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Microbial diversity limits soil heterotrophic respiration and mitigates the respiration response to moisture increase

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ABSTRACT

Decline in soil biodiversity associated with anthropogenic activities has raised concerns about the consequences for ecosystem functions. It remains uncertain how important soil microbial diversity is relative to abiotic factors, and how they interact, in driving ecosystem processes. Here we present results of a soil microcosm experiment in which microbial diversity and moisture conditions were independently manipulated. Loss of microbial diversity led to higher rates of soil microbial respiration, and the diversity effect was maintained over time during the course of the experiment. Higher moisture also enhanced soil respiration; but the moisture effect reduced over time, more rapidly in microcosms of higher microbial diversity. Overall, loss of microbial diversity enhanced soil respiration to a greater extent than moisture elevation, and also exacerbated the response of soil respiration to water addition. Loss of negative species interactions in microcosms of lower microbial diversity might be the major reason for the diversity effects observed in this study. Our results suggest that the integrity of soil microbial communities be crucial for the maintenance of soil carbon storage function.

1. Introduction

Loss of species diversity may cause significant changes in ecosystem functions, with its effects being of comparable magnitude of that of many abiotic environmental factors including nutrient availability and temperature (Tilman, 1999; Loreau et al., 2001; Reich et al., 2001; Hooper et al., 2012; Tilman et al., 2012; Allison et al., 2013; Eisenhauer et al., 2013; Boyero et al., 2014; Bradford et al., 2014; Steinauer et al., 2014). Meanwhile, species diversity may interact with the abiotic factors as drivers of ecosystem processes. Species diversity may, on one hand, alleviate the impacts of stressful environmental factors such as drought (Tilman and Downing, 1994; Yachi and Loreau, 1999; Mulder et al., 2001; Gonzalez and Loreau, 2009; Awasthi et al., 2014), and on the other hand, enhance ecosystem responses to 'positive' perturbations including nutrient enrichment (Reich et al., 2001; He et al., 2002; Fridley, 2003). This can result from a 'sampling effect' as more diverse communities are more likely to contain particular species that are very resistant to environmental stress or highly responsive to improved conditions (Aarssen, 1997; Huston, 1997), or niche complementarity and facilitation among species because wider ranges of functional traits in species-richer communities can positively affect ecosystem performance under novel environmental conditions (Tilman et al., 1997; Loreau, 2000).

Microbes, particularly those inhabiting topsoil, are characterized by tremendous diversity which is now threatened by anthropogenic activities including agricultural intensification and land use changes (Maeder et al., 2002; de Vries et al., 2013; Paula et al., 2014). While it seems reasonable to expect functional redundancy among hyper-diverse microbes (Torsvik et al., 2002; Nannipieri et al., 2003; Allison and Martiny, 2008; Cleveland et al., 2014), experimental studies that manipulated microbial communities often, although not always, found negative consequences of microbial diversity loss for ecological functions such as nitrogen cycling or biomass production (Degens, 1998; Wertz et al., 2007; Hol et al., 2010; Peter et al., 2011; van Elsas et al., 2012; Philippot et al., 2013; Wagg et al., 2014). However, it remains uncertain how important microbial diversity is relative to abiotic factors, and how they interact, in driving ecosystem processes (but see Degens, 1998; Wertz et al., 2007; Awasthi et al., 2014).

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Here we experimentally address the dependency of soil microbial respiration on microbial diversity and soil moisture. Knowledge of the determinants of soil microbial respiration is crucial for a better understanding of global carbon cycles under future climatic conditions (Spehn et al., 2000; Curiel Yuste et al., 2007; Curiel Yuste et al., 2010; Suseela et al., 2012; Zhou et al., 2012; Li et al., 2013). There has been a rich literature on how abiotic factors, including temperature and moisture, affect soil respiration; however, microbial diversity and composition have often been confounding factors as they co-vary with the abiotic factors (Carney et al., 2007; Cleveland et al., 2007; Allison et al., 2013; Karhu et al., 2014; Matulich and Martiny, 2014; Whitaker et al., 2014; García-Palacios et al., 2015). It is possible that higher species diversity allows microbial communities to achieve more reliable ecosystem functions under different moisture regimes. We carried out a microcosm experiment where microbial diversity and soil moisture were manipulated independently, to address their interaction in driving soil heterotrophic respiration.

