for further analyses. Taxonomic
5 information was analyzed using the naïve Bayesian rRNA classifier in RDP (Wang et
6 al., 2007). The Shannon diversity index was calculated based on Complete Linkage
7 Clustering data for operational taxonomic units (OTUs), with an evolutionary distance
8 of 0.03. The distribution of shared OTUs among the communities was obtained using
9 the Mothur program (Schloss et al., 2009). Non-metric multi-dimensional scaling
10 (NMDS) based on the distribution of shared OTUs was plotted by using the PRIMER
11 V6 software (Clarke & Gorley, 2006). The Mantel tests as implemented in PRIMER
12 V6 software was used to analyze the relationships between bacterial communities,
13 phylogenetic groups and soil properties. Principal component analysis (PCA) to
14 determine the relationship between bacterial community and soil properties was
15 carried out using R v.3.2.1.

16

17 **3 Results**

18

19 **3.1 Soil respiration, SOC, and SON**

20

21 Data on soil respiration CO₂-C in samples taken from three elevations and incubated
22 at different temperatures are shown in Fig. 1. Under the same temperature, the soil
23 samples collected at higher elevation, especially those from 1,800 m, had a
24 significantly higher soil respiration rate than those obtained at lower elevation. The
25 soil respiration rate increased with temperature within each elevation. At 35°C, the
26 soil respiration rate decreased significantly with incubation time. At 15°C and 20°C,

1 the respiration rates of some soil samples slightly increased in the early incubation
2 period (until day 28 [d28]) (Fig. 1). Because the respiration rate was stabilized after
3 d72, respiration rate analyses were conducted only up to this time point.

4 At d0, the SOC and SON contents of the soils increased significantly with
5 elevation (Fig. 2). Compared to d0, the concentration of SOC in the high-elevation
6 soils (1,800 m) decreased, while those at 600 and 1,200 m increased after 112 days
7 (d112) of incubation at three temperatures. Incubation at higher temperature (35°C)
8 resulted in higher SOC content than that at lower temperatures (15°C and 20°C). In
9 most samples, SON content increased until d28 of incubation, but was decreased at
10 d112.

11

12 **3.2. Community diversity at different temperatures**

13

14 The soil bacterial diversity at three elevations at different incubation temperatures
15 was determined based on an OTU cutoff of ≤ 0.03 . Based on the Shannon diversity
16 index, the bacterial diversity of soils incubated at 35°C decreased after long
17 incubation (d112). Under incubation at 15°C or 20°C, the bacterial diversity slightly
18 increased at d7 and d28, and decreased at d112 (Fig. 3). Analysis of the β -diversity
19 revealed that though incubated with different temperature, the communities at the
20 same elevation formed a cluster different from those at other elevation
21 (Supplementary Fig. 1).

22

23 **3.3 Community composition at different incubation temperatures**

24

25 Before incubation, *Acidobacteria* and *Proteobacteria* were the two most abundant
26 phyla in soils from all three elevations, together representing more than 60% of the

1 soil bacterial communities (Table 1). Within the *Proteobacteria*, *α-Proteobacteria*
2 were predominant (Table 1). At 1,800 m, *Bacteroidetes* accounted for 8% of the
3 community, while they comprised only 2–4% of the communities at the two other
4 elevations. The relative abundance of *Actinobacteria* was 4–6%, and the other
5 phylogenetic groups represented less than 3% of the communities.

6 Bacterial groups of the soil communities showed different responses to the
7 incubation temperature. The relative abundance of *Acidobacteria* at 600 and 1,200 m
8 gradually decreased over the entire incubation period at all temperatures (Fig. 4a-4f).
9 At 1,800 m, it increased during the first seven days of incubation at 35°C, and
10 decreased thereafter at all temperatures (Fig. 4i). The relative abundance of
11 *α-Proteobacteria* showed similar trends; it gradually decreased at 600 and 1,200 m
12 over the entire incubation period at different temperatures, except at d7 at 600 m,
13 20°C, and at d7 at 1,200 m, 35°C (Fig. 4a-4f). At 1,800 m, the changes in abundance
14 were different. *α-Proteobacteria* were elevated at d7 and d112, but were lower at
15 d28 of incubation at 15°C and 20°C. Their abundance decreased over time under
16 incubation at 35°C (Fig. 4g-4i). With regard to *γ-Proteobacteria*, their relative
17 abundance mostly increased over incubation, except in soils sampled at 1,800 m
18 under incubation at 35°C, in which it was increased at d7, but decreased at d28 and
19 d112 (Fig. 4i). The relative abundance of *Chloroflexi* also increased over incubation,
20 except in samples taken at 600 m, incubated at 15 °C, on d112. Some other phyla
21 demonstrated inconsistent changes under increased temperature. The abundances of
22 *Actinobacteria* at 1,200 m and 1,800 m increased at higher temperature (Fig. 4d-4i),
23 while it decreased in samples taken at 600 m (Fig. 4a-4c). Likewise, *Bacteroidetes*
24 showed inconsistent changes after different incubation times and temperatures (Fig.
25 4a-4i).

