



## Data in Brief

## GeoChip profiling of microbial community in response to global changes simulated by soil transplant and cropping



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## ABSTRACT

Microbe plays an important role in driving biogeochemical cycles, thus it is of great interest to understand microbial responses and feedbacks to global changes. We have recently analyzed functional potentials of soil microbial community via a high-throughput, microarray-based metagenomic tool named GeoChip 3.0 to illustrate microbial responses to global changes simulated by soil transplant and/or maize cropping. Here we describe detailed experimental design, data collection and pre-processing to support our published studies by Liu et al. [5] and Zhao et al. [14].

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## Specifications

Organism/cell line/tissue	Soil
Strains	Soil microorganisms
Sequencer or array type	GeoChip 3.0 ( <a href="#">GPL17825</a> )
Data format	Raw data:TXT, processed data: SOFT, MINIML and TXT
Experimental factors	Climate effect, cropping effect
Experimental features	Profiling microbial functional response to global changes simulated by soil transplant and maize cropping
Consent	n/a
Sample source location	Hailun, Heilongjiang Province, China (E126°38' and N47°26'), Fengqiu, Henan Province, China (E114°24' and N35°00'), Yingtan, Jiangxi Province, China (E116°55' and N28°15')

## Experimental design, materials and methods

## Site description

The experiment at three long-term agricultural experimental stations (namely the N, C and S sites) across northeast to southeast China was set up and operated by the Institute of Soil Science, Chinese Academy of Sciences ([Fig. 1](#)). This experiment belongs to an integrated project (The Soil Reciprocal Transplant Experiment, SRTE), which serves as a platform for a number of studies evaluating climate and cropping effects on soil microbial diversity and its agro-ecosystem functioning [[8](#)]. The N site, situated at Hailun, Heilongjiang Province (E126°38' and N47°26') in the northeastern China, has a cold temperate monsoon climate with an annual average temperature and precipitation of 1.6 °C and 60 mm, respectively ([Table 1](#)). The C site, located about 1700 km away from the N site at Fengqiu, Henan Province (E114°24' and N35°00'), has a warm temperate monsoon climate with an annual average temperature and precipitation of 13.8 °C and 128.8 mm, respectively. The S site, located about 800 km away from the C site at Yingtan, Jiangxi Province (E116°55' and N28°15'), has a middle subtropical monsoon climate with an annual average temperature and precipitation of 18.4 °C and 132.4 mm, respectively.

SRTE was performed with three types of agricultural soils from the local area of each of the three experimental stations: pH-neutral black

## Direct link to deposited data

<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE51592>.

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**Fig. 1.** Map of the three experimental stations in this study. Abbreviations: N – Hailun, Heilongjiang Province, C – Fengqiu, Henan Province, S – Yingtan, Jiangxi Province.

soil at the N site, pH-alkaline Chao soil at the C site, and pH-acidic red soil at the S site, which belong to *Phaeozem*, *Cambisol* and *Acrisol* in the FAO soil classification system [3] (Table 1). *Phaeozem* soil, occupying approximately  $5.9 \times 10^5 \text{ km}^2$  in China as one of the three zonal *Phaeozem* areas in the world [2], is the most fertilized soil with a high organic matter content of 48.6 g/kg soil and pH of 6.3 [9]. *Cambisol* soil covers an area of about  $1.3 \times 10^5 \text{ km}^2$ , which was derived from alluvial sediments of the Yellow River, the second largest river in China. It is a sandy loam soil with an organic matter content of 9.5 g/kg soil and pH of 7.7 [1,7]. *Acrisol* soil, accounting for about  $2.0 \times 10^6 \text{ km}^2$  of southern China, was derived from quaternary red clay with a low pH of 4.0 and an organic matter content of 9.9 g/kg soil [6,11].

#### Experimental design

In October 2005, eighteen plots with a size of 1.4 m in length  $\times$  1.2 m in width  $\times$  1.0 m in depth were established in each station. The plots were surrounded by 20 cm brick walls and paved with sand underneath to be isolated from surrounding environment.

