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Spatial distribution of microbial community composition along a steep slope plot of the Loess Plateau

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ABSTRACT

Spatial heterogeneity of soil microbes introduces great uncertainty to our understanding of microbe-mediated soil carbon cycling, yet was few studied on sloping lands. Along a steep-slope grassland (35°) on the Chinese Loess Plateau, soils of 0-10 cm were sampled in 2016 at three slope positions (upper, middle and bottom) to determine microbial community composition (by Illumina Hiseq sequencing) and function (enzymes involved in carbon cycling, the in situ soil respiration and temperature sensitivity). The bacterial alpha-diversity were greater at middle- and bottom- than at upper slope position, while fungal alpha-diversity varied little across slope positions. The bacterial phylum Proteobacteria was 9.7% and 19.4% lower but Acidobacteria was 36.5% and 41.3% greater at bottom- than at upper- and middle- slope positions. The fungal community transitioned from being Basidiomycota-dominant (relative abundance of 46.8%) at upper slope position to Zygomycota-dominant (relative abundance of 36.5%) at bottom slope position. The β -D-glucosidase activity generally declined down the slope while β -p-xylosidase and cellobiohydrolase activities hiked at middle slope position. All the enzyme activities were suppressed at bottom slope position. Soil respiration increased by 49.1% (P < 0.05) while temperature sensitivity decreased by 13.2% (P < 0.05) down the slope. Both bacterial richness (OTU) and diversity (Shannon diversity index) positively correlated with soil respiration. The copiotrophic groups (Acidobacteria and Zygomycota) negatively and oligotrophic groups (Proteobacteria and Basidiomycota) positively correlated with temperature sensitivity of soil respiration. Our findings revealed divergent responses of soil bacterial and fungal communities along the slope and highlighted the importance of microbial information in predicting the spatial variability of soil respiration in hillslope ecosystems.

1. Introduction

Soil microorganisms are a main component of the terrestrial biosphere and play key role in mineralization and sequestration of soil organic carbon (SOC), which regulates soil CO_2 flux (Schimel and Schaeffer, 2012; Six et al., 2006; Trivedi et al., 2016). The spatial heterogeneity of soil microbes poses great challenge to accurately estimating CO_2 flux in global carbon (C) cycling (Singh et al., 2010; Wagg et al., 2014). So far, the heterogeneity of soil microbes has been extensively studied in forest (Chen et al., 2016; Churchland et al., 2013; Li et al., 2015), grassland (Budge et al., 2011; Chen et al., 2017; Li et al., 2017a) and farmland (Dungait et al., 2013; Helgason et al., 2014; Xiao et al., 2017b), but less in hillslope ecosystems. Actually, more than 60% of the global land area are slopes with gradients $> 8^{\circ}$ (Berhe and Kleber, 2013). On sloping lands, soil microbial communities and the microbe-mediated C cycling processes are regulated by water erosion (Huang et al., 2013; Li et al., 2015) through soil water and substrate differentiation (Hu et al., 2016; Wang et al., 2017b). This further brings about considerable uncertainties when estimating soil respiration at global scale (Davidson et al., 2006; Xu and Qi, 2001).

For a certain soil, the microbial community composition and functioning are generally controlled by soil microclimate (Yuste et al., 2014; Yuste et al., 2011; Zhang et al., 2005), substrate quantity and quality (Chen et al., 2016; Fierer et al., 2003; Heitkötter et al., 2017), as well as plant growth and root activity (Hu et al., 2010; Li et al., 2017b; Singh et al., 2010). Soil water and substrate distribution on sloping lands are

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very likely to experience evident changes even over short distance (Du et al., 2015; Helgason et al., 2014; Li et al., 2015), mostly as a result of erosion-induced water and material transport and redistribution (Hu et al., 2016; Wang et al., 2017b; Wang et al., 2017c). Soil water directly alters physical environment for soil organisms and their accessibility to substrate (Jassal et al., 2008; Suseela et al., 2011) and indirectly affects soil microbes by changing the input of plant C into the rhizosphere (Bardgett et al., 2008; Singh et al., 2010). Soils at lower part of a slope tend to have relatively better soil water condition due to more inflow and less outflow (Wang et al., 2017b), as well greater water holding capacity (Wei et al., 2014). The available substrates are also likely to be redistributed by water erosion through selective erosion and deposition processes of light/fine particles along slopes (Lal and Pimentel, 2008; Polyakov and Lal, 2008), namely depleting SOC-rich soil fractions from upper part of the slope and depositing them at lower part (Hu et al., 2013; Wang et al., 2017b). The varying soil water and substrate conditions along the slope can further lead to distinctive vegetation coverage, above- and below-ground plant biomass on different topographic positions (Wang et al., 2015; Xu and Wan, 2008), which in turn generates a variety of substrates for different microbial communities through fresh C supply (Fuchslueger et al., 2014; Li et al., 2017a).

In this study, spatial variations in microbial diversity, community composition and enzyme activity at upper, middle and bottom slope positions on the sloping grassland were investigated. In addition, soil respiration, soil temperature and soil moisture were *in situ* measured for three years. The objectives were to 1) characterize the spatial variation of microbial diversity, community composition and enzyme activities along the steep slope; 2) identify the influential environmental factors of soil microbial community; and 3) explore potential links between microbial diversity, community composition, enzyme activities and soil respiration on the eroding slope. We hypothesized that 1) microbial diversity, abundance and activity would increase down slope due to greater soil water and substrate availability; 2) the deposition of soil water and substrate would result in increased soil respiration and decreased Q_{10} downslope.