1 The changes in relative abundance of some abundant genera are shown in Fig. 5.
2 The relative abundance of acidobacterial-GP1 generally decreased over the entire
3 incubation period at all temperatures, except at 35°C (Fig. 5i). The α -proteobacterial
4 *Bradyrhizobium* showed similar trends, while its relative abundance at 1,800 m
5 increased at all three incubation temperatures (Fig. 5g-5i). Within β -Proteobacteria,
6 the relative abundance of *Burkholderia* at 1,200 m decreased over incubation, except
7 in samples incubated at 15°C in the first seven days (Fig. 5d). With regard to
8 γ -Proteobacteria, *Dyella* in samples from 1,200 m decreased under all three
9 incubation temperatures (Fig. 5d-5f), and its abundance increased mostly in
10 communities at 600 m and 1,800 m (Fig. 5a-5c, 5g-5i). The relative abundance of
11 *Mucilaginibacter* of *Bacteroidetes* at 1,800 m increased greatly in samples incubated
12 at 35 °C, on d28 and d112 (Fig. 5i).

13 NMDS analysis based on the distribution of shared OTUs also revealed the
14 variability in bacterial structure under different incubation times and temperatures
15 (Fig. 6). The bacterial community at 1,800 m formed a different cluster from those at
16 600 and 1,200 m. Incubation at higher temperature (35°C) led to a bacterial structure
17 different from those at 15°C and 20°C. Incubation time also changed the bacterial
18 structure. The bacterial structure under long incubation (at d112) was different from
19 those at d7 and d28.

20 PCA analysis revealed the correlation between bacterial structure and
21 environmental factors. When incubated at 35°C, bacterial structure correlated with
22 temperature and soil respiration CO₂-C, while at 15°C and 20°C, bacterial structure
23 correlated with incubation time (Fig. 7).

24

25 **4 Discussion**

26

1 The present study revealed that the SOC content was higher at high incubation
2 temperature and decreased with increasing elevation after long incubation. The soil
3 respiration CO₂-C rate was greater at higher elevation. Similarly, a previous study in
4 tundra soils using different incubation temperatures reported higher respiration rate
5 at high temperatures (Stark et al., 2015). Incubation at increasing temperatures
6 enhanced the soil microbial activity and led to an increase in soil respiration in forest
7 mesocosms (Lin et al., 2001). In our study, the respiration rate decreased after long
8 incubation. This could be due to the exhaustion of labile compounds after microbial
9 decomposition (Zhou et al., 2016). The decrease in bacterial diversity at high
10 elevation and high incubation temperature, calculated from abundance data of the
11 phylogenetic groups, could also be the result of nutrient exhaustion after long
12 incubation. In addition, the correlation between soil respiration and bacterial
13 structure in the soil samples under incubation at 35°C suggests the adaption and high
14 activity of bacterial communities at higher temperature.

15 In d0 samples, which represent the original composition of the bamboo soils, the
16 bacterial diversity was higher in the 1,800 m soils, followed by the 600 and 1,200 m
17 soils. Communities with higher diversity are reportedly more resistant to
18 environmental changes (Loreau and de Mazancourt 2013). In a study by Ren et al.
19 (2015) in rice paddies, the diverse soil communities were more resistant to elevated
20 CO₂ and temperature than the less diverse foliar bacterial communities. The
21 increasing concentration of recalcitrant C with increasing elevation (Wang et al.,
22 2016) could be helpful in providing more carbon resources to the community at high
23 elevation. Together, these findings indicate that bamboo soil bacterial communities
24 with higher diversity could be more capable to maintain soil community and
25 function when exposed to climatic changes and subjected to management at high
26 elevation (1,800 m).

1 The bacterial community structure varied over different incubation periods and
2 temperatures. Based on the abundance data of phylogenetic groups, the communities
3 at the three elevations formed different clusters as compared to the results of our
4 previous study (Lin et al., 2015). The different soil bacterial structures at different
5 elevations can be explained by differences in soil management. The effects of
6 management practices on soil microbial community can persist over time (Keiser et
7 al., 2011). Recently, bamboo shoot harvest and timber production has moved from
8 about 600 m to 1,200 m in the study area. Soils at different elevation have distinct
9 soil SOC and SON contents, which could result in different forces to alter bacterial
10 communities. Incubation temperature also had an effect on community structure.
11 Warming in the experimental field in a previous study in the Arctic environment
12 caused a significant increase in the abundance of fungi and bacteria (Yergeau et al.,
13 2012). The quantity of SOC and CO₂ flux has been shown to increase under
14 warming condition (Zhang et al., 2005; Zhou et al., 2011). Increasing temperature
15 increased relative bacterial growth in arable soils from southern Sweden
16 (Bárcenas-Moreno et al., 2009), and particularly, the abundance of genes involved in
17 labile carbon degradation in a tall-grass prairie ecosystem in Central Oklahoma,
18 USA (Zhou et al., 2011), and led to C loss. In the present study, the shifts in bacterial
19 communities at three elevations could reflect differences in nutrient availability,
20 including SOC and SON, and bacterial activity under different incubation
21 temperatures and at distinct time points during incubation.

22 Bacterial community structure under incubation at 35°C was affected by
23 temperature, while under incubation at 15°C and 20°C, it correlated with incubation
24 time (Fig. 7). Warming has been shown to change the bacterial structure of alpine
25 meadow soils (Xiong et al., 2014) and to cause thermal-adaption functional shift of
26 microbial communities (Rousk et al., 2012). Recent studies have observed changes

1 in temperature sensitivity of microbial communities along incubation time. Shifts in
2 microbial communities in response to warming occur after a few years (Yergeau et
3 al., 2012) or even only a few months (Xiong et al., 2014). However, some studies
4 revealed no significant community changes over time owing to warming (Allison et
5 al., 2010; Zhou et al., 2011). The present work revealed community structure
6 differences after incubation for only about four months, suggesting that the bacterial
7 communities in bamboo soils at elevation are highly sensitive to temperature
8 changes, even though they faced a relative short-time warming condition.

