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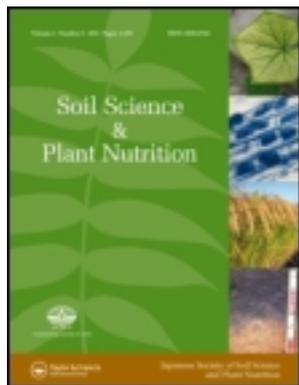


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## ORIGINAL ARTICLE/SHORT PAPER

## Carbon and nitrogen contents and greenhouse gas fluxes of the Eurasian steppe soils with different land-use histories located in the Arkaim museum reserve of South Ural, Russia

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### Abstract

The effects of different land-use histories on contents of soil carbon (C) and nitrogen (N) and fluxes of greenhouse gases [carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>), and nitrous oxide (N<sub>2</sub>O)] measured using the closed chamber method were investigated in the Arkaim museum reserve located in the South Ural of Russia. A natural forest site (NF) and two grassland sites that had different land-use histories (CL: cropland until 1991; PST: pasture until 1991; both sites have been fallow for 18 years) were selected for soil sampling and gas flux measurements. The vegetation in NF was mainly *Betula pendula* Roth. with steppe cherry and grassy cover. Perennial grasses (*Stipa* spp., *Festuca* spp. and others) have been planted in CL and PST since 1991 to establish reserve mode, and the projective cover of these plants were >90% in both sites in 2009. Soil samples were taken from the A horizon in the three sites, and additionally samples of the O horizon were taken from NF. The contents of soil C and N [total C, total N, soluble organic C, soluble N and microbial biomass C (MBC)] in the O horizon of NF were the largest among all investigated soils ( $p < 0.05$ ). Additionally, the total C, total N and MBC in PST were significantly larger than in CL ( $p < 0.05$ ). Positive CO<sub>2</sub> fluxes (i.e., CO<sub>2</sub> efflux) in all three investigated sites were observed. The CO<sub>2</sub> efflux in NF was significantly larger than in CL and PST (129, 30 and 25 mg C m<sup>-2</sup> hour<sup>-1</sup>, respectively,  $p < 0.05$ ), although there was no significant difference in values of CO<sub>2</sub> efflux between CL and PST. There were no significant differences in the fluxes of CH<sub>4</sub> and N<sub>2</sub>O among NF, CL and PST ( $p > 0.05$ ). Our current research indicated that, in soils of the Eurasian steppe zone of Russia, total C, total N and MBC were affected not only by current land-use (i.e., fallow grassland vs. natural forest) but also by past (until 18 years ago) land-use.

**Key words:** Chernozem, effect of land-use difference, Eurasian steppe, greenhouse gas, soil C and N.

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### INTRODUCTION

Several studies have investigated properties of soils with different land-use (e.g., Kadono *et al.* 2008; Kaschuk *et al.* 2011; Zak *et al.* 1994). However, few studies have investigated properties of soils that are currently under

the same land-use but have different land-use histories. Investigation of soils with different past land-use may enable us to know how the past human activity affects the reserve site, where human activities have been restricted or inhibited for preservation of natural ecosystems and/or archeological monuments.

Temperate semi-arid grasslands are important as a global sink and source of greenhouse gas, because they cover approximately 10% of the global land area (White *et al.* 2000). Although the North American prairies and the Eurasian steppes are the two historically dominant temperate semi-arid grassland ecosystems, most studies of greenhouse gas flux in the grasslands have been conducted in North America (Mosier *et al.* 1991). Of the Eurasian steppes, only the typical steppe of Inner Mongolia has received attention in recent years (Holst *et al.* 2007, 2008; Chen *et al.* 2011).

Arkaim, belonging to the administrative region of Chelyabinsk in South Ural, Russia, is archeologically important because, in 1987, a unique fortified settlement and some archeological monuments established about 4000 years ago (i.e., during the Bronze age), were discovered. In particular, since 1991, Arkaim has been distinguished as a museum reserve to preserve the archeological monuments and the natural ecosystem. Since then, in the Arkaim museum reserve (hereafter referred to as the Arkaim), human activities such as crop production and pasturing of livestock have been prohibited.

