**Soil and ecosystem respiration responses to grazing, watering and experimental warming
chamber treatments across topographical gradients in northern Mongolia**

Anarmaa Sharkhuu^{1,2}, Alain F. Plante¹, Orsoo Enkhmandal², Cédric Gonneau³, Brenda B.
Casper³, Bazartseren Boldgiv², Peter S. Petraitis³

¹ Department of Earth & Environmental Science, University of Pennsylvania, Philadelphia, PA
19104-6316, USA

² Department of Biology, National University of Mongolia, Ulaanbaatar 14201, Mongolia

³ Department of Biology, University of Pennsylvania, Philadelphia, PA 19104-6018, USA

Corresponding author: A.F. Plante

Department of Earth & Environmental Science, University of Pennsylvania

Hayden Hall, 240 South 33rd Street, Philadelphia, PA 19104-6316

Telephone: 215-898-9269

Fax: 215-898-0964

Email: aplante@sas.upenn.edu

1. Introduction

Global soil respiration, that is the efflux of heterotrophic- and plant-respired carbon dioxide (CO₂) from the soil surface to the atmosphere, is the second largest C flux (98 ± 12 Pg C yr⁻¹) in terrestrial carbon cycling (Bond-Lamberty and Thomson, 2010). A relatively minor disturbance could trigger the loss of significant amounts of CO₂ to the atmosphere and potentially create a positive feedback to climate change. Hence, understanding responses of the terrestrial carbon cycle to climate change and land-use at the landscape scale has become an important goal in terrestrial ecosystem ecology (Luo, 2007).

The net effects of climate and land-use changes on soil and ecosystem respiration will depend not only on independent effects of climate and land-use variables, but also their interactive effects. Results of experiments and modeling show that experimental treatments could have strong interactive effects on CO₂ effluxes (Luo et al., 2008; Selsted et al., 2012), while other experiments suggest that the interactive effects of warming and precipitation are minor compared to the independent main effects of treatments (Zhou et al., 2006). Hence, it is necessary to evaluate the interactive effects of climate change with changes in land-use and other environmental factors along with their main effects to accurately predict ecosystem responses.

For instance, in addition to altering vegetation composition (Frank et al., 1995), grazing removes live biomass, affects soil temperature and moisture (Klein et al., 2005), as well as a number of soil physical properties such as bulk density (Kölbl et al., 2011), all of which can indirectly or directly affect ecosystem and soil respiration. Soil respiration rates also vary across the landscape in response to spatial variation in microclimate, topography, soil and vegetation characteristics and disturbance regime (Luo and Zhou, 2006). Soil and ecosystem respiration typically respond positively to temperature increases (Rustad et al., 2001; Wu et al., 2011) and

negatively to decreases in soil moisture under unsaturated conditions (Harper et al., 2005; Liu et al., 2002). Temperature and moisture vary with topographic gradients, resulting in spatial variability in CO₂ production and efflux (Pacific et al., 2008; Sotta et al., 2006). In addition, plant species diversity (Fu et al., 2004), productivity (Nippert et al., 2011) and nutrient availability (Casper et al., 2012; Fisk et al., 1998; Hook and Burke, 2000) also vary with topography, with possible consequences for ecosystem and soil respiration (Bardgett et al., 2009; Chapin III et al., 2009). Variations in these landscape features can directly impact respiration by regulating substrate supply, or indirectly by altering the temporal dynamics of soil moisture (Liancourt et al., 2012). Thus, topographically-induced conditions may either exacerbate or negate effects of climate change and land-use.

The numerous studies that have been designed to test the response of carbon fluxes to climate change and land-use are predominantly centered on temperate areas of North America and Europe, while colder, drier areas are underrepresented (as are also tropical areas). Northern Mongolia lies in the transition zone between the Siberian boreal forest and the Eurasian steppe, where boreal forest and semi-arid steppe co-exists within close proximity. Northern Mongolia currently acts as a net carbon sink (Lu et al., 2009), but the balance may shift due to climate change and land-use. Over the last 40 years, the area has experienced a significant increase (1.8 °C) in mean annual temperature (Nandintsetseg et al., 2007), greater than the global average temperature increase (IPCC, 2007). In the future, air temperature in this region is projected to increase by 2-3 °C by the end of 2070-2080 (Sato and Kimura, 2006), and simultaneously, soil moisture is predicted to decrease due to the temperature increase and precipitation decrease (Sato et al., 2007; but see IPCC 2007). Moreover, livestock numbers in this region (Khankh soum) have increased from 13.7 thousand sheep units in 1972 to 32.8 thousand sheep units in 2014

(National Statistical Office of Mongolia, 2015), thereby increasing grazing pressure. It is uncertain, and important to assess, how the ecosystem- and landscape-scale carbon balance of this area might change in response to climate change and intensification of grazing pressure.

