Biological properties of paleosols and present-day soils in Arkaim and its surrounding area, south Urals, Russia

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Abstract
We investigated biological properties of paleosols in comparison to those of present-day soils in Arkaim, Kizilskoe and Sintachta, south Urals, Russia. Soil microbial biomass C and N were detected not only in present-day soils but also in paleosols. The past cultivation history such as plowing and differences in parent materials (e.g., illite-montmorillonitic vs. kaolinitic) had significant effects on microbial biomass and activities of microbes. These results indicated that effects of human activity in the past (20 years ago) on soil microbes still remained and varied depending on the type of parent material. Soils originated from a riparian topography emitted more CO_2 and CH_4 as compared with extremely dried soils in grassland landscapes in Arkaim. N_2O flux was positive (1.3 mg N m^2 /h) on average in a soil with middle water content (28%), while almost zero in soils with minimum (11%) and maximum water content (33%).

Key Words
Greenhouse gasses, land-use change, microbial biomass, paleosol.

Introduction
On the brink of the third and second millennia B.C., a strong center of cultural genesis formed in the South Urals, Russia, with the powerful copper-ore base of the Trans-Urals plain that brought achievements in the field of metallurgy and consequent rapid economic and military developments in this area. The places of Arkaim, Kizilskoe and Sintachta, belonging to this area, are the examples of typical ancient fortresses developed in the period. We conducted a field investigation and sampling of paleosols and present-day soils in Arkaim, Kizilskoe and Sintachta, as a part of research collaboration between JSPS and RFBR Joint Research program. In this paper, we report biological properties of paleosols and present-day soils in relation to greenhouse gaseous emissions as affected by human activities, such as plowing, parent materials, and soil water content.

Materials and methods
Study area
The studied area is located in the Eurasian steppe zone. Fifteen soils located in Arkaim, Kizilsokoe and Sintachta in the administrative region of Chelyabinsk in south Urals, Russia (52^o N, 58 -60^o E) were investigated. Soils were sampled in August 2009, by taking samples from the upper two horizons: surface and sub-surface. Paleosols in this region were buried under the ancient embankment and artificial mounds. Each paleosol was sampled after removing upper present-day soil. The distance between paleosol and present day soil of each site was within 1 km. Soils plowed 20 years ago were sampled as plowed soil in Arkaim. Distances between plowed and unplowed soils were within 1 km. Soils with different water contents were sampled in riparian grassland near Arkaim.

Chemical and microbial biomass analysis
Fresh soil samples were sieved (2 mm) and analysed for soil soluble carbon (C) and nitrogen (N) and microbial biomass C and N within no longer than 1 month. Soil microbial biomass C and N were determined by chloroform-fumigation extraction method (Vance *et al.* 1987). Fresh soils were pre-incubated aerobically for 10 days and fumigated with alcohol-free chloroform at 25^oC for 24h. Then fumigated and non-fumigated soils were extracted with 0.5M K_2SO_4, and soluble organic C and soluble N concentrations were determined using a TOC analyser (TC5000, Shimadzu, Kyoto, Japan) and the peroxide-disulfate digestion/colorimetric method (Hayashi *et al.* 1997). Soil water content was determined by the oven drying method (Foster 1995).

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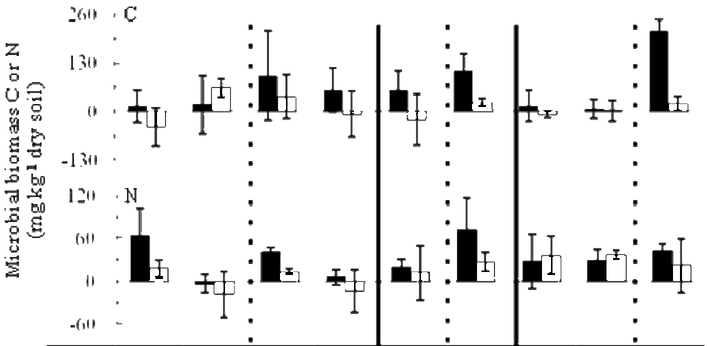
DNA analysis
Total DNA from the samples was extracted using the FastDNA SPIN for Soil Kit (Qbiogene, California, USA) according to the manufacturer's protocol, and then extracted DNA was stored at -20 ^{o}C. *nirK* gene and 16S rRNA gene of methanogenic archaea in soils with different water contents were amplified by the PCR method using with a thermal cycler (Dice TP600, Takara bio Inc., Shiga, Japan) and suitable primer for each gene and DNA polymerase (Ex taq DNA polymerase Hot start version, Takara Bio Inc.).