9 The responses of phylogenetic abundances to temperature differed. As for
10 *Acidobacteria*, the abundance generally decreased with increasing temperature. This
11 is in accordance with previous studies showing decreases in the relative abundance
12 of *Acidobacteria* in warming soils (Xiong et al., 2014; Yergeau et al., 2012).
13 *Acidobacteria* are known as slow-growing (oligotrophic) bacteria that prefer low
14 nutrient availability (Fierer et al., 2007) and possess high maximum growth
15 efficiency (Roller and Schmidt, 2015). Warming conditions in the soil could increase
16 substrate availability and might favor fast-growing (copiotrophic) microorganisms,
17 which are more sensitive to nutrient availability (Männistö et al., 2016). Thus, the
18 decreases in the abundance of *Acidobacteria* could reflect their interactions with
19 copiotrophic species. The abundance of *Acidobacteria* could also be limited by high
20 temperatures (Stark et al., 2015).

21 Under increased temperature, some phyla in our study responded differently
22 from previous studies. Increasing α -*Proteobacteria* abundance has been observed in
23 short warming conditions (Xiong et al., 2014) and in a range of Antarctic
24 environments (Yergeau et al., 2012). α -*Proteobacteria* are mostly fast-growing
25 (copiotrophic) bacteria, and are known to be positively correlated with soil available
26 C pools (Nemergut et al., 2010). The decreases in the abundance of

1 *α-Proteobacteria* in the present study could reflect the decrease in SOC content,
2 which was exhausted by soil respiration CO₂-C after incubation. *Bradyrhizobium* of
3 *α-Proteobacteria* at 1,800 m increased at all three incubation temperatures. This
4 genus includes species capable of nitrogen fixation and may significantly contribute
5 to soil function (Yarwood et al., 2009). The increase in their abundance might
6 explain the elevated SON. Moreover, these bacteria are plant growth-promoting
7 bacteria, stimulating plant growth by fixing N₂, increasing the availability of
8 nutrients in the rhizosphere, positively influencing root growth and morphology, and
9 promoting other beneficial plant–microbe symbioses (Vessey, 2003). Their response
10 to incubation temperatures indicates their potential roles in bamboo growth and
11 responses to application of fertilizers under climatic change.

12 The increase in abundance of *γ-Proteobacteria* after incubation in our study
13 differed from that in the soil community sampled at elevated soil temperature.
14 *γ-Proteobacteria* showed a lower relative abundance under elevated temperature
15 than in the ambient temperature control (Ren et al., 2015). Within *β-Proteobacteria*,
16 the abundant genus *Burkholderia* is nutritionally versatile and is commonly found in
17 rhizosphere soils. Their functional diversity, including nitrogen fixation and plant
18 growth promotion (Coenye and Vandamme, 2003), could help maintain soil
19 community stability. In addition, *Actinobacteria* and *Bacteroidetes* showed variable
20 responses at different temperatures. These phyla also prefer nutrient-rich
21 environments (Nemergut et al., 2010). Differences in vegetation and litter quality
22 among the study sites might explain this variation. *Actinobacteria* are involved in
23 the organic matter degradation. Under climatic changes, managements of bamboo
24 forests need to consider the responses of *Actinobacteria* to temperature, especially
25 that in N fertilizers, since the abundance of this phylum was positive affected by N
26 fertilization treatments (Zhou et al., 2015). The *Mucilaginibacter* species of

1 *Bacteroidetes* are capable of degrading polysaccharides (Pankratov et al., 2007);
2 thus, the increase in their abundance can be explained by the increase in plant
3 residues in the soil. A previous study suggested the relationship of elevation and
4 temperature with the decomposition of recalcitrant C (Wang et al., 2016a). After
5 decomposition of labile C, the availability of recalcitrant C can strongly affect the
6 community. Moreover, based on a literature survey by Ho et al. (2017), there is little
7 consistency in oligotrophic and copiotrophic phyla of bacterial communities. The
8 microorganisms can display a variety of metabolic characteristics and adjust
9 between high and low substrate use efficiency, to adapt to environmental changes.
10 Therefore, shifts in the relative abundances of bacterial taxa may not necessarily
11 indicate their life strategies as an oligotroph or copiotroph, but might just reveal the
12 response of a community to such local factors (Ho et al., 2017). Further study,
13 including more comprehensive temperature gradients and more detailed time-course
14 analysis, will be necessary to elucidate the exact influences of temperature on soil
15 communities.

16 *Acidobacteria* and α -*Proteobacteria*, which comprised more than 10% of the
17 communities before incubation, tended to decrease after incubation. Groups with
18 lower abundance before incubation, especially, γ -*Proteobacteria*, showed an
19 increasing trend after incubation. These patterns were similar to those shown in
20 communities of a rice paddy and desert soils (Wang et al., 2012; Ren et al., 2015), in
21 which numerically dominant bacterial phyla/classes were reduced, while originally
22 rare groups increased in relative abundance after exposure to environmental changes.
23 These results suggest that shifts of bacterial populations facing environmental
24 changes may follow predictable patterns.

25

26 **5 Conclusions**

1

2 Our results revealed that an increase in temperature could result in increased soil
3 respiration CO₂-C and consumption of SOC and SON contents, which directly or
4 indirectly influence the bacterial diversity and structure of bamboo soils at different
5 elevations. In addition, the different responses of bacterial groups to the temperature
6 changes suggest the adaptation of soil communities to global warming-related
7 climatic changes. This study highlights the need for further research on the
8 physiologic and ecologic roles of soil bacterial members, such as *Acidobacteria*, α -
9 and γ -*Proteobacteria*, in climatic change in forest ecosystems.

