

# Spatial variations of soil respiration and temperature sensitivity along a steep slope Version 2

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## Abstract

Spatial heterogeneity of soil respiration and its temperature sensitivity poses great challenge to accurately estimating carbon flux in global carbon cycling, which have been researched mostly on flatlands but few times in hillslope ecosystems. On an eroded slope (35°) of the semiarid Loess Plateau, soil respiration, soil moisture and soil temperature were measured *in situ* at upper and lower slope positions in triplicates from 2014 until 2016, with soil biochemical and microbial properties also being determined.

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## Protocol

Select the experimental site.

### Step 1.

This study was conducted on a typical ridge slope in Wangdonggou watershed (35°13' N-35°16' N, 107°40' E-107° 42' E; elevation 946-1226 m; area 8.3 km<sup>2</sup>), which is located in the typical eroded tableland-gully region of the southern Loess Plateau in the middle reaches of the Yellow River in northern China.

Experimental design.

### Step 2.

To avoid the influences 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 × 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 had a catchment water base, a water sink, and two water tanks (A and B) for the measurement of runoff and sediment. The water base was tilted inwardly towards the centre to help water and sediment produced in the plot flow into the water sink. Two cylindrical steel buckets with an inner diameter of 80 and 90 cm and a height of 125 cm were used as the water tanks. Nine holes with the same diameter were arranged at a depth of 40 cm in water tank A. The middle hole was connected to water tank B, and the other eight holes were arranged synnetrically for the drainage of water. The 20 m length plot was divided into two parts by the 10 m boundry, above which was the range of upper part of the plot and down which was range of lower part of the plot. The positions with respect to the plot are referred as: upper slope position (upper) and lower slope position (lower), which was in similar design with that of one previous study [48].

## Soil respiration measurement

### Step 3.

For each plot, three PVC soil collars (20 cm in diameter and 12 cm in height) at each slope position to collect soil respiration data. They were at least 50 cm apart from each other to representatively cover the upper or lower slope position.

During the three years of observation period (2014, 2015 and 2016), the respiration rates of surface soil ( $R_s$ ) were measured every seven days, by mounting a soil CO<sub>2</sub> flux system (a portable chamber of 20 cm in diameter, Li-8100, Lincoln, NE, USA) onto the polyethylene collars. When effective rainfall events occurred, measurements were conducted immediately afterwards, and continued for at least three days to collect the possible pulses of CO<sub>2</sub> emissions stimulated by rainfall events [36, 41]. Given the limited soil respiration activities in cold winter, CO<sub>2</sub> flux was only measured once a month during December, January and February. For each measurement, it was conducted between 9:00 am and 11:00 am [2], and all visible living organisms were removed prior to measurements. Soil respiration at each slope position was calculated from the mean of three collar measurements (the measurement at three collars in each slope position differed by less than 15% at any measurement period).

## Soil biochemical analysis

### Step 4.

To obtain basic soil properties, six soil samples were taken in the parallel positions beside each plot (three cores at upper slope position and three cores at lower slope position). Each sample consisted of three subsamples which randomly collected at topsoil (0–20 cm). Immediately after sampling, each sample was passed through a 2.0 mm sieve and divided into three portions: one portion stored at –80 °C for DNA extraction, one portion stored at 4 °C for less than four days to measure soil microbial biomass carbon content (SMBC), soil dissolved organic carbon (DOC) and soil nitrate (NO<sub>3</sub>-N) and ammonium (NH<sub>4</sub>-N) nitrogen content, and the third portion was air dried and then crushed to pass through a 0.15 mm sieve to determine soil organic carbon (SOC).

## Data analysis

### Step 5.

Statistical significance was defined as  $P \leq 0.05$ . All the statistical analysis was performed using SPSS 20.0 software (SPSS Inc., Chicago, USA). The figures were generated using Sigmaplot 12.5 software (Systat Software Inc., San Jose, CA, USA).