气温上升对草地土壤微生物群落结构的影响

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摘要:在 20 世纪内,全球气温已经上升了 0.6 C,并预计到本世纪末仍将上升 1.4～5.8 C。全球气候变暖对生态系统的潜在影响,生态系统对气温上升的反馈已成为国际生态学界的研究热点,而且所研究的系统也已经从过去简化的模拟系统到复杂的真实生态系统。但是,现有对真实生态系统的研究大部分集中在地上植物群落和土壤气体交换等领域,对在土壤有机碳分解和保护中起决定作用的土壤微生物研究较少。为此,在美国大平原地区进行人工提高气温(上升 1.8 C),来研究土壤微生物对气温上升的反应。结果表明:增温对土壤微生物的总生物量没有显著效应,但可以提高微生物的 C∶N 比。另外,磷脂肪酸分析发现,气温上升显著降低土壤微生物量中的细菌比重,提高真菌的份额,从而显著提高了群落中真菌与细菌的比值。而且,通过对土壤微生物底物利用方式和磷脂肪酸特征的主成份分析,发现增温导致了土壤微生物群落结构的转变。可见,气温上升可能是通过提高土壤微生物中真菌的优势,而导致群落结构的变化。该变化将可以提高微生物对土壤有机碳的利用效率,并利于土壤有机碳的保护。

关键词:气温上升;土壤微生物;群落结构;全球变化;草地

Impacts of experimental atmospheric warming on soil microbial community structure in a tallgrass prairie

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Abstract: Global surface temperature is predicted to increase by 1.4 to 5.8 C by the end of this century. However, the impacts of this projected warming on soil carbon balance and budget of terrestrial ecosystems are not clear. One major source of the uncertainty stems from warming effects on soil microbes, which exert dominant influence on soil organic carbon decomposition and storage in terrestrial ecosystems. We have therefore conducted an experiment in a tallgrass prairie ecosystem in the US Great Plains to study soil microbial responses to artificial warming of about 1.8 C. Our data showed that warming did not induce significant differences in soil microbial biomass size, but increased microbial biomass C∶N ratio. Also, warming caused an increase in bacterial contribution and a decrease in fungal contribution to the total microbial PLFAs, consequently inducing an increase in the ratio of fungi to bacteria within the whole soil microbial community. Moreover, principle component analysis of substrate utilization patterns and the profiles of phospholipid fatty acids showed that warming caused a shift in soil microbial community structure. Together, our results indicate that this shift in microbial community structure induced by experimental warming may be attributed to the increase in soil fungal dominance and the decrease in bacterial dominance. The observed shift

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in soil microbial community structure may increase microbial carbon use efficiency and benefit organic carbon protection in the soil.

Key words:climatic warming; microbial community; global change; grassland

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1 Introduction

Climatic warming has increased global mean temperature by about 0.6 C over the 20st century and will continue to increase it by 1.4～5.8 C by the end of 21st century[1]. Ecologists from around the world have conducted experiments to investigate the effects of global warming on terrestrial ecosystems and ecosystem feedbacks to warming. Most of those experiments have mainly focused on water and surface energy balance, vegetation structure, primary productivity, exchange of greenhouse gases, nutrient cycling, soil or ecosystem respiration and soil carbon dynamics. Few relevant studies have been specifically devoted to the potential impacts of elevated temperature on soil microbes, which exert dominant influence on the net carbon (C) balance of terrestrial ecosystems by controlling soil organic matter (SOM) decomposition and plant nutrient availability[2,3]. An understanding of microbial responses to warming in situ is of critical significance in predicting terrestrial ecosystem feedbacks and their carbon budget in a warmer climate.

Warming may influence soil microbes through changing their physical and chemical soil environments. Saleska *et al*.[4] and Wan *et al*.[5] demonstrated that experimental warming caused an increase in soil temperature and a decrease in soil moisture. These effects may induce changes in physiology and growth of some specific groups within soil microbial communities[6], because the tolerances of soil temperature and moisture are different between specific microbial groups[7,8]. In addition, warming has been reported to stimulate plant growth[9,10] and to alter plant community composition[11,12]. These effects will have profound impacts on the quantity and quality of carbon inputs to the soil, which in turn is likely to have been a major key factor in controlling the microbial community. Moreover, the increased plant growth rate induced by warming[9,10] may stimulate the competition for nutrient between plant and soil microbes. Therefore, it is expected that global warming may influence soil microbial activities and community, but data confirming it are scarce.

