Applied Soil Ecology 94 (2015) 40–48

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Temperature sensitivity of soil bacterial community along contrasting warming gradient

Jinfeng Wu [a], Jinbo Xiong [a,*], Changju Hu [a], Yu Shi [b], Kai Wang [a], Demin Zhang [a]

[a] Faculty of Marine Sciences, Ningbo University, Ningbo 315211, China
[b] Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China

ARTICLE INFO

Article history:
Received 27 September 2014
Received in revised form 29 April 2015
Accepted 30 April 2015
Available online 20 May 2015

Keywords:
Contrasting temperature
Bacterial community
Turnover rate
Temperature relationship
Indicator species

ABSTRACT

Soil microbial communities potentially mediate the feedbacks to climate change, and thus, the understanding of microbial ecology is central to predict future global warming. However, the direct warming effects on microbial communities are uncertain due to the confounding effects of plant traits and soil heterogeneities. Here, we exposed alpine meadow soil microcosms to temperature between 10 and 40 °C for 28 days, to evaluate the temperature relationship of the bacterial community structure and microbial functions. Our results showed that sustained exposure to contrasting temperature produced compositionally and functionally distinct microbial communities. The degree of these changes was dependent on the magnitude of warming, as shown by a consistent increase in the dissimilarities and a shift of the carbon (C) use pattern along the temperature gradient. We found that the bacterial community temporal dynamics followed a gradual process of succession, and the turnover rate was substantially accelerated ($P = 0.012$) by warmer temperatures; every 1 °C elevation in soil temperature was estimated to increase the turnover by 0.001. Consistently, contrasting temperature was found to be the dominant factor explained 6.1% of the total variation of the microbial community structures; however, the incubation duration and the interaction between incubation temperature and duration explained 5.4% and 4.1% of the variation, respectively. As anticipated, the shift in bacterial community rapidly translated to a similar change in enzymes activities and C utilization. In addition, potential indicator species were screened; these species characterized the temperature gradient. Collectively, our findings indicate that direct warming effects on soil bacterial community could substantially alter certain function and that contrasting temperatures would accelerate the bacterial community turnover–temperature relationship in this alpine meadow soil.

1. Introduction

High-altitude alpine meadow and tundra soils store large amounts of organic carbon (Davidson and Janssens, 2006; Wang et al., 2002) and are experiencing considerably greater than average increases in temperature by ongoing global warming (Liu and Chen, 2000; Solomon, 2007). Soil microorganisms mediate the carbon cycle feedback to climate warming through decomposition processes (Melillo et al., 2002; Singh et al., 2010; Zhou et al., 2012). In addition, the activities of the microbial community regulate the carbon exchange between the soil and atmosphere, thus potentially determining the trajectory of climate change (Bardgett et al., 2008). For these reasons, understanding the effects of warming on soil microbial communities at sensitive regions is not only indispensable to a comprehensive understanding of basic ecology but is also critical to improve the prediction of climate models (Treseder et al., 2012; Zhou et al., 2012).

Currently, there is still controversy on the soil temperature–respiration relationship (Davidson and Janssens, 2006; Wang et al., 2002), though most field experiments state an initial increase soil respiration in response to climate warming (Peng et al., 2009; Xiong et al., 2014a; Zhou et al., 2012). For example, it has been reported that elevated warming declines 50% of the soil respiration (Allison and Treseder, 2008), while Bradford et al. (2010) find minor warming effect. These divergent response could result of the co-limitation of labile soil carbon pool over time (Melillo et al., 2002), changing the substrate allocation of plants (Zhou et al., 2012) through thermal adaption (Rousk et al., 2012), and shifts in the microbial community (Zhou et al., 2012). Additionally, much of this uncertainty may be due to the complex interactions that occur among the microbes, vegetation, and physical soil environment in the context of climate change (Bardgett et al., 2008; Davidson and

* Corresponding author. Tel.: +86 574 87600164; fax: +86 574 87608347.
E-mail address: xiongjinbo@nbu.edu.cn (J. Xiong).

http://dx.doi.org/10.1016/j.apsoil.2015.04.018
0929-1393/

Janssens, 2006; Insam et al., 1996). Indeed, several studies have shown that the responses of the soil microbial community and activity to warming are ecosystem dependent due to differences in soil types and plant traits (Na et al., 2011; Peng et al., 2009). Similarly, current evidence suggests that warming alters the bacterial phylogenetic and functional compositions by the indirect effects of plant and edaphic variable alterations (Xiong et al., 2014b; Zhou et al., 2012). In addition, higher temperature does not invariably contribute greater changes in certain taxa (Xiong et al., 2014b) and bacterial communities (Rinnan et al., 2009) to warming gradient. For this reason, it is unclear whether the effects of warming on microbial composition are magnitude dependent.