Gas analysis
Carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) fluxes in the field were determined by the closed chamber method (Inubushi *et al.* 2003). The concentrations of CO_2, CH_4, and N_2O in sampled vials were analysed using gas chromatographs (GC 14B, Shimadzu) equipped with a thermal conductivity, electrons capture, and a flame ionization detectors, respectively. Each gas flux was calculated from the change in each gas flux concentrations over time.

Results and discussion
Chemical and microbial characteristic in paleosol and present-day soil
In paleosol and present-day soil of Arkaim, Kizilskoe and Sintachta, soil water contents were in the range of 1.6-5.8%. Soil microbial biomass was detected not only in present-day soil but also in paleosol, with larger values in present-day soil than in paleosol (Figure 1). Microbiological investigation of 100 steppe paleosols of Russia showed that in paleosols microbial communities preserved their existed communities from the time of archaeological monument construction (Blagodatskaya *et al.* 2003; Khomutova *et al.* 2004). Inubushi *et al*. (2005) discussed existence of microbial biomass in the buried humic horizon (i.e. ancient surface layer) of Andisol in Japan. They investigated vertical distribution of microbial biomass in Andisol profile and found higher amount of microbial biomass in deeper soil layers near the buried humic horizon. They reasoned that significant amount of biologically available organic matter remaining under semi-anaerobic conditions, which reversed to aerobic conditions during pre-incubation, or due to substrates leaching from surface layers and accumulating in deeper layers. In Arkaim and surrounding area, however, later reason is unreasonable because evaporation (450-650 mm /year) excedes precipitation (300-360 mm /year). Therefore, microbial biomass in paleosol in this study may be due to aerobic reverse of biologically available organic matter remaining in semi-anaerobic conditions during pre-incubation.

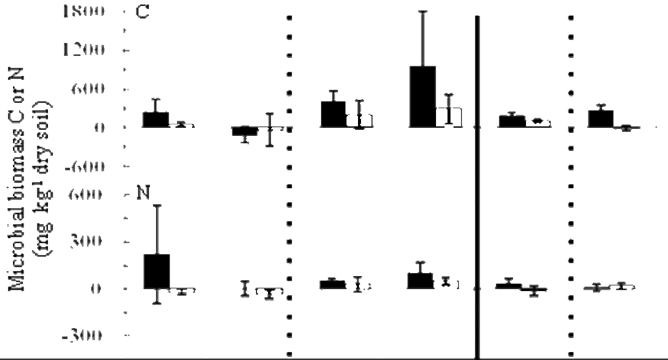
Effect of human activity on soil chemical and microbial characteristics
In soils of illite-montmorillonitic and kaolinitic parent material, soil water contents were in the range of 4.0-7.4%. Soluble C and N in surface of forest soil of illite-montmorillonitic parent material were the highest (336 and 63 mg/kg dry soil, respectivly). Microbial biomass of unplowed soils tended to be larger than of plowed soils (Figure 2). Figure 3 shows various gaseous fluxes as indicators of microbial activities in plowed or unplowed soils of illite-montmorillonitic or kaolinitic parent material. CO_2 was emitted in all types of soil. In soils of illite-montmorillonitic parent material, CO_2 emission in forest soil was larger than that emission in plowed and unplowed grass soils. In soils of kaolinitic parent material, however, there were no significant difference between CO_2 emissions in plowed and unplowed soil (p>0.05, t-test). CH_4 was taken in most soils. Soils of unplowed forest and grass on illite-montmorillonite parent material took in larger amount of CH_4 than plowed grass on the same parent material. However on kaolinitic parent material, the plowed soils took in the larger amount of CH_4 contrast with unplowed. Positive N_2O flux was only detected in plowed grass of illite-montmorillonitic parent material. Additionally, in soils of kaolinitic parent material, taken N_2O by plowed soil was larger than unplowed soil. These differences of microbial biomass and activities between plowed and unplowed soils indicated that effects of human activity (e.g., the plow more than 20 years ago) on soil microbes still have been remaining. Additionally, results of differences of microbial condition affected by human activity between illite-montmorillonitic and kaolinitic soils indicated that effects of human activity on soil microbes varied with the type of parent material.