In the current study, the contents of soil carbon (C) and nitrogen (N) and flux of greenhouse gases [carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>), and nitrous oxide (N<sub>2</sub>O)] were measured in three sites in the Arkaim, to investigate the soil properties as affected by both current and past land-use.

## MATERIALS AND METHODS

The study area is located in the Arkaim museum reserve (52°N, 58–60°E) and in the Eurasian steppe zone, belonging to the administrative region of Chelyabinsk in South Ural, Russia (Fig. 1a). The Arkaim is located 400 km to the south-southwest of Chelyabinsk at the confluence of the Utayganka and Big Karaganka (a tributary of the Ural) rivers. The regional soils are characteristic of the moderately freezing lands with continental climate. The average annual air temperatures are from 1 to 3°C (the average January temperature is from –16 to –18°C, and the average July temperature is from 19 to 20°C). The sum of temperatures exceeding 10°C equaled 1950–2300°C, and the frost-free period ranged from 111 to 125 days over a year. The annual precipitation in the region was from 300 to 360 mm (45% of all precipitation occurred in the summer as

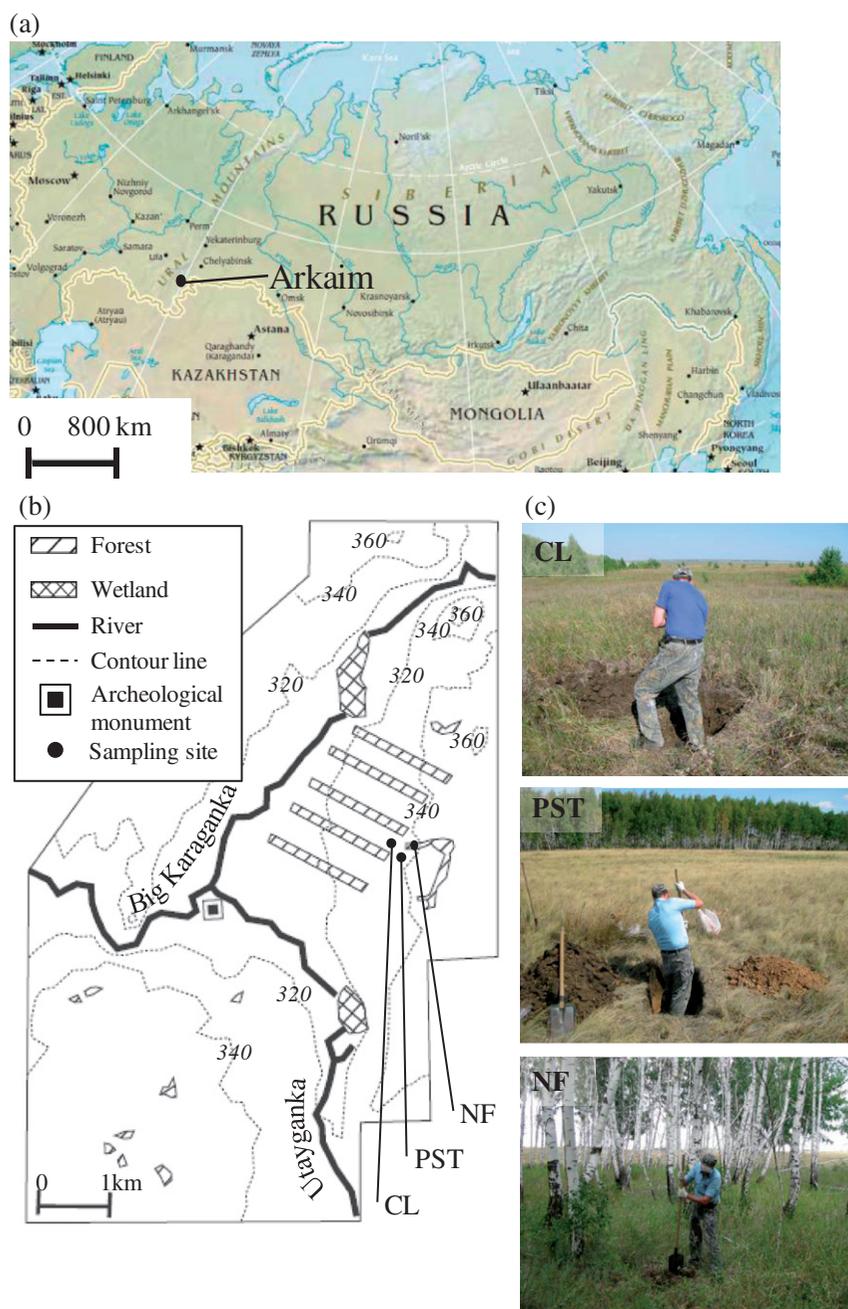
storm rain fall, and 10–12% occurred in winter). The maximum thickness of snow cover was usually 0.25 m. The freezing depths of soils ranged from 0.8 to 2.0 m, and the frozen state was kept up to five months. The annual transpiration ranged from 450 to 650 mm. In some years, dry winds and deflation caused severe damage to the soils and crops.