There is evidence that the phylogenetic composition and function are closely linked (Langille et al., 2013) under warming disturbance (Xiong et al., 2014; Zhou et al., 2012), though functional redundancy are proposed to exist among soil bacterial taxa (Allison and Martiny, 2008; Rousk et al., 2009). Given that the soil temperature–respiration relationship is controversial among previous works (Allison and Treseder, 2008; Davidson and Janssens, 2006; Wang et al., 2002), it appears that microbial community structure and composition offer an alternative and sensitive index for evaluating the temperature–microbial community relationship. In addition, by employing microcosms, it avoids heterogeneous edaphic factors that mask the direct warming effects, such as heterogenic substrate among biological sites in field, thus enabling us to observe a real response of the community (He et al., 2014; Insam et al., 1996). Herein, to focus on the direct warming effects, we incubated the soils at different temperatures (10–40 °C) for four weeks to evaluate the temperature relationship of the bacterial community structure and functional abilities, including carbon use patterns and enzyme actives. It has been reported that the relative bacterial growth rate (Bárcenas-Moreno et al., 2009) and mineralization (Birgander et al., 2013) were consistently increased along the incubated temperature gradient of 5–30 °C, thus we predicted that a higher temperature would select thermal-resistant bacteria, resulting in a consistent response (that is, a consistent change in the abundance for a given bacterial taxa) to warming gradient. If this is the case, indicator species can be identified to indicate this responsive pattern. Further, we predicted that shifts in the soil bacterial community would translate into changes in microbial function.

2. Materials and methods

2.1. Soil and incubation conditions

After the growing season, soil samples were randomly collected from 10 respective sites on 5 November 2013, at the depth of 0–15 cm in an alpine meadow near the Beilu River Research Station (92°56′03″E, 34°49′22″N, 4635 m above sea level) on the Tibetan Plateau, China. The region is a summer-grazed zone and is dominated by alpine meadow vegetation, including *Carex moorcroftii*,*Kobresia capillifolia*, and *Kobresia pygmaea*. The soil is classified as Cambisols under FAO/UNESCO taxonomy (Wang et al., 2007a) with a rich organic content of 8.75% and a pH of 7.91 (Wang et al., 2007a; Xiong et al., 2014b). The mean annual temperature is −3.8 °C (range: −27.9 to 19.2 °C), and the mean annual relative humidity is 57% (Wang et al., 2007a). The mean annual precipitation is 290.9 mm, with over 95% falling during the warm growing season (from May to October).

Visible plant roots and residues were removed and the fresh soil sieved through a 2 mm mesh sieve, and then was well homogenized. For each temperature treatment, an aliquot (100 g fresh weight) soil was incubated in plastic pots with lids following a similar approach to other warming incubation studies (Bárcenas-Moreno et al., 2009; Birgander et al., 2013). Five replicates were then incubated at 10 (T10), 20 (T20), 30 (T30) or 40 (T40) °C. The lids were removed to aerate the pots every other day. At every aeration, soil moisture was monitored gravimetrically and adjusted to its original moisture content of 16%. After 28 days, soil samples from each incubation temperature (CK, T10, control, T10, T20, T30 and T40) were measured to assess the responses of bacterial community composition and C use pattern to warming gradient; an additional five replicates were measured at day 0 for the control (the initial bacteria community). In addition, to assess the temporal dynamics of the bacterial response to warming, we additionally collected soil samples (5 g for bacterial community analysis) from T20 and T30 after incubation for 3, 7, 14 and 21 days. All assays were performed in five replicates, yielding a total of 65 assays (5 assays at day 0 for control) for the bacterial community analysis: 2 temperatures × 5 time points × 5 repeats = 50. At day 28, the additional 10 assays were from incubations at T30 and T40. The C use pattern was measured for samples incubated for 28 days (4 temperatures × 5 repeats + 5 control = 25).

2.2. Analysis of community-level physiological profile (CLPP) and enzyme activities

To determine the carbon (C) use patterns of the soil microbial community, EcoPlates™ (Insam and Rangger, 1997) containing $_{31}$C sources and control wells (without substrate) with three replicates in a 96-well plate were used according to the manufacturer's instructions. The soil suspension was prepared as previously described, that is, five grams soil from each sample was added to 45 ml double-distilled water (ddH_2O) and incubated at 4 °C with shaking (200 rpm) for 45 min and was then left standing for 30 min (Xiong et al., 2012). The samples were then serially diluted to 10^{-4} based on a pilot experiment, and an aliquot of 100 μl of the diluted suspension from each soil sample was then inoculated into each EcoPlate well and incubated at 25 °C for 168 h. The color of each well was manually measured by a microplate reader every 12 h during the incubation. The mean of the C use capacities across the 31 different substrates, which was calculated using the differences between the optical densities (O.D.s) of the wells containing individual carbon sources and the 'water control', was represented by the average well color development (AWCD) for the replicate (Insam and Rangger, 1997; Ros et al., 2008). The C use capacities of the 31 different substrates were treated as responsive variables to calculate functional diversity of each sample with Shannon diversity index, and functional similarity among the samples with Bray–Curtis similarities, using the data obtained at 120 h.

The soil urease and phosphatase activities were measured by a modified indophenols blue reaction (Tabatabai and Bremner, 1972) and a colorimetric estimation of the *p*-nitrophenol released by phosphatase activity, respectively (Tabatabai and Bremner, 1969).

2.3. DNA extraction, bacterial 16S rRNA gene amplification and MiSeq sequencing

Soil DNA was extracted using a FastDNA® Spin kit (Bio 101, Carlsbad, CA, USA) from 0.5 g of wet soil according to the manufacturer's protocol. The genomic DNA extracts were quantified with a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, USA) and stored at −80 °C until amplification.

An aliquot (50 ng) of DNA from each sample was used as a template for bacterial 16S rRNA gene amplification. Briefly, the bacterial hypervariable domain V4 was amplified with region-specific primers (515F: 5′-GTGCCAGCMGCCGCGGTAA-3′ and 816R: 5′-GGACTACVSGGGTATCTAAT-3′) that included the Illumina flow-cell adapter sequences (Bates et al., 2011). The forward and reverse amplification primers each contained a 6-base barcode sequence.