Few experiments have been conducted in northern Mongolia to address the response of carbon efflux to direct and interactive effects of grazing and climate change despite its substantial land area. Otgonsuren et al. (2008) used multiple valleys along the shore of Lake Hövsgöl as a means of assessing the effect of grazing intensity and topography on soil CO₂ fluxes, though the experimental design was less than optimal due to confounded variables. We conducted a number of simultaneous field experiments to determine how ecosystem and soil respiration might respond to independent and interactive effects of soil temperature and soil moisture, grazing manipulations, and topographic position on a single slope in a single valley. Climate was manipulated using passive open-top chambers (OTCs) similar to those used in the International Tundra Experiment (Marion et al., 1997). Experimental blocks with OTCs and control plots were installed on opposite ends of a topographic gradient. Grazing was manipulated by fencing and crossed with OTC treatments on the lower slope. Soil moisture was altered by weekly watering and crossed with OTC treatments on the upper slope. In this study, we aimed to answer: (1) how do topography, watering, grazing and climate manipulation affect soil and ecosystem respiration through changes in soil temperature and moisture, and (2) how does climate manipulation interact with topography, watering and grazing to affect soil temperature, moisture and these same measures of respiration?

2. Methods

2.1. Study site

The study site is located in the Dalbay valley, in the Lake Hövsgöl International Long-Term Ecological Research (ILTER) site, in northern Mongolia (51° 01.405' N, 100° 45.600' E; 1670 m asl). The mean annual temperature of this region is -4.5 °C, with the coldest average temperature of -21 °C in January and the warmest average temperature of 12°C in July (Nandintsetseg et al., 2007). Mean annual rainfall ranges between 290-300 mm in lower altitudes (Namkhajantsan, 2006). The study area is located on the southern fringe of Siberian continuous permafrost. Forests on north-facing slopes and riparian areas in valley bottoms are underlain by permafrost, but steppe areas on south-facing slopes are permafrost free. The dominant soil texture in the steppe is sandy loam, and steppe soils are classified as non-calcareous dark Kastanozems (Aridic Borolls or Typic Ustolls) (Batkishig, 2006).

Soil moisture and soil depth gradients exist on the south-facing slope, where our experimental plots were located because of natural topographical variation. The upper slope (elevation 1800 m a.s.l. and incline ~20°) has a shallower A horizon and less soil moisture (mean summer soil volumetric water content was 8.4%) compared to the lower slope (elevation 1670 m a.s.l. and gentle to flat slope), a deeper A horizon, and mean summer soil volumetric water content of 14%. These gradients drive nutrient availability, vegetation composition and plant cover percentage (Casper et al., 2012). Vegetation cover is semi-arid steppe characterized by grasses (*e.g.*, *Festuca lenensis*, *Helictotrichon schellianum*, *Koeleria macrantha*, *Agropyron cristatum*), sedges (*e.g.*, *Carex pediformis*, *Carex dichroa*) and forbs (*Potentilla acaulis*, *Aster alpinus*, *Artemisia commutata*). The upper slope has less total plant cover (64%), which is

dominated by *Potentilla acaulis*, while the lower slope is characterized by greater total plant cover (78%) dominated by *Carex* spp.

Since the study area is a part of the Hövsgöl National Park, grazing is not as intensive as in other valleys in the region. Still, the steppe on the south-facing slope is used as pasture, particularly in autumn and winter, by two herder families, and vegetation in some parts of the riparian area are harvested for hay. The number of livestock in the immediate area is equivalent to approximately 300 sheep-head. Cattle, yaks, and horses are the main grazers on the lower slope, while sheep and goats forage mainly on the upper slope.

2.2. Experimental design and measurements

Experimental treatments were grouped in fifteen blocks on the south-facing slope of the Dalbay valley. Eight 9×13 m blocks, spaced approximately 40 m apart, were located on the lower slope. Each was divided into a 9×4 m section, which was exposed to fall/winter grazing, and a 9×9 m section with year-round fencing. Seven 9×9 m blocks, fenced year round and likewise approximately 40 m apart, were located on the upper slope (Fig. 1). All fifteen blocks had two hexagonal open-top passive warming chambers (OTCs) and two control plots, enabling us to cross the OTC treatment with water addition on the upper slope and with the grazing treatment on the lower slope. Because OTCs increased soil temperature and decreased soil moisture (see Results), we refer to them as climate manipulation chambers hereafter. An OTC was 1.5 m wide at the bottom, measured between parallel sides, and had 40 cm tall slanted sides, such that the opening at the top measured 1.0 m between parallel sides. Control plots without OTCs had the same hexagonal footprint. We conducted the study during the 2009, 2010 and

2011 growing seasons. We consistently installed OTCs in the same locations in the beginning of June and retrieved at the end of August each year.