In August 2009, investigations were carried out at three sites with different land-use histories: 1. a natural forest site (NF); 2. continuous cropland until 1991 and fallow afterward (CL), and 3. continuous pasture until 1991 and fallow afterward (PST) (locations of the sites are shown in Fig. 1b). Soils in the current research were classified as ordinary Chernozem (CL and PST) and Phaeozem (NF), humus-rich soils associated with each other. NF, CL and PST developed on a gentle slope 330–340 m above sea level. Until 1991, cropping of wheat (*Triticum* spp.), perennial grasses (*Bromopsis inermis* (Leys) Holub. and *Medicago caerulea* Less. ex Ledeb. and others) and vegetables had been carried out for about 40 years in CL. NF forms in the bottom of the slope at its discontinuity and contains relatively more soil moisture. The vegetation cover of NF was composed mainly of *Betula pendula* Roth. with steppe cherry and grassy cover in 2009 (Fig. 1c; Okitsu *et al.* 2011). Perennial grasses have been planted in CL and PST since 1991 to establish reserve mode, and the projective cover of these plants was >90% in both sites in 2009. In 2009, the vegetation in CL and PST was composed mainly of *Stipa* spp., *Festuca* spp., drought-tolerant worm wood (i.e., *Artemisia* spp.) and different species of grasses (in CL, there was also observed *Bromopsis inermis*) (Fig. 1c).

In the three sites, soil samples were taken from the A horizon (0–20 cm in CL and PST, and 0–11 cm in NF, respectively). In NF, samples of the O horizon (–4–0 cm) were also taken. All soil samples were air-dried over 2 weeks and passed through a 2-mm mesh size sieve.

Soil water pH [pH(H<sub>2</sub>O)] was determined using a pH meter (pH meter D-52; Horiba Ltd., Kyoto, Japan) at soil to water ratio of 1:2.5, after shaking for 1 hour. Soil electrical conductivity (EC) was measured using an EC meter (EC meter CM-14 P; TOA Electronics Ltd., Tokyo, Japan) at soil to water ratio of 1:5, after shaking for 1 hour. Total C and N in air-dried soil were measured using a C/N analyser (MT500; Yanaco New Science Inc., Kyoto, Japan).