10

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15 molecular analyses.

16

17 *Competing financial interests.* The authors declare that they have no competing
18 interests.

19

20 *Author Contributions.* YTL performed statistical analyses; ZJ built statistical models;
21 CYC interpreted ecological rationale; ZJ and CYC formulated the study hypothesis
22 and developed the methodology. YTL wrote, and ZJ and CYC edited the manuscript.
23 All authors read and approved the final manuscript.

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1 **Table 1.** Relative abundances (%) of different phylogenetic groups in the bamboo
 2 soil bacterial communities at different elevations.

3

Phylogenetic groups	600 m	1200 m	1800 m
<i>Acidobacteria</i>	46.9	43.6	39.8
<i>Actinobacteria</i>	5.5	6.9	4.3
<i>Bacteroidetes</i>	4.3	2.0	8.1
<i>Chloroflexi</i>	3.3	3.4	1.7
<i>Firmicutes</i>	1.1	0.6	0.7
<i>Gemmatimonadetes</i>	0.8	1.2	1.8
<i>Nitrospirae</i>	1.2	0.6	0.5
<i>Alphaproteobacteria</i>	11.4	13.0	15.3
<i>Betaproteobacteria</i>	3.2	1.4	4.4
<i>Gammaproteobacteria</i>	3.1	1.9	2.2
<i>Deltaproteobacteria</i>	1.4	0.9	1.3
<i>Proteobacteria, others</i>	6.2	6.3	7.1
<i>Others</i>	11.6	18.2	12.8

4

5

1 **Figure legends**

2

3 **Figure 1.** Respiration CO₂-C rate in soils sampled at three elevations under
4 incubation at (a) 15 °C, (b) 20 °C, and (c) 35 °C. Error bars represent standard
5 deviation.

6

7 **Figure 2.** Concentration of (a-c) soluble organic carbon (SOC) and (d-f) nitrogen
8 (SON) in bamboo soils sampled at three elevations and incubated at (a, d) 15 °C, (b,
9 e) 20 °C and (c, f) 35 °C. Error bars represent standard deviation.

10

11 **Figure 3.** Changes in bacterial diversity of soil community at 600 m, 1,200 m, and
12 1,800 m incubated at different temperatures.

13

14 **Figure 4.** Changes in relative abundance of phylogenetic groups of bamboo soil
15 bacterial communities at (a) 600 m, 15 °C, (b) 600 m, 20 °C, (c) 600 m, 35°C, (d)
16 1,200 m, 15 °C, (e) 1,200 m, 20 °C, (f) 1,200 m, 35 °C, (g) 1,800 m, 15 °C, (h)
17 1,800 m, 20 °C and (i) 1,800 m, 35 °C. Abbreviation: Acid: *Acidobacteria*; Actino:
18 *Actinobacteria*; Bac: *Bacteroidetes*; Chloro: *Chloroflexi*; Firm: *Firmicutes*; Gem:
19 *Gemmatimonadetes*; Nitro: *Nitrospirae*; α , β , γ , δ : α -, β -, γ - and δ -*Proteobacteria*.

20

21 **Figure 5.** Changes in relative abundance of abundant genera of bamboo soil
22 bacterial communities at (a) 600 m, 15 °C, (b) 600 m, 20 °C, (c) 600 m, 35°C, (d)
23 1,200 m, 15 °C, (e) 1,200 m, 20 °C, (f) 1,200 m, 35 °C, (g) 1,800 m, 15 °C, (h)
24 1,800 m, 20 °C, and (i) 1,800 m, 35 °C.

25

26 **Figure 6.** NMDS analysis of bamboo soil bacterial communities sampled at three

1 elevations and incubated at different temperatures. Circles, triangles, and diamonds
2 represent communities at 600 m, 1,200 m, and 1,800 m elevation, respectively. The
3 analysis was based on the distribution of OTUs formed at an evolutionary distance
4 of 0.03.