Fences were installed in June 2009 and left year-round except we removed fencing on three sides of the 9×4 m section of each block on the lower slope in mid-August of each year, when all OTCs were also removed. Grazers had access to this section only until fencing and OTCs were again installed in early June the following year. Each grazed and non-grazed section of a block contained one OTC and one control plot. The grazing experiment was conducted on the lower slope only because this is where biomass and normal grazing pressure are greatest. The litter biomass in the non-grazed plots was consistently twice as much as the litter biomass in the grazed plots (Spence et al., 2014). Soil moisture was manipulated on the drier, upper slope by supplemental watering, where fencing was left in place year round. There, one OTC and one control plot in each block did not receive any additional watering, while another OTC and another control plot received a weekly watering treatment equal to 4.5 mm of rainfall per week. The watering experiment was conducted on the upper slope only because this is where water limitations to primary productivity were most apparent. Thus, for the lower slope, the grazing and climate manipulation treatments were fully factorial while on the upper slope, the experimental design with watering and OTCs was fully factorial. Additionally, because otherwise unmanipulated control plots and OTCs were located on both the upper and lower slope, the climate manipulation treatment was fully crossed with topographic location (elevation), and we make use of that experimental design in the analysis presented here.

We created areas of bare soil within each of our experimental plots, where we measured soil respiration (see below). OTCs were always oriented with one side facing towards the north and the parallel side facing south. We created a triangular 0.55 m^2 area of bare soil in either the

west or east corner, chosen randomly, of the hexagonal area defined by an OTC (Fig. 1). The same triangular bare soil area was created in each paired control plot. In these areas, we removed all aboveground vegetation including any mosses or lichens that may have been present (Liancourt et al., 2012), and trenched to 20 cm to exclude roots. We kept the areas vegetation-free by weekly hand weeding and trenching.

We measured soil temperature and soil moisture (volumetric soil water content, % VWC) of the surface (0-6.3 cm) in every experimental plot within each block on a daily basis using a calibrated WET-2 sensor connected to a HH2 handheld device (DeltaT Devices Ltd., Cambridge England). These daily measurements were made between 10 am and 12 pm in both vegetated and bare areas of every plot in all treatments. The total number of days in which these measurements were made varied among years: 41 in 2009, 71 in 2010 and 25 in 2011 due to differences in the length of the experimental season. As a result, the total number of measurements made (days $\times$ treatments $\times$ blocks) was 3923 in 2009, 6912 in 2010 and 1875 in 2011. An in-depth study of vegetation effects on soil moisture is reported by Liancourt et al. (2012). Vegetation decreased soil moisture by 1.5% VWC and temperature by 0.6 °C on the lower slope plots in 2009. However, the vegetation effect was not statistically significant in other years, and no statistically significant interactions between climate manipulation and presence/absence of vegetation were observed. We anticipated that any non-significant difference in temperature or moisture between vegetated and non-vegetated areas would be temporally inconsistent and masked by much larger variability among plots and treatments. Therefore, we did not use temperature and moisture data sets collected from the bare areas from further analyses in the current study, and used only data from the vegetated areas.

CO₂ efflux measured in the vegetated area is hereafter referred to as *ecosystem respiration* because it includes CO₂ efflux originating from both heterotrophic and autotrophic sources. In contrast, CO₂ efflux measurements made on the bare soil area of plots is referred as *soil respiration* because it includes only dying root and microbial respiration. Soil and ecosystem respiration were measured between 10 am and 3 pm, using a portable infra-red gas analyzer (IRGA) and soil respiration chamber (EGM-4 + SRC-1, PP Systems Inc.) in bare and vegetated areas. The SRC-1 chamber has a metal collar that was driven 1-2 cm into the ground, and respiration was measured twice in each plot on a given day, and averaged for statistical analyses. Each measurement lasted three minutes. The order in which blocks were visited for measurements was completely randomized, resulting in 4 biweekly visits to each plot in 2009, 5 visits in 2010, and 3 visits in 2011. Respiration measurements were taken 2-5 days after watering in the upper plots to avoid capturing only the immediate pulse in respiration. As a result, the total number (visits × treatments × blocks) of soil and ecosystem respiration measurements taken was 80 in 2009, 140 in 2010 and 84 in 2011.