Soil microbial biomass C (MBC) and N (MBN) were determined by the chloroform-fumigation extraction method (Vance *et al.* 1987). Air-dried soil samples were adjusted to 20% ( $W_{\text{water}}/W_{\text{drysoil}}$ ) of water content. These soils were then pre-incubated aerobically for 3 d at 25°C in darkness. After pre-incubation, a portion (ca. 10 g) of this pre-incubated soil was fumigated



**Figure 1** Location of the Arkaim in Russia (a), locations of archeological monument and sampling sites in the Arkaim (b) and view of sampling sites (c). Sampling sites were a natural forest site (NF) and two fallow grassland sites that had different land-use histories until 1991 and were fallow afterward: continuous cropland (CL) and continuous pasture (PST). Values on contour lines (b) are height above sea level (in meters). The five forest bands in (b) are the remains of windbreakers for cropland. Original map of (a) from the World Factbook (Central Intelligence Agency 2011).

with alcohol-free chloroform at 25°C for 24 h. Another portion of the unfumigated soil was incubated at 25°C for 24 h without chloroform. Then each of the fumigated and unfumigated soils was extracted with 50 mL of 0.5 M potassium sulphate ( $K_2SO_4$ ). Contents of organic C and both organic and inorganic N in the extracted liquid were determined using a TOC analyser

(TOC-5000, Shimadzu Co. Ltd., Kyoto, Japan) and the peroxide-disulfate digestion/colorimetric method (Hayashi *et al.* 1997), respectively. Then MBC and MBN were calculated by the following equations:

$$MBC = (C_f - C_{uf}) \times K_{ec}$$

$$MBN = (N_f - N_{uf}) \times K_{en}$$

where  $C_f$  and  $C_{uf}$  are the total contents of organic C in fumigated and unfumigated extracts, respectively;  $N_f$  and  $N_{uf}$  are the total content of N as organic and inorganic forms in fumigated and unfumigated extracts, respectively, and  $K_{ec}$  and  $K_{en}$  are the efficiency of extraction of microbial C and N from soil ( $K_{ec}$ , 2.22;  $K_{en}$ , 2.41), respectively.  $C_{uf}$  and  $N_{uf}$  were determined as soil soluble organic C (SOC) and soluble N (SN).

The fluxes of  $CO_2$ ,  $CH_4$ , and  $N_2O$  in the fields were determined using 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 detector, a flame ionization detector and an electron capture detector, respectively. Gas flux was calculated from the change in each gas flux concentration over time and chamber volume and temperature.

To determine statistical significances, one-way analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) tests for multiple comparisons were used (IBM SPSS statics 19, IBM, New York, USA).

## RESULTS AND DISCUSSION

As shown in Table 1, lower pH in both O and A horizons of NF was observed as compared with those of the CL and PST A horizons. Soil EC ranged from 6.4 to  $17.7\text{ mS m}^{-1}$  (Table 1). The contents of total C and total N in the O horizon of NF were significantly larger than those of the other sites ( $p < 0.05$ ; Table 1). Contents of total C and total N in the A horizon of NF were close to those of CL and smaller than those in PST soil (Table 1). In NF, the contents of SOC and SN in the O and A horizons were significantly larger than those in CL and PST ( $p < 0.05$ ; Table 1). Of the two fallow grasslands, total C and total N contents in PST were significantly larger than those in CL ( $p < 0.05$ ; Table 1). There were no significant differences in SOC and SN between CL and PST (Table 1). As shown in Fig. 2, the highest MBC ( $1647\text{ mg kg}^{-1}$  dry soil) was observed in the O horizon of NF, while the lowest MBC ( $340\text{ mg kg}^{-1}$  dry soil) was found in the A horizon of NF ( $p < 0.05$ ). Furthermore, MBC in PST was significantly higher than that in CL