Expected effects of warming on grass growth may greatly affect soil microbes and microbially mediated processes, since grasses allocate a high percentage of their photosynthetic products to the belowground. Also, the tallgrass prairies are temporally very dynamics with C_3 plants dominant in winter and spring, and C_4 species dominant in summer and early fall. Warming may alter plant growing period resulting in changes in grass community dynamics, which in turn likely affects soil microbes. Therefore, we conducted an experiment in a tallgrass prairie ecosystem in the US Great Plains to study soil microbial responses to artificial warming of about 2 C. The objectives of this study were to evaluate the effects of experimental warming on soil microbial community structure.

2 Materials and methods

2.1 Site description

The experimental site is located at the Great Plain Apiaries (34°58′54″N,97°31′14″W), Oklahoma, USA. The grassland is dominated by C_4 grasses and C_3 forbs. Mean annual temperature is 16.3 C with January being the coldest month (3.3 C) and July the warmest (28.2 C). Mean annual precipitation is 914 mm. The soil is Vernon clay loam, part of the Nash-Lucien complex, which is characterized as having a low permeability rate, high available water capacity and a deep, moderately penetrable root zone. The soil pH is 6.7 with an C∶N of 26.3 and total N of 0.11 g N kg^{-1} soil.

2.2 Experiment design

There were five pairs of 2×2 m plots. One plot in each pair had been warmed continuously with infrared heaters since November 1999, and the other was the control. In each warmed plot, a single 165×15 cm infrared heater was suspended 1.5 m above the ground. In the control plot, a 'dummy' heater of the same shape and size as the infrared heater was suspended 1.5 m high in order to simulate the shading effects of the heater. The distances between the individual sets of paired plots varied from 20 to 60 m. Experimental warming significantly increased daily mean air temperature (up 1.2 C) and daily mean soil temperature (at 8 cm)[12].

2.3 Soil sampling

Soil samples were collected from the topsoil (0～15 cm) of all the plots in September 2001, and May and September 2002. Soil sample was passed through a sieve (4 mm dia), and visible living plant material (e. g. roots) was removed. Two subsamples of each sieved soil were obtained, one kept in the refrigerator at 4 C for routine biological analyses and another at −20 C, for phospholipid fatty acids (PLFA) analysis.

2.4 Determination of soil microbial biomass

Soil microbial biomass C (MBC) and N (MBN) were determined using the method of fumigation-extraction (48 h fumigation)[13]. Soil extractable organic C in the K_2SO_4 extracts before and after the fumigation was quantified using a total C analyzer (Shimadzu TOC-5050A, Shimadzu Co., Kyoto, Japan). Soil extractable inorganic N in the non-fumigated and fumigated soils was measured on a flow injection analyzer (Lachat Quickchem Systems, Milwaukee, WI) after alkaline persulfate digestion[14]. The differences in organic C and total extractable inorganic N between the fumigated and non-fumigated soils were assumed to be released from lysed soil microbes. The released C and N were converted to MBC and MBN, respectively, using k_{ec} 0.33[15] and k_{en} 0.45 [16].

2.5 Substrate utilization patterns

Function groups in carbon source utilization of soil microbial community were examined using BIOLOG microplates. Subsamples of moist soil (5 g dry wt equivalent) collected in September 2001 and 2002 were put in 50 ml autoclaved DI water and sharked for 30 minutes. The suspensions were diluted with sterile Ringer solution (final dilution 10^{-3}). Each well of BIOLOG EcoPlate (Biolog Inc, 3938 Trust way, Hayward, CA 94545, U.S.A.) was inoculated with 125 μl of the diluted soil extracts and incubated at 25 C in the dark. The color formation at 590 nm was measured every 24 h for 7 days using an automatic plate reader (Bio-Tek Instrument Inc, U.S.A.). Absorbency from the control was subtracted from the wells containing substrates. In order to minimize the effects of different inoculation densities data from the 72 h reading were normalized by dividing the absorbency for each well by the average absorbency for the whole plate [17].