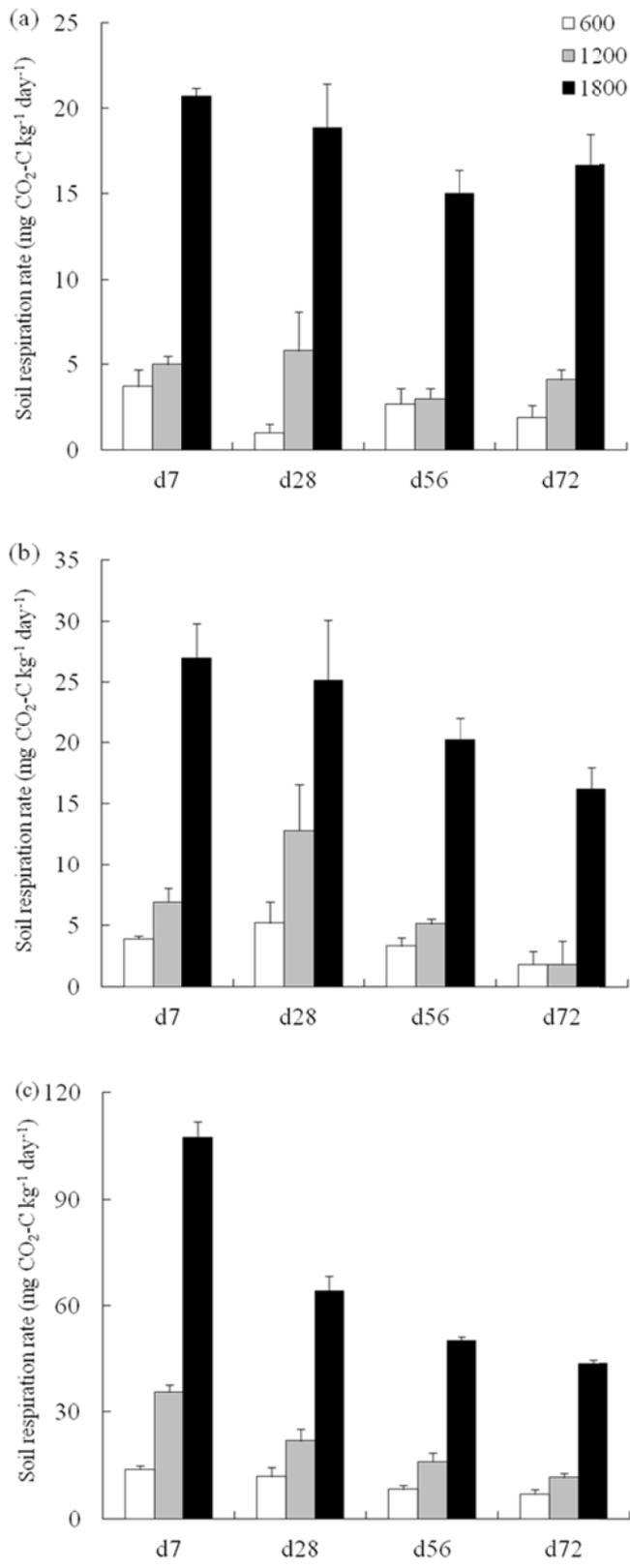
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6 **Figure 7.** PCA analysis of bamboo soil bacterial communities and environmental
7 properties. Symbols are the same as in Fig. 6.