**Table 1** Chemical and physical properties of soils with different land-use histories, located in the Arkaim

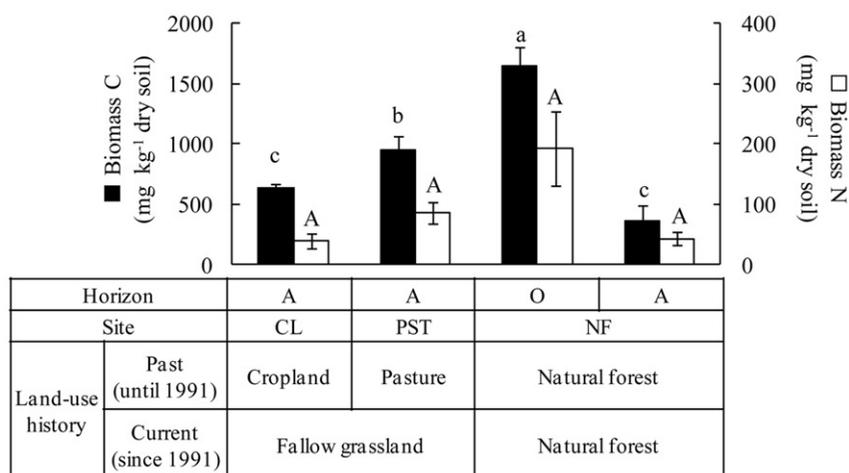
Land-use history		Site	Soil horizon	pH (H <sub>2</sub> O)	EC (mS m <sup>-1</sup> )	Total C (g kg <sup>-1</sup> dry soil)	Total N (g kg <sup>-1</sup> dry soil)	C/N ratio	SOC (mg kg <sup>-1</sup> dry soil)	SN (mg kg <sup>-1</sup> dry soil)
Current†	Past‡									
Fallow grassland	Cropland	CL	A	7.2	15.1	31 d	2.7 c	12	139 c	32 c
	Pasture	PST	A	6.7	9.4	37 b	3.1 b	12	167 c	33 c
Natural forest	Natural forest	NF	O	5.9	17.7	105 a	7.3 a	14	884 a	196 a
			A	5.2	6.4	33 c	2.5 c	13	423 b	73 b

Values with different letters among each column differ significantly by the Tukey honestly significant difference (HSD) tests ( $p < 0.05$ ,  $n = 3$ ).

†Current means land-use history since 1991.

‡Past means land-use history until 1991.

Abbreviations: EC, electrical conductivity; C, carbon; N, nitrogen; SOC, soil soluble organic carbon; SN, soluble nitrogen; CL, continuous cropland; PST, continuous pasture; NF, natural forest.



**Figure 2** Microbial biomass carbon (C) (closed bars) and nitrogen (N) (opened bars) in soils with different land-use histories, located in the Arkaim. Error bars show standard deviations. Different lower-case letters are used for statistical differences in biomass C, and different capital letters are for biomass N [Tukey's honestly significant difference (HSD) tests,  $p < 0.05$ ,  $n = 3$ ].

(952 and 630 mg kg<sup>-1</sup> dry soil in PST and CL, respectively) ( $p < 0.05$ ; Fig. 2). Although there was no significant difference, the value of MBN was greatest in the O horizon of NF and lowest in CL (145 and 40 mg kg<sup>-1</sup> dry soil in NF and CL, respectively; Fig. 2).

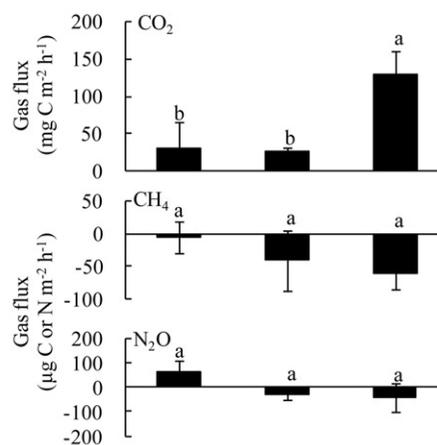
As mentioned above, the contents of soil C and N (total C, total N, SOC, SN, MBC) in the O horizon of NF were the highest among all investigated soils (Table 1 and Fig. 2). These high contents of C and N in NF can be attributed to organic matter input (i.e., leaves and litter fall) in forest soil that is usually greater than in grasslands. *Betula* spp., dominant in NF, are deciduous species. Large contents of C and N (i.e., total C, total N, SOC, SN, MBC and MBN) in forest soil were also observed in other sites of the Eurasian steppe zone (Kadono *et al.* 2008) or semi-arid areas of North America (Zak *et al.* 1994). For example, Kadono *et al.* (2008) reported that organic C and total N were found to be 46 and 4 in forest soils, 28 and 3 in grassland soils, and 20 and 2 in crop lands, respectively (unit is g kg<sup>-1</sup> dry soil). However, in the West Siberian region in the same climatic zone as in Arkaim, larger content of soil C and N was revealed in virgin Chernozem compared with soil of *Betula* forest located (Bazilevich 1965).