2. Materials and methods

2.1. Soil collection and treatment

Soil was collected from the top 15 cm of a semiarid grassland (Xilingol, Inner Mongolia, China; 43°32′N, 116°30′E), where *Leymus chinensis* was the dominant plant species. The soil was sieved to <2 mm, homogenized, and divided into microcosms of 160 g (equivalent dry mass) in 250 mL Schott Duran bottles. These microcosms were sterilized by 100 kGy gamma irradiation (Hongyisifang radiation technology Co., Ltd, Beijing, China), with soil sterility checked by enumeration of culturable bacteria on nutrient agar plates (3 g L^{-1} beef extract, 10 g L^{-1} peptone, 5 g L^{-1} NaCl and 15 g L^{-1} agar). Microcosms were then inoculated with suspensions of the same soil that had not been sterilized. Inocula were prepared by homogenizing 50 g of soil (equivalent dry mass) in 100 mL of sterile demineralized water by grinding and vortexing, followed by serial dilutions in sterile demineralized water. Sterile soil microcosms were inoculated with dilutions to create inocula equivalent to 10^{-2}, 10^{-4}, and 10^{-6} g of non-sterile soil per g sterile soil, 18 replicates per treatment level. Soil microcosms were incubated for recovery for 1 month at 25 °C (which is near the mean topsoil temperature at the soil collection site in June and July), with bottle lids loosened and moisture content adjusted to 10% gravimetric water content (water/dry soil; near the moisture content of our source soil) regularly by addition of sterile water. Previous studies showed that the dilution disturbance could lead to a significant decrease in microbial diversity (Wertz et al., 2006; Peter et al., 2011; van Elsas et al., 2012). In the present study, experimental microcosms were incubated for recovery for one month, a period that is likely long enough for a full recovery in microbial activity (Zhang and Zhang, 2015).

Analyses of soil property and microbial composition were carried out immediately after the 1-month recovery incubation (with six replicate microcosms randomly chosen from each level of dilution treatment for the measurements). Microcosms recovering from different levels of dilution treatments showed no detectable difference in total carbon or nitrogen content (Appendix A: Methods A1, Fig. A1). Pyrosequencing analyses of bacterial and fungal species composition (see detailed experimental procedures in Appendix A: Methods A2) confirmed that increasing levels of dilution treatments led to progressively decreased bacterial and fungal diversity (Fig. 1; Figs. A2 and A3). The microcosms under the 10^{-2}, 10^{-4}, and 10^{-6} dilution treatments are hereafter referred to as high-, intermediate- and low-diversity microcosms, respectively.

2.2. Experimental setup and measurement of soil respiration

After the 1-month recovery growth, microcosms were incubated for another 46 days under three different moisture conditions. The 18 microcosms at each level of microbial diversity were randomly assigned into three groups, six replicates per group. Each group was exposed to one of the following three moisture treatments: low (10% gravimetric water content, near 30% of the water-holding capacity), high (20%), or fluctuating moisture (changing between 10% and 20%). For the low- or high-moisture microcosms, water content was maintained at 10% or 20% by adding sterile water at a 4-day interval (at day 1, 5, etc.). For the fluctuating-moisture microcosms, moisture content was increased to 20% at day 1. Then moisture was measured at a 4-day interval; when the average moisture of the microcosms became <10%, moisture of every microcosm was increased abruptly to 20% (pulse water addition was performed at day 1, 17 and 37; Fig. A4).

Respiration rate of each microcosm was measured at a 4-day interval (at day 2, 6, etc.), with a Li-Cor 840A CO_2/H_2O analyzer (Li-Cor Environmental, Lincoln, NE, USA). Before measurement, each microcosm (bottle) was placed in water bath at 25 °C and sealed with a rubber stopper. The rubber stopper had two ports that were connected with the gas analyzer, thus the bottle and the analyzer became a closed system. The analyzer maintained an air flow through this closed system, and recorded temperature, air pressure, CO_2 and water content every second. For each microcosm, we ran the measurement for 70 s; data from 31 to 70 s were used for CO_2 efflux calculation (fluctuations in air pressure and CO_2 concentration may be observed at the beginning of the 70-s measurement, but then air pressure would remain constant and CO_2 concentration increases gradually and linearly). The slope of CO_2 concentration against time (dCO_2/dt) was calculated, and the amount of CO_2 evolved from the soil per second is the product of the slope and the volume of the system. The volume of the system was measured by injecting a known quantity of air with known CO_2 concentration into the system that had been CO_2-free (by flushing N_2) and measuring the final CO_2 concentration, with the dilution factor in CO_2 concentration equal to the ratio of injected air volume to the system volume (Curiel Yuste et al., 2007). Soil respiration rate was expressed as μmol per g dry soil per second.

2.3. Data analysis

There were three explanatory variables for soil respiration rates: level of microbial diversity, moisture treatment, and time. However, time may not be a meaningful explanatory variable for the fluctuating-moisture microcosms, as its effect can be confounded with that of moisture changes. To avoid this issue, we carried out two separate analyses. First, the average respiration rate over time was calculated for each microcosm, and analyzed using ANOVA, with diversity level and moisture treatment as two categorical explanatory variables. Second, respiration rates of the low- and high-moisture microcosms at all points in time were analyzed using linear mixed-effects model, with diversity level and moisture treatment as two categorical explanatory variables, time as a continuous explanatory variable, and microcosm ID as a random factor.

2.4. Accession numbers

The pyrosequencing data have been deposited in NCBI Sequence Read Archive under accession number SRP057044.