2.6 Phospholipid Fatty Acid

Phospholipid fatty acids were extracted and quantified from 5 g freeze-dried soil collected in September 2001 and 2002 using a procedure previously described by Bossio *et al.*[18]. The separation and identification of extracted PLFAs were carried out according to the standard protocol of the Sherlock Microbial Identification System $V_{3.1}$ (MIDI, 1999) and a Gas Chromatograph (Hewlett Packard 5890A, Hewlett-Packpard Co. Pessylvania, USA). The following fatty acids i14∶0, i15∶1c, i15∶0, a15∶0, i16∶1c, i16∶0, 16∶1ω7c, i17∶0, 17∶1ω6c, a17∶0, 17∶0cy, 18∶1ω7c, 18∶1ω5c and 19∶0cy were chosen to represent the PLFAs of the bacterial group[19,20]. Also three fatty acids (16∶1ω5c, 18∶2ω6,9c and 18∶1ω9c) were used to represent the fungal group[20～22]. Data from the PLFAs was presented in units of percent of the total amounts detected within a sample. Total percentages of PLFAs identified for each microbial group were calculated to present their relative contributions to the total microbial biomass. In addition, the ratio of fatty acid relative contribution of fungi to bacteria (F/B) was also included in the data analysis.

2.7 Statistic Analysis

Statistical significance of warming treatments was evaluated by analysis of variance (ANOVA) at $P=0.05$ level. In addition, substrate utilization patterns (BIOLOG data) and PLFA profiles were analyzed using principal component analysis (PCA) to detect warming effects on soil microbial community structure. For all statistical analyses, the SPSS V. 10.0 (SPSS Inc., Chicago, Illinois, USA) software package was used.

3 Results

3.1 Soil microbial biomass and C∶N ratio

There were no significant difference in microbial biomass C and N between the treatment and control during the three sampling dates (Fig. 1 a and b). However, warming caused a consistent increase in microbial biomass C∶N ratio during the two years. Microbial biomass C∶N was 24% higher in the warming than in the control plots ($P<0.05$) in September 2001, though the data were not statistically significant as compared to the control in May and September 2002 (Fig. 1c).

3.2 Soil microbial community structure

Analysis of BIOLOG data using principal component analysis (PCA) showed that warming induced changes in substrate utilization patterns of microbial communities (Fig. 2). The first two principal components accounted for 62 and 59%

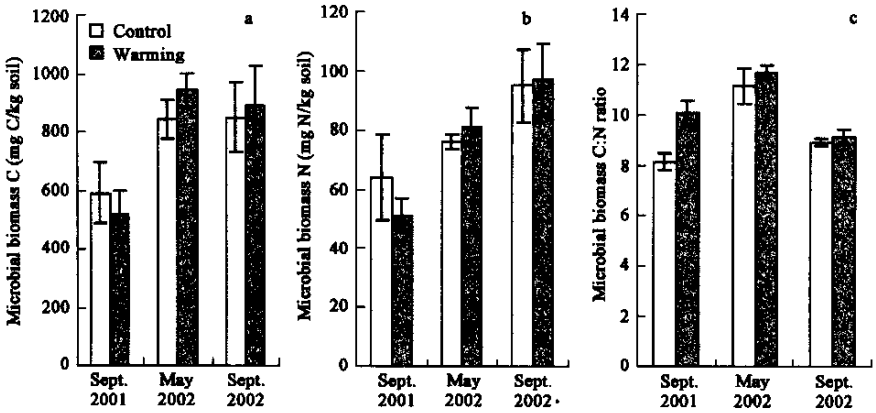

Fig. 1　Effects of warming on soil microbial biomass

(a) Biomass carbon, (b) biomass nitrogen and (c) biomass C : N ratio ($LSD_{0.05}=0.68$); Values are means ± 1SE with the sample size $n=3$ in 2001 and $n=5$ in 2002

of the total variance in September 2001 (Fig. 2a) and 2002 (Fig. 2b), respectively ($PC_1=36\%$ and $PC_2=26\%$ in 2001, $PC_1=41\%$ and $PC_2=18\%$ in 2002). PC_2 clearly separated the microbial communities in the warming from those in the control plots ($P<0.05$).