2.3. Data analysis

We conducted three separate analyses (experiments) to evaluate main treatment effects. First, we analyzed the effects of topography (upper versus lower slope) to determine how topographical variation alters microclimate and CO₂ efflux. In this analysis, we included data (soil temperature, soil moisture, ecosystem and soil respiration) measured in the unmanipulated, *i.e.*, non-watered, OTC and control plots on the upper slope and the unmanipulated, *i.e.*, non-grazed, OTC and control plots on the lower slope. In a second analysis, we focused on the watering effect crossed with chamber treatment, and therefore data (soil temperature, soil

moisture, ecosystem and soil respiration) measured in the upper slope blocks only were used. The third analysis examined the grazing effect crossed with chamber treatment, and hence data from only the lower slope blocks were used. Because the grazing treatment was not started until Fall 2009, respiration measurements are only for 2010 and 2011. Soil temperature and moisture measurements were only made in grazed plots in 2011. Each analysis examined the effect of OTCs and their interactions with one other treatment (topography, watering or grazing).

Treatment effects on soil temperature, soil moisture, ecosystem and soil respiration were evaluated for each year separately using two-way, repeated-measures ANOVA with measurement dates included as a within-subject factor (R, v. 3.1.2). Treatment, measurement date and all interactions were included as fixed factors, and block as a random factor for all three analyses. Blocks were nested within the slope factor only in the first analysis. When a main treatment effect was consistent among years for any response variable (*e.g.*, soil respiration), we report a mean for the three years. If a main treatment effect was not consistent among years, response variables are reported separately by year. Detailed results of the statistical analyses (*e.g.*, d.f., F, *P*-values) are reported in the Supplementary Materials.

To test broader responses of soil and ecosystem respiration to soil moisture and temperature, linear regression models were used. We used the model selection method and weighted partial regression coefficients to test the relative importance of environmental variables instead of stepwise multiple regression analyses because soil moisture and temperature were highly correlated with each other. We choose to use Akaike Information Criteria (AIC) as a model selection method. Linear regression models were ranked according to secondary AIC (AIC_c) and a confidence set for the best model given the data and model set using ΔAIC_c and cumulative weights. The models included in the confidence set were those for which ΔAIC_c was

the lowest or cumulative AIC weight was < 0.95 . To test the relative importance of each environmental variable, standardized partial regression coefficients (b') were computed. The b' of a given environmental variable is weighed by Akaike weights of corresponding models, and reported. Model selection and estimation of coefficients of environmental variables were performed using the R statistical software (R Development Core Team, 2011) with the AICcmodavg package (Mazerolle 2012). In a first set of simpler regression models, we excluded data from treated plots (watered, grazed, OTC) and used only data from un-manipulated plots. In a second set of more complex regression models, we included data from the manipulated plots and included slope position, watering, grazing and OTC treatments as dummy variables.

3. Results

Years — The repeated measures ANOVA of environmental variables (e.g., soil temperature and moisture) and soil and ecosystem respiration data showed that time or seasonality (as determined by day of year) had no effect. That is, the interaction terms including day of year (DOY) were not statistically significant (see Supplementary Materials). This is likely attributable to larger variability among plots and few repeated measures within each year. As a result, data were averaged within each year and are subsequently reported as annual/seasonal means. Averaged across all treatments (*i.e.*, topography, watering, grazing and chamber), the largest seasonal mean ecosystem respiration rate ($0.93 \text{ g CO}_2 \text{ m}^{-2} \text{ h}^{-1}$) was measured in 2009. An on-site weather station showed 2009 to have the lowest seasonal mean air temperature (9.8°C) and greatest precipitation (200 mm) compared to 2010 and 2011 (Supplemental Figure S1). The lowest seasonal mean ecosystem respiration rate ($0.74 \text{ g CO}_2 \text{ m}^{-2} \text{ h}^{-1}$) occurred in 2011, which was the hottest and driest summer (10.9°C and 137 mm). Similarly, the mean seasonal soil respiration

rate was $0.70 \text{ g CO}_2 \text{ m}^{-2} \text{ h}^{-1}$ in 2009 (the wettest summer) and $0.40 \text{ g CO}_2 \text{ m}^{-2} \text{ h}^{-1}$ in 2011 (the driest and hottest summer). The average contributions of soil respiration to ecosystem respiration were 79.5% in 2009 and 60.5% in 2010 and 2011 (Table 1).

Climate manipulation — Averaged across the main treatments (*i.e.*, topography, watering and grazing) and years, mean soil temperature was $0.6 \text{ }^{\circ}\text{C}$ warmer in OTCs than in control plots, and mean soil moisture was 2.5 percentage points drier in OTCs than in control plots. Ecosystem respiration rates were $0.08 \text{ g CO}_2 \text{ m}^{-2} \text{ h}^{-1}$ less in OTCs than in control plots. Similarly, mean soil respiration rates were $0.06 \text{ g CO}_2 \text{ m}^{-2} \text{ h}^{-1}$