8

2 **Figure 1.**

3

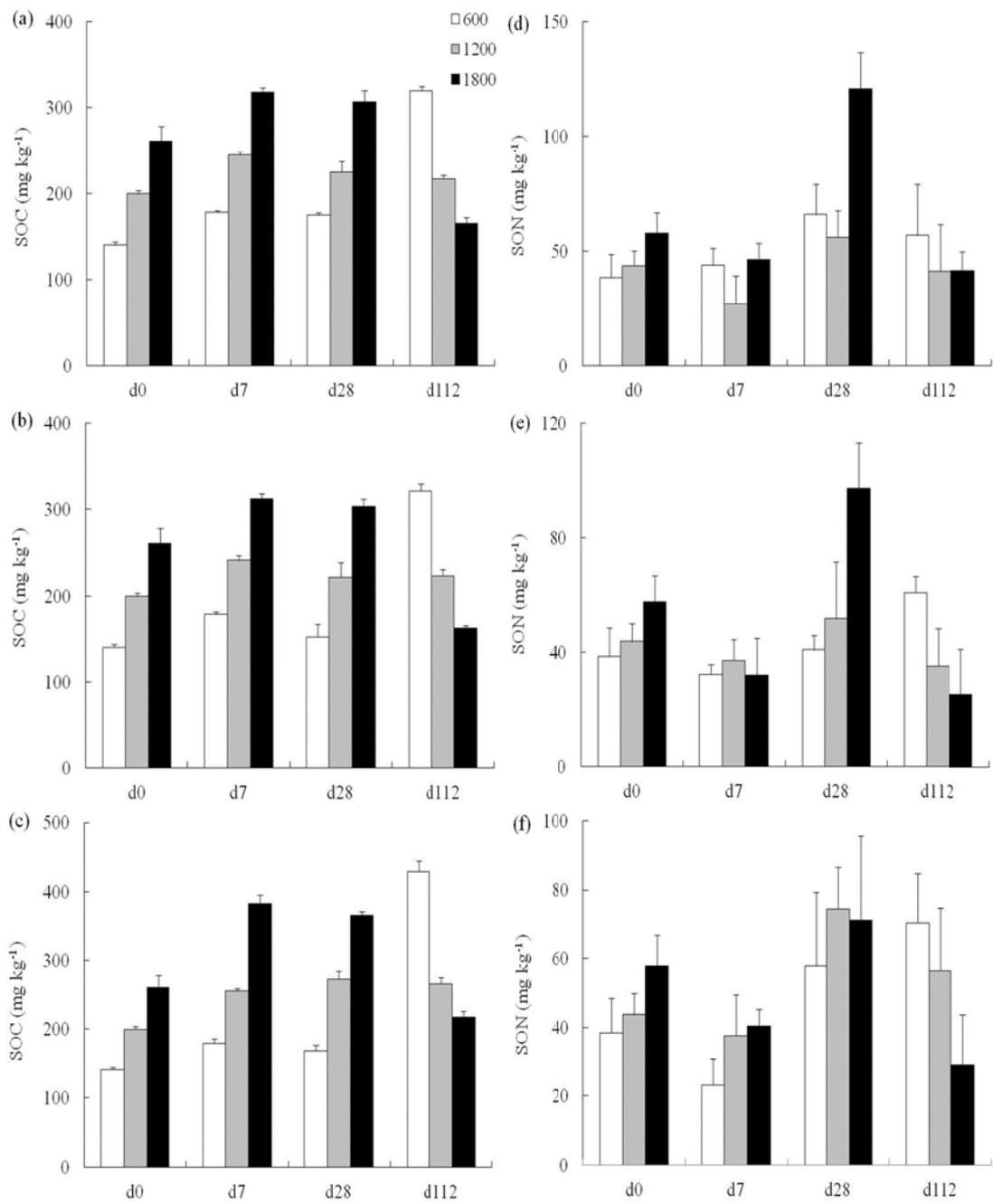


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2 **Figure 2.**

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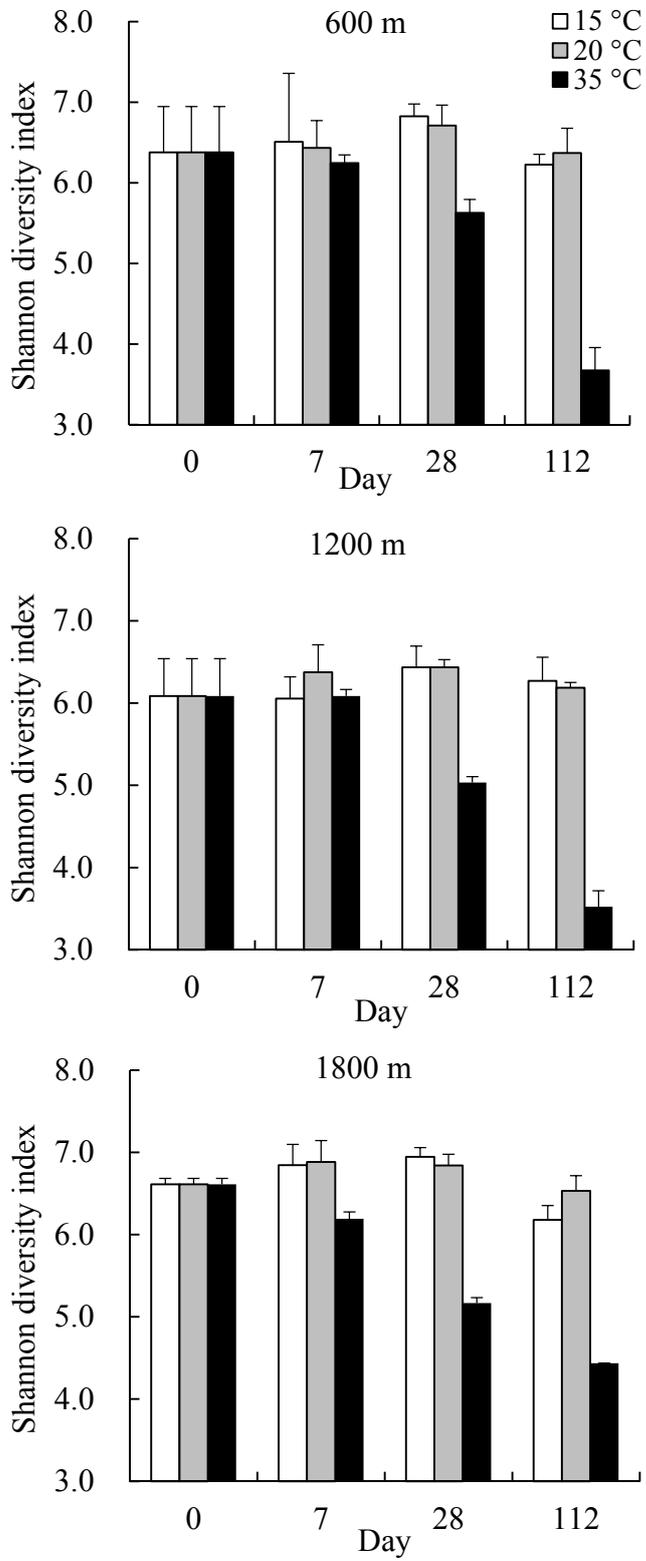