The contents of total C, total N, and MBC in PST were significantly higher than those in CL (Table 1 and Fig. 2). Additionally, in the 1992 aftermath of the establishment of reserve mode, total C contents in the A horizon of CL, PST and NF were 29, 35 and 33 g kg<sup>-1</sup> dry soil, respectively (Prikhodko *et al.* 2006). In other words, total C contents in CL and PST increased ca. 2 g kg<sup>-1</sup> dry soil during 17 years. Large soil C and N contents in PST may be attributed to more organic matter inputs in PST until 1991, and during 1991–2003 after the establishment of reserve mode (Ermolaev 1999; Plekhanova *et al.* 2003). Until 1991, PST may have received greater quantities of organic matter input by plant biomass above and below ground, and dung and urine through the ruminant processing of forage. It is known that dung and urine decompose more rapidly because of their low C/N ratio (Franzluebbers *et al.* 2000). On the other hand, CL had received fewer organic matter inputs due to plant material removal with yield. The productivity of CL was supported by organic fertilizers (2–3 tons/ha) and perennial grasses (about 20% in the rotation) until 1991. During 1991–2003 after establishment of reserve mode, the organic matter input in PST may have also been larger than in CL because of more projective plant cover (area covered by plant within a site/total area within the same site) of perennial grasses and cereal crops on the soil surface in PST compared with CL during this period (Ermolaev, 1999 and Plekhanova *et al.*, 2003). Perennial grasses including cereal crops have larger productivity of plant biomass above and below ground

than one- and two-year plants. CL grew mainly one- and two-year weed plants, and projective cover of perennial grasses and cereal crops was less than 60% during the first 2–3 years after 1991, although projective cover of perennial plants in PST was already near 80% in 1992 (Ermolaev 1999). Then, in CL, one- and two-year weed plants were replaced with perennial grasses and cereal crops, and projective cover of these plants was about 85% in 2003 (Plekhanova *et al.* 2003). In 2009, the state of the vegetation of CL was almost the same as in PST, with near 100% projective plant cover (90–95% in CL and 95–100% in PST, as we can see in the field without calculation, Fig. 1c), although there was slightly higher productivity in PST.

Therefore, it was thought that the large contents of total C, total N and MBC in PST compared with CL investigated in 2009 could be attributed to large amounts of organic matter input until 1991 and during 1991–2003 after the establishment of reserve mode. Additionally, amounts of organic material input of CL and PST until 1991, and during 1991–2003, were thought to be affected by differences of land-use in CL and PST until 1991. Thus, it was thought that the large contents of total C, total N and MBC in our PST compared with CL investigated in 2009 were affected by differences of land-use in CL and PST until 1991.