The PLFA fingerprints showed similar results from the analysis of BIOLOG data (Fig. 3). It showed that warming caused significant dissimilarities in soil microbial community structure. The first two principal components explained 87 and 65% of total variance in September 2001 (Fig. 3a) and 2002 (Fig. 3b), respectively ($PC_1=66\%$ and $PC_2=21\%$ in 2001, $PC_1=45\%$ and $PC_2=20\%$ in 2002). The PLFA profiles of the control and warming plots could be distinctly separated by PC_1 ($P<0.05$) in 2001 and by PC_2 in 2002 ($P<0.05$).

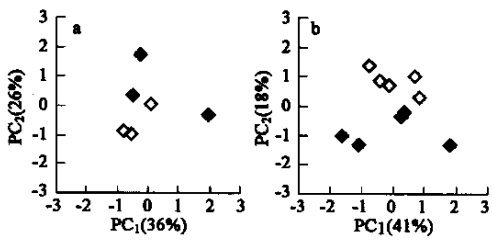

Fig. 2　Principal component analysis (PCA) plots of BIOLOG data in September 2001(a) and September 2002(b)

Open symbol, control plot; infilled symbol, warming plot; PC_1 and PC_2 explain 36% and 26% of the total variance in 2001, and 41% and 18% in 2002, respectively; The sample size is 3 and 5 in 2001 and 2002, respectively

3.3　Relative contributions of fungi and bacteria to total microbial biomass

Warming significantly affected the relative contributions of fungi and bacteria, measured as their percentage of PLFA (Fig. 4 a and b). It enhanced fungal contribution by 28 and 13% ($P<0.05$), and reduced bacteria contribution by 13 and 6% ($P<0.05$) in both sampling times. Consequently, warming resulted in increases in the ratios of fungi to bacteria by 63 and 22% ($P<0.05$) in the two sampling dates of 2001 and 2002, respectively (Fig. 4c).

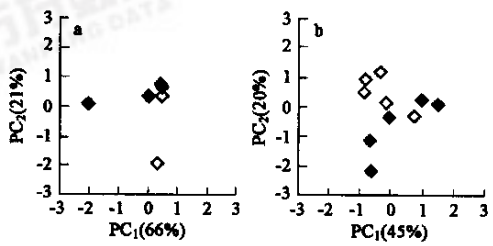

Fig. 3　Principal component analysis (PCA) plots of all PLFAs signatures detected in September 2001(A) and September 2002(B)

Open symbol, control plot; infilled symbol, warming plot; PC_1 and PC_2 explain 66% and 21% of the total variance in 2001, and 45% and 20% in 2002, respectively; The sample size is 3 and 5 in 2001 and 2002, respectively

4　Discussion

The distinct separations in the substrate utilization patterns (BIOLOG) and the PLFA profiles indicate that three-year experimental warming caused significant changes in soil microbial community structure. Also, the increases in the ratios of microbial biomass C:N and fungal to bacterial PLFA induced by warming suggest that warming benefited soil fungi over bacteria. Together, these results indicate that warming may induce a shift in soil microbial community structure facilitating

fungi over bacteria in unmanaged tallgrass prairies.