The three types of soil hereinbefore were transported to the plots at each station, each for six plots. Aboveground vegetation was removed before transport. Soil block was excavated in five 0.2 m depth layers with each layer sufficiently mixed and then deposited sequentially to a plot. To analyze the cropping effect, maize was grown since spring of

2006 to three of the six plots. The maize type was Haiyu 6, Zhengdan 958 and Denghai 11 at the N, C and S site, respectively. The other three plots remained to be bare fallow. None of the plots was fertilized or irrigated. Weeds were manually removed.

Samples were designated according to the sites (Table 2). Bare fallow soil samples transferred from the N site to the N, C or S site were named as N, NC or NS, respectively. Similarly, samples from the C site to the C, N or S site were named as C, CN or CS, and from the S site to the S, C or N site were named as S, SC or SN, respectively. Soil samples with maize cropping were distinguished from bare fallow samples by a suffix of m, that is, Nm, NCm, NSm, Cm, CNm, CSm, Sm, SSm and SNm.

#### Sampling

In the late summer (August–September) of 2009, soil samples were collected within 2 days after harvesting maize. For each plot, ten soil cores from surface soil (0–15 cm) were collected by a steel auger of 2 cm diameter. Rhizospheric soil in cropped plots was avoided. Soil was composited by using a point-quarter method as previously described [4], resulting in an approximately 500 g soil. Each sample was separated into two sterile plastic bags. One was transported to the lab in liquid nitrogen and stored at  $-80^\circ\text{C}$  until DNA extraction. The other one was transported on ice and stored at  $4^\circ\text{C}$  for geochemical analyses.

**Table 1**

Site description.

Site	Location	Annual temperature ( $^\circ\text{C}$ )	Annual precipitation (mm)	Longitude and latitude	Climate	Soil type	Soil pH	Soil organic matter (g/kg)
S	Yingtan, Jiangxi	18.4	132.4	28°15'N, 116°55'E	Middle subtropical	<i>Acrisol</i>	$4.0 \pm 0.38$	$9.9 \pm 0.70$
C	Fengqiu, Henan	13.8	128.8	35°00'N, 114°24'E	Warm temperate	<i>Cambisol</i>	$7.7 \pm 0.20$	$9.5 \pm 0.55$
N	Hailun, Heilongjiang	1.6	60.0	47°26'N, 126°38'E	Cold temperate	<i>Phaeozem</i>	$6.3 \pm 0.17$	$48.6 \pm 5.08$

**Table 2**

Sample denotation.

Sample name	Original location	Target location	Cropping	Sample name	Original location	Target location	Cropping
N	N	N	No	Nm	N	N	Yes
NC	N	C	No	NCm	N	C	Yes
NS	N	S	No	NSm	N	S	Yes
C	C	C	No	Cm	C	C	Yes
CN	C	N	No	CNm	C	N	Yes
CS	C	S	No	CSm	C	S	Yes
S	S	S	No	Sm	S	S	Yes
SC	S	C	No	SCm	S	C	Yes
SN	S	N	No	SNm	S	N	Yes

### Soil DNA extraction and purification

Microbial genomic DNA was extracted by a freeze-grinding method [12,15]. Briefly, 5 g of soil was mixed with 2 g of sterile silica sand and grinded in liquid nitrogen until the sample started to thaw. The freezing and grinding steps were repeated twice. After collecting the sample into a fresh tube, 16.5 mL of extraction buffer, mixed by 0.1 M NaH<sub>2</sub>PO<sub>4</sub>, 0.1 M Na<sub>2</sub>HPO<sub>4</sub>, 0.1 M ethylene diamine tetraacetic acid (EDTA), 0.1 M Tris-HCl, 1.5 M NaCl and 1% cetyltrimethyl ammonium bromide (CTAB), and 61 μL proteinase K (10 mg/mL) was added. The mixture was incubated at 37 °C for 30 min, followed by another 2 h incubation at 65 °C with an addition of 1.83 mL 20% sodium dodecyl sulfate (SDS). The sample was then centrifuged for 20 min at 3600 × g, 25 °C to retrieve the supernatant. To extract more DNA, the remaining soil in the tube was re-suspended with 6 mL extraction buffer and 0.67 mL 20% SDS. After centrifugation, supernatant was collected and combined with a previous supernatant, which was then mixed with an equal volume of chloroform: isoamyl alcohol (24: 1 (v/v)) and continuously inverted for 5–10 min. Supernatant was collected into a fresh conical tube after centrifugation at 3700 × g for 20 min, followed by an addition of 0.6 volume of 2-isopropanol. The liquid was kept at –80 °C overnight and thawed in a 37 °C water bath on the second day. The supernatant was discarded after centrifuging the tube at 25 °C, 15,000 × g for 20 min. DNA pellet was then washed with 1 mL ice-cold 70% ethanol and dissolved in 200 μL nuclease-free water.