As shown in Fig. 3, there were positive CO<sub>2</sub> fluxes (i.e., CO<sub>2</sub> efflux) in all three investigated sites. And the value



Site		CL	PST	NF
Land-use history	Past (until 1991)	Cropland	Pasture	Natural forest
	Current (since 1991)	Fallow grassland		Natural forest

**Figure 3** Carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>) and nitrous oxide (N<sub>2</sub>O) fluxes in soils with different land-use histories, located in the Arkaim. Error bars are standard deviations. Different letters are used for statistical differences in gas fluxes among sites [Tukey's honestly significant difference (HSD) tests,  $p < 0.05$ ,  $n = 3$ ].

of the CO<sub>2</sub> efflux in NF was significantly higher than in the two fallow grasslands (129, 30 and 25 mg C m<sup>-2</sup> hour<sup>-1</sup> in NF, CL and PST, respectively;  $p < 0.05$ ) (Fig. 3). Large CO<sub>2</sub> efflux in NF reflected a large content of soil C and N (total C, total N, SOC, SN and MBC) in the O horizon of NF (Table 1 and Fig. 2). On the other hand, contrary to total C, total N and MBC in CL and PST (Table 1 and Fig. 2), there was no significant difference between CO<sub>2</sub> efflux in CL and PST. It is known that root/rhizosphere respiration can account for as little as 10% to more than 90% of total in-situ soil respiration (i.e., CO<sub>2</sub> efflux) depending on vegetation type and season (Hanson *et al.* 2000). Therefore, this result (i.e., differences between CL and PST were not so much CO<sub>2</sub> flux but microbial biomass) might be due to the large contribution of root/rhizosphere respiration to total CO<sub>2</sub> efflux in CL and PST with the same plant biomass.

We observed negative CH<sub>4</sub> fluxes (i.e., CH<sub>4</sub> influxes) in all soils (from -5 to -60 μg C m<sup>-2</sup> hour<sup>-1</sup>; Fig. 3). On the other hand, N<sub>2</sub>O fluxes were positive in CL and negative in PST and NF (60, -30 and -41 μg N m<sup>-2</sup> hour<sup>-1</sup> in CL, PST and NF, respectively) (Fig. 3). However, there were no significant differences in fluxes of CH<sub>4</sub> and N<sub>2</sub>O among NF, CL and PST ( $p > 0.05$ ). Temperate semi-arid grasslands, such as prairies and steppes, are generally considered as CH<sub>4</sub> sinks (Mosier *et al.* 1991). Values of our CH<sub>4</sub> flux fell within the range previously reported in native prairies of North America (-10.8 μg C m<sup>-2</sup> hour<sup>-1</sup>, annual average; Mosier *et al.* 1991) and Inner Mongolian steppes ungrazed since 1979 (-42.9 μg C m<sup>-2</sup> hour<sup>-1</sup>, annual average of 2008; Chen *et al.* 2011). Previously reported N<sub>2</sub>O fluxes were 14.6 μg N m<sup>-2</sup> hour<sup>-1</sup> (annual average) in native prairies of North America (Mosier *et al.* 1991), and 1.6 μg N m<sup>-2</sup> hour<sup>-1</sup> (average during growing season of 2005) in Inner Mongolian steppes ungrazed since 1979 (Holst *et al.* 2007). Although these previous studies suggested that temperate semi-arid grasslands could behave as an N<sub>2</sub>O source, Holst *et al.* (2007) pointed out that a negative N<sub>2</sub>O flux was also observed at several times during growing season. Therefore, our negative N<sub>2</sub>O flux was not so extraordinary because our measurement season was in August as well as during the growing season. Additionally, Holst *et al.* (2008) pointed out the importance of flux measurements of greenhouse gasses in the non-growing season (i.e., winter and spring), because freeze-thawing driven N<sub>2</sub>O flux could contribute a large part of the annual N<sub>2</sub>O emissions in the steppe ecosystem. Thus, future work in the Eurasian steppes of Russia will require more frequent and continuous measurements of gas fluxes.

Our current research indicated that, in soils of the Eurasian steppe zone of Russia, total C, total N and microbial biomass were affected not only by current

land-use (i.e., fallow grassland vs. natural forest) but also by past land-use (until 18 years ago). However, it is still unknown how much the effects of past human activity remain in the fallow grasslands of the Arkaim, because of the lack of virgin (i.e., natural) grasslands as a reference for fallow. Therefore, in future works, it is necessary to investigate not only fallow, but also natural, grasslands.

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