Various mechanisms may have attributed to the observed shift of the microbial community structure toward an increasing contribution of fungi[4,5,10,11]. Firstly, warming-induced increase in plant growth and decrease in soil N availability may benefit fungi over bacteria. Experimental warming has been reported to enhance aboveground biomass by more than 30% in the present tallgrass prairie[12]and the stem-wood growth by more than 50% in a mid-latitude hardwood forest[10]. The stimulated-growth, on the one hand, may significantly increase C inputs to soil as plant litter, root exudation and fine root turnover, which will benefit soil microbe growth. On the other hand, the enhanced plant growth may accelerate soil nutrient uptake by the plant and decrease soil N availability[12], which in turn may intensify nutrient competition between plant and soil microbe. This may result in nutrient limitation of microbes. Fungi can relocate nutrients due to their filamentous nature and recycle limited nutrients (especially N) via cytoplasm translocation. This feature may give fungi competitive advantages over bacteria for exploitation of available C and nutrients[23]. Consequently, an increase in C inputs and a decrease in N availability to soil microbes likely contribute to the enhancement of fungal dominance in the whole soil microbial community.

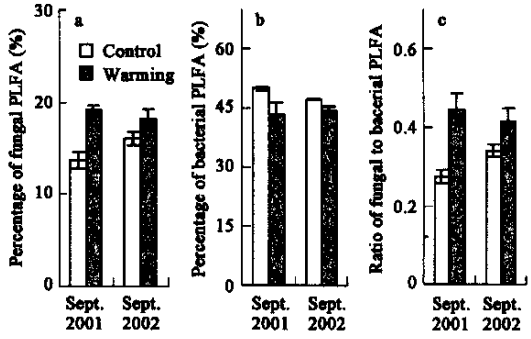

Fig. 4 Effects of warming on relative contributions of specific groups to the total microbial biomass

(a) percentage of fungal PLFA ($LSD_{0.05}=2.6$), (b) percentage of bacterial PLFA ($LSD_{0.05}=2.7$) and (c) the ratio of fungal to bacterial PLFA ($LSD_{0.05}=0.08$); Valuse are means ± 1SE with the sample size $n=3$ in 2001 and $n=5$ in 2002

Secondly, soil fungi likely benefit from the changes in plant community composition induced by the elevated temperature. In the present experiment, three-year warming significantly enhanced aboveground biomass of C_4 plants by about 37%～57% but did not cause any change in the aboveground biomass of C_3 plants. This difference in the stimulated biomass growth between C_3 and C_4 plants resulted in a decrease in the quality of C inputs to the soil, since C_4 plant has higher C∶N ratio as compared to C_3 plant. Sturm *et al.*[11] also reported a widespread increase in shrub abundance induced by the warming in Alaskan Arctic during the past 50 years, suggesting an increase in quantity and a decrease in quality of plant litter, because shrub plants have higher C∶N as compared to grasses. High C∶N ratio of plant litter likely benefits soil fungi growth over bacteria in soils. Therefore, results from the present experiment indicate that long-term warming may enhance fungal dominance within soil microbial community through increasing soil temperature and drying and through enhancing plant growth and the C∶N ratio of total plant biomass in natural terrestrial ecosystems.

Thirdly, warming may enhance fungal contribution to microbial community through altering soil temperature and moisture conditions. Luo *et al.*[9] demonstrated that the present experimental warming increased mean soil temperature by 1.8℃, and decreased soil moisture by 6.4%. These stresses of soil temperature and moisture likely facilitate fungi to survive better, since soil fungi rely on more aerobic conditions[24] and are more tolerant to high soil temperature and drying due to their filamentous nature[25].

An increase in fungal abundance may shift the decomposition process from bacterial-based to fungal-based channels. This transformation in decomposition channels probably results in an increase in soil C use efficiency due to differences between bacteria and fungi in their cell structure and physiology[26]. The major components of bacterial membranes are phospholipids, which are energy-rich, readily decomposable substrates for many soil organisms[27]. Nevertheless, fungal cell walls contain more polymers of melanin and chitin, which can persist in soil for years and account for significant pool of soil organic matter. In addition, fungi have greater C assimilation efficiency as compared to bacteria[28]. Hence, the greater C utilization efficiency in fungi may lead to more organic C being transformed into more recalcitrant humic materials. Furthermore, Fungal hyphae have long been recognized to enmesh microaggregates (<250 μm) into macroaggregates (>250 μm)[29,30]. Increasing fungal contribution to the microbial community may also contribute to organic C protection through facilitating the formation and stabilization of soil aggregates.

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