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1 **Figure 3.**

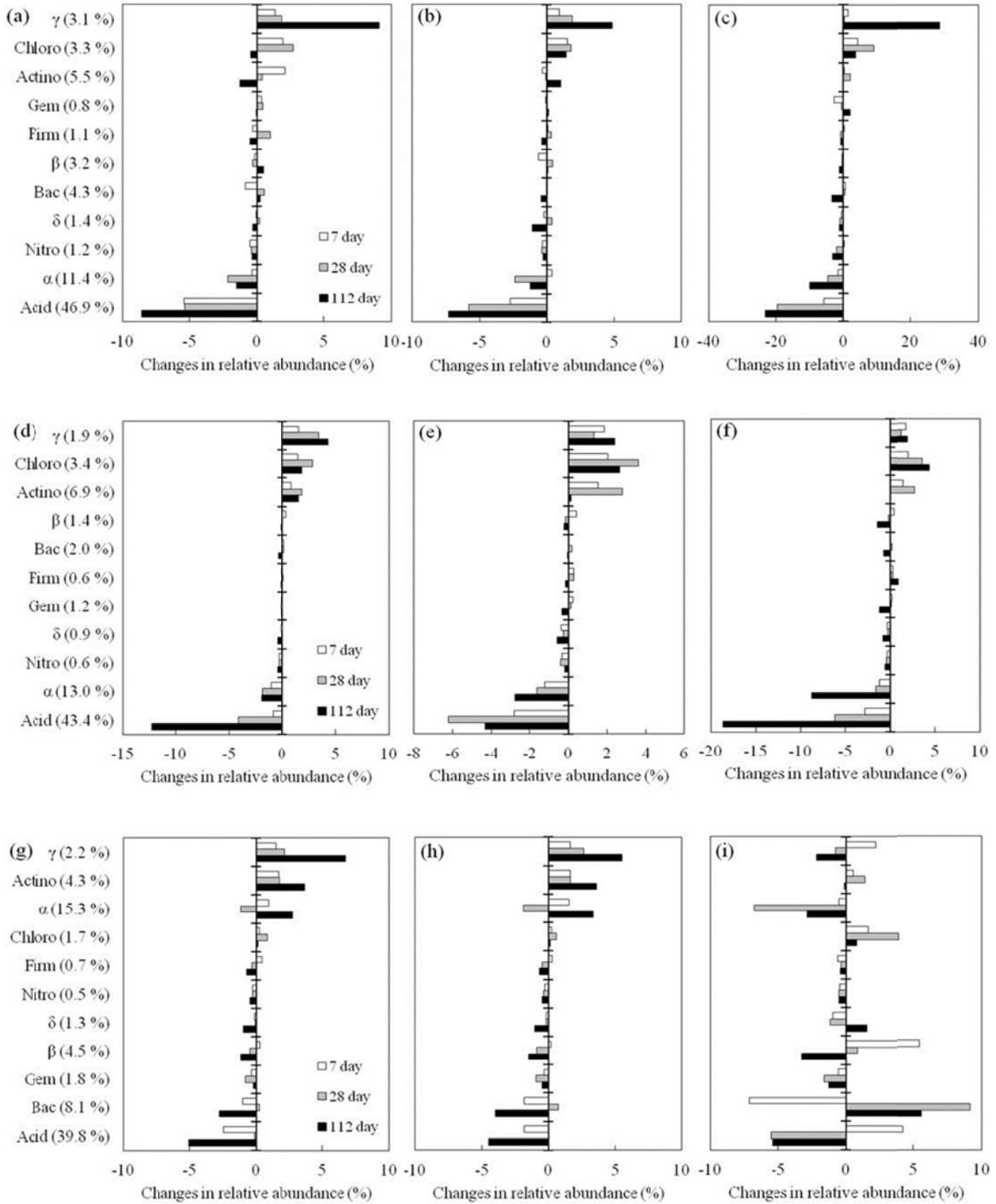
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2 **Figure 4.**

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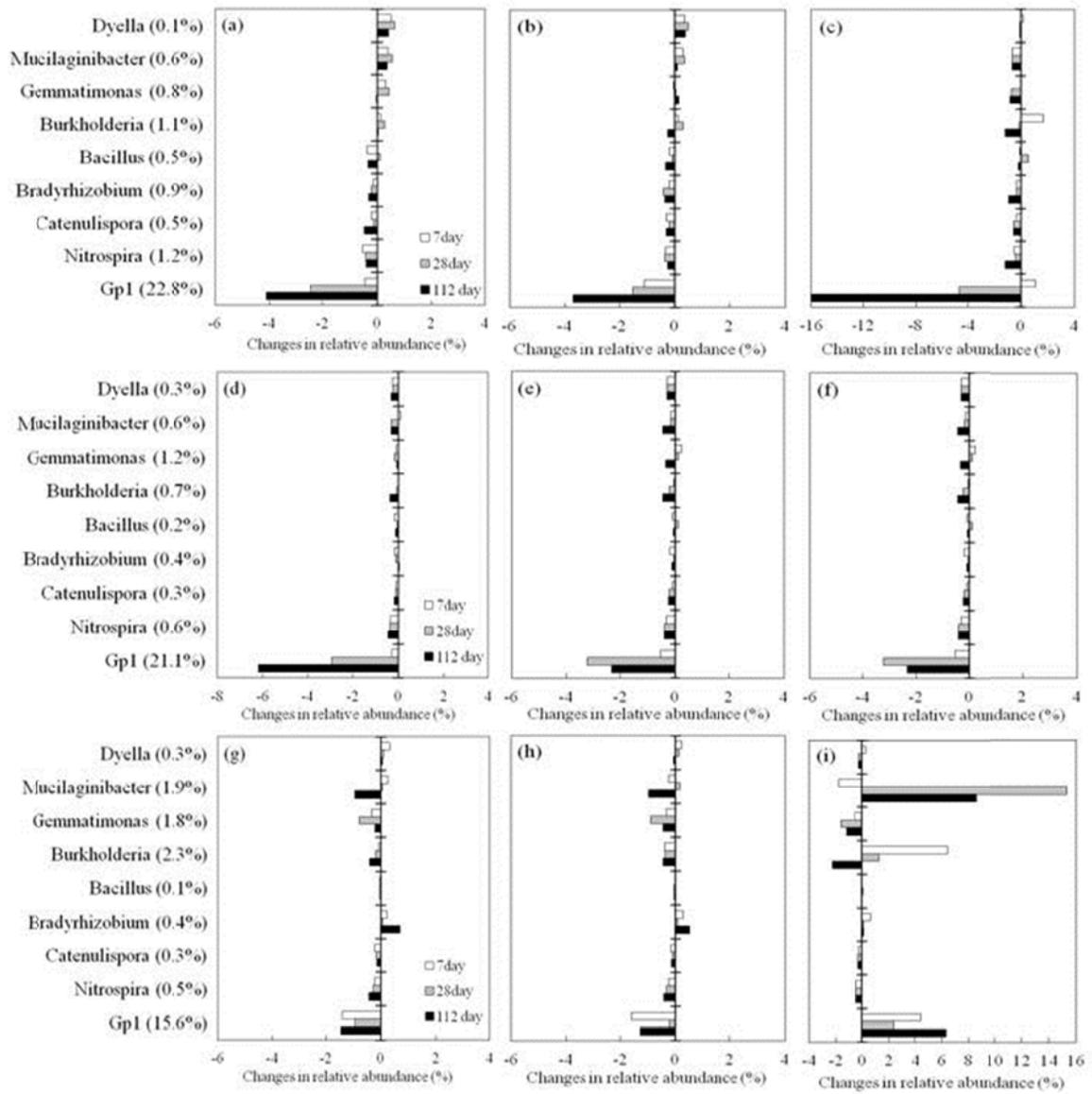