2. Materials and methods

2.1. Study site

This study was conducted on a ridge slope in Wangdonggou wa-107°40′ E-107° tershed (35°13′ N-35°16′ N, 42′E; elevation 946-1226 m; area 8.3 km²), which is located on the southern Loess Plateau in the middle reaches of the Yellow River, northern China (Fig. 1a). Soils in this region have been heavily weathered with low SOC (Guo et al., 2012), thus highly erodible with poor cohension. Regional soil erosion, average annual soil erosion rate is $50-200 \text{ t ha}^{-1}$ (Fu et al., 2005; Xiao et al., 2017a), has greatly reduced crop yield and altered regional hydrologic regimes (Zhang et al., 2015; Zhu et al., 2014), resulting in fragmented terrain (Wang et al., 2017b). In general, sloping land and gullies account for two thirds of the studied Wangdonggou watershed (Fig. 1b), with about 60% of the slopes in length < 60 m and more than 41.2% of the slopes with gradient $> 25^{\circ}$ (Li and Su, 1991). The region has a mean temperature of 9.4 °C (1957–2016). The average annual precipitation is 560 mm, 60% of which falls between July and September. Average annual sunshine duration is 2330 h, annual total radiation is 484 kJ cm^{-2} , and average frost-free period is 171 days. The meteorological data were provided by the State Key Agro-Ecological Experimental Station established in 1984 in Changwu County. According to the American soil classification system, the studied soil is a uniform loam of loess deposits belonging to Calcic Cambisols, which originate from parent material of calcareous loess.

2.2. Experimental design

A natural steep slope (35°) of 50 m long covered by grass was

selected as experimental site (Fig. 1c). Three quadrats of $1 \text{ m} \times 1 \text{ m}$ were established on each slope position, with the grass species, herb coverage be surveyed. The grass composition are Bothriochloa ischaemum L., Lespedeza davurica and Artemisia gmelinii, among which the Bothriochloa ischaemum L. is the dominant species. The coverage of each soil sampling site are around 50% at upper slope position, 75% at middle slope position and 100% at bottom slope positions. To exclude edge effects, three plots were established in the central position of the slope in 2014. To avoid the influence of slope aspect and differences in original soil properties on soil respiration, all three plots were established on the same slope with similar soil properties and therefore, had the same aspects. Each plot was 20 m \times 5 m with the longest side in the direction of the slope gradient. Plots were separated 150 m apart and separated by a brick wall of 15 cm in height, 40 cm in depth and 6 cm in thickness to prevent the inflow of runoff outside the plots and the outflow of runoff inside the plots. Each plot was divided into three parts: upper slope position (2.5-7.5 m), middle slope position (7.5–12.5 m), and bottom slope position (12.5–17.5 m). In March 2014, three polyethylene soil collars were placed on each slope position (20 cm in diameter and 12 cm in height) deep to 10 cm to in situ collect the soil respiration data. To representatively cover the targeted slope position, the polyethylene collars at each slope position were at least 50 cm apart from each other.

2.3. Measurements of soil respiration, soil temperature and soil moisture

During the three years of observation period (2014, 2015 and 2016), soil respiration rates (R_s) were measured every seven days between 9:00 am and 11:00 am (Iqbal et al., 2009), by mounting a soil CO₂ flux system (a portable chamber of 20 cm in diameter, Li-8100, Lincoln, NE, USA) onto the polyethylene collars (Jiang et al., 2015; Wang et al., 2017a). At the same time with the soil respiration measurement, soil temperature (using a Li-Cor thermocouple probe) and soil moisture (using a Theta Probe ML2X with an HH2 moisture meter) at 10 cm depth were also measured in three directions (0°, 120° and 240°), each 10 cm away from the collar.

2.4. Soil sampling and soil chemical analysis

Three composite soil samples were taken at each part of the plot (upper, middle and bottom slope positions). Each composite soil sample consisted of three subsamples collected randomly at topsoil (0-10 cm), using a soil auger of 3 cm in diameter on October 1 of 2016. Each composite soil sample was then passed through a 2.0-mm sieve and divided into three subsamples: one part stored at -80 °C for DNA extraction, a second part stored at 4 °C and at field moisture for less than 4 days to measure soil enzyme activities, soil microbial biomass carbon content (SMBC), soil dissolved organic carbon (DOC) and soil nitrate (NO3-N) and ammonium (NH4-N) nitrogen content. The third part of the soil sample was air dried and then crushed to pass through a 0.15 mm sieve to measure SOC and soil total nitrogen content (TN). The SOC was determined using the K₂CrO₇-H₂SO₄ oxidation method (Sparks et al., 1996). To measure DOC content, the field-moist soil samples (equivalent to 15 g oven-dried soil) were extracted with 60 ml of 0.5 M K₂SO₄ (soil to solution ratio 1:4) for 1 h. After centrifuged at 4000 rpm for 25 min, the supernatant was filtered through a 0.45 mm membrane filter and measured in a Total Organic Carbon Analyzer (TOC-VCPH, Shimadzu, Japan) (Fujii et al., 2011; Vance et al., 1987). The soil nitrate (NO₃-N) and ammonium (NH₄-N) nitrogen were extracted with KCl $(1 \text{ mol } L^{-1})$ and determined by colorimetry using a Bran & Luebbe II AutoAnalyser (Fernández-Escobar et al., 2009). Soil pH was determined with a digital pH meter (Woonsocket, RI, USA) using a soil-to-water ratio of 1:2.5(w/v).

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