Microbial characteristics of soils with different water content
In soils with different water content, CO_2 and CH_4 emissions were larger in soil with more water content. N_2O flux was positive in average only in a soil with middle water content (28%), while almost zero in soils with minimum (11%) and maximum (33%) water content. Total DNA from soils with different water content were extracted and amplified by PCR method using with primers for *nirK* and 16S rRNA gene of methanogenic archaea. DGGE analysis is undergoing.



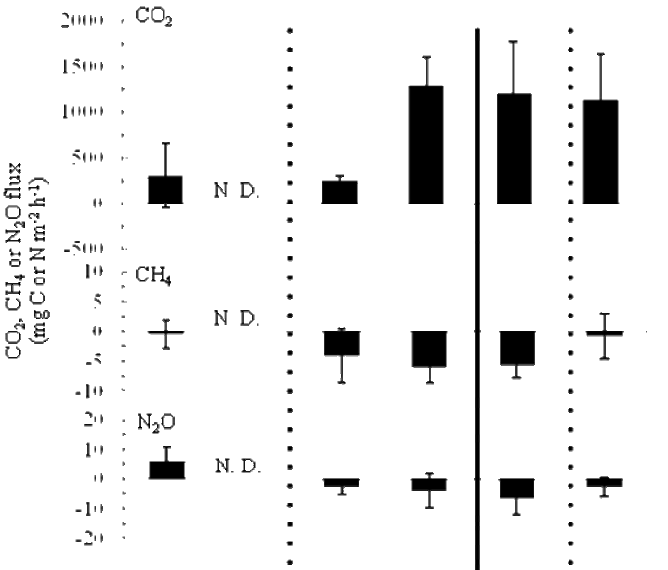
Site No	5	9	6-4	6-6	10	11	12-2	12-4	13
Soil	Paleosol		Present-day		Paleosol	Present-day	Paleosol		Present-day
Site	Arkaim				Kizilskoe		Sintachta		

Figure 1. Microbial biomass C and N in surface (■) and subsurface (□) layer of paleosol or present-day soil of Arkaim, Kizilskoe or Sintachta.



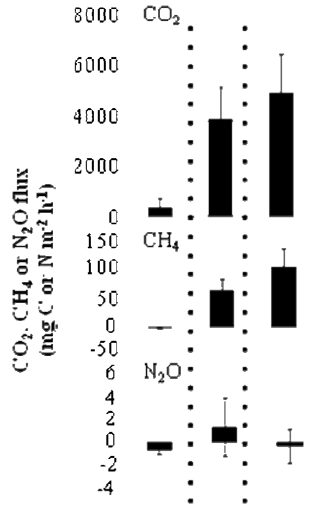
Site No	1	2	3	4	S-2	S-3
Human activity	Plowed		Unplowed		Plowed	Unplowed
Vegetation	Grass			Forest	Grass	
Parent material	illite-montmorillonitic				Kolinitic	

Figure 2. Microbial biomass C and N in surface (■) or subsurface layer (□) of plowed or unplowed soil of illite-montmorillonitic or kaolinitic parent material of Arkaim.



Site No	1	2	3	4	S-2	S-3
Human activity	Plowed		Unplowed		Plowed	Unplowed
Vegetation	Grass			Forest	Grass	
Parent material	illite-montmorillonitic				Kolinitic	

Figure 3. CO_2, CH_4 and N_2O flux in plowed or unplowed soil of illite-montmorillonitic or kaolinitic parent material of Arkaim. N. D. means not determined.



Site No	14-1	14-2	14-3
Water content (%)	11	28	33

Figure 4. CO_2, CH_4 and N_2O flux in soils with different water content of Arkaim.

Conclusions
The presence of microbial biomass in the paleosol horizon of Arkaim, Kizilskoe and Sintachta may be due to the aerobic reverse of biologically available organic matter remaining in semi-anaerobic conditions during pre-incubation. In soils of illite-montmorillonitic and kaolinitic parent material, differences of microbial biomass and activities between plowed and unplowed soils indicated that effects of human activity (e.g., the plow more than 20 years ago) on soil microbes remain. Additionally, results of differences of microbial condition affected by human activity between illite-montmorillonitic and kaolinitic soils indicate that effects of human activity on soil microbes varied with the type of parent material. In soils with different water content, CO_2 and CH_4 emissions were larger in soil with more water. N_2O flux was layer (1.3 mg N m^2 /h) on average in a soil with middle water content (28%), while it was almost zero in soils with minimum (11%) and maximum (33%) water contents.

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