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2 **Figure 5.**

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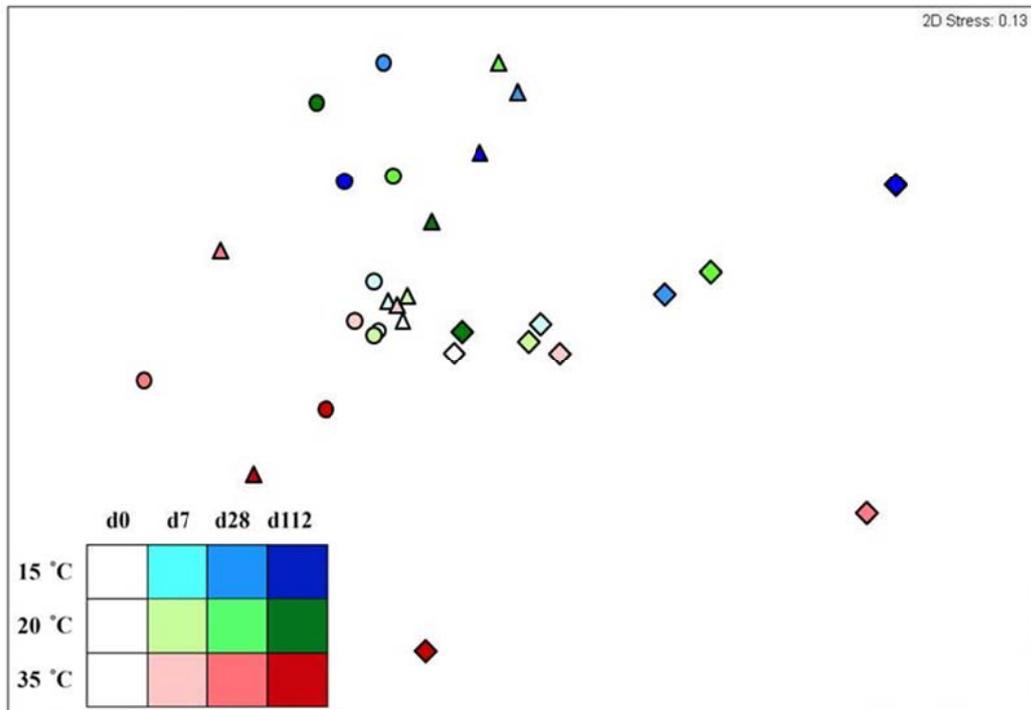
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2 **Figure 6.**

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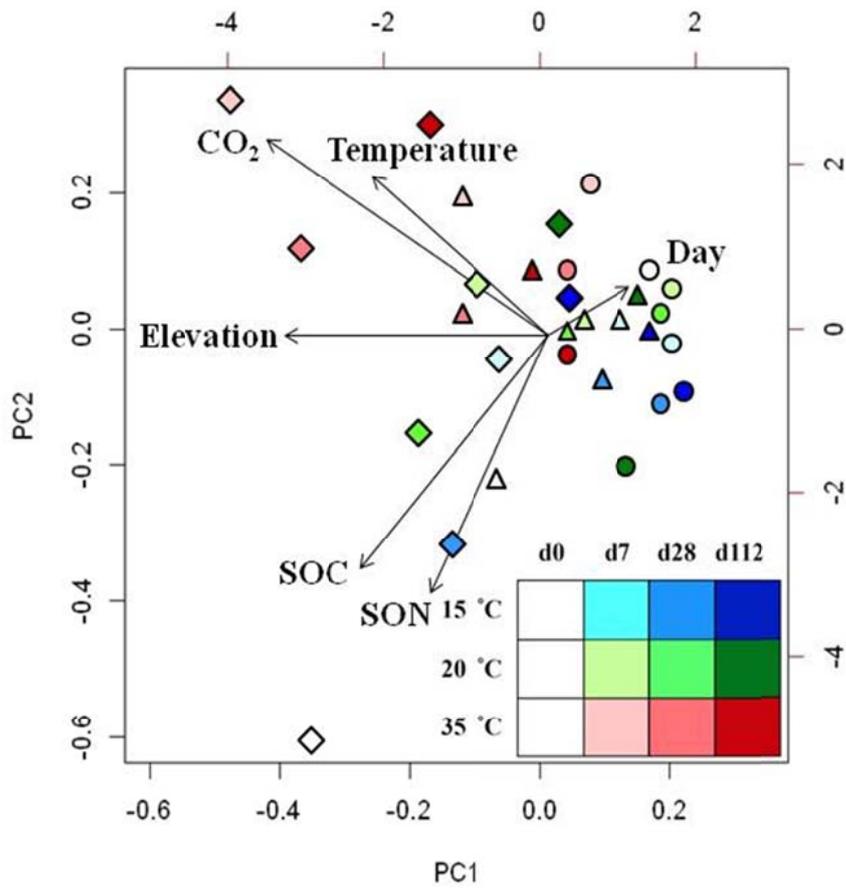


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2 **Figure 7.**

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