Crude DNA was purified using 0.5% low melting point agarose gel electrophoresis at 30 V for 14 h. The gel slice with the DNA band was collected into a 2 mL tube and melted at 65 °C. Then an equal volume of water, Tris-saturated cold phenol (pH 8.0), and chloroform:isoamyl alcohol (24:1 (v/v)) was added sequentially to extract DNA from the gel matrix. After centrifugation at 8 krpm for 5 min, the aqueous (top) layer was transferred to a new tube. An equal volume of isobutanol was then added to extract DNA, which was repeated until the aqueous volume was reduced to ~400–500 μL. Finally, 0.1 volume of 3 M NaOAc (pH 5.2) and 2 × volume of cold 100% ethanol were added. The mixture was incubated at –20 °C overnight and centrifuged at 12 krpm for 30 min to precipitate DNA. Then DNA pellet was washed by 95% ethanol. DNA was resuspended in 50 μL nuclease-free water after air-drying the pellet.

### DNA quality and quantification

The quality of purified DNA was assessed using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE). Typical DNA samples of good quality have the ratios of A<sub>260</sub>/A<sub>230</sub> > 1.7 and A<sub>260</sub>/A<sub>280</sub> around 1.8. For samples with low A<sub>260</sub>/A<sub>230</sub> (< 1.7), an additional step of desalting was carried out to further purify DNA until the final A<sub>260</sub>/A<sub>230</sub> over 1.7. DNA concentrations were quantified by a PicoGreen method using a FLUOstar Optima (BMG LABTECH, Jena, Germany).

### DNA labeling and GeoChip hybridization

DNA was labeled with the fluorescent nucleic acid dye Cy5 by a random primer method [10]. To this end, approximately 1 μg of DNA was mixed with 20 μL random primers, incubated at 99.9 °C for 5 min and then chilled on ice immediately. Then a reaction mixture containing 2.5 μL water, 2.5 μL dNTP, 1 μL Cy5 dUTP (Amersham, Piscataway, NJ, USA) and 80 U of the large Klenow fragment (Invitrogen, Carlsbad, CA, USA) was added to the DNA/random primer mixture and incubated in a PCR cycler at 37 °C for 3 h, 95 °C for 3 min, and then chilled to 4 °C. Labeled DNA was purified with a QIAquick PCR purification kit (Qiagen, Chatsworth, CA, USA) following the manufacturer's instructions. Then DNA was dried at 45 °C for 45 min in a SpeedVac (ThermoSavant, Milford, MA, USA).

Labeled DNA was hybridized on GeoChip 3.0 slides. Firstly, DNA was rehydrated with 120 μL hybridization solution master mixture containing 50% formamide, 3 × SSC, 10 μg of unlabeled herring sperm DNA (Promega, Madison, WI, USA) and 0.1% SDS and then denatured at 95 °C for 5 min. The mixture was immediately transferred to 50 °C until it was loaded onto a microarray slide. Finally, microarray slides with DNA mixture were hybridized on a TECAN Hybridization Station HS4800 Pro (TECAN, US) at 45 °C for 16 h and then washed and dried. The slides were scanned with a ScanArray Express Microarray Scanner (Perkin Elmer, Boston, MA, USA) at 633 nm and spot intensity was determined using the ImaGene version 6.0 (Biodiscovery, El Segundo, CA, USA).

### Data analyses

Raw data from ImaGene were processed before statistical analyses as previously described [5,13,14]. Firstly, spots with signal to noise ratio [SNR = (signal mean – background intensity) /background standard deviation] < 2.0 were removed as poor-quality spots. Spots present only once in three replicates were also removed. Subsequently, the data were logarithmically transformed and then normalized by dividing by the mean value of each slide. The processed data were submitted to GEO with the accession number GSE51592 (<http://www.ncbi.nlm.nih.gov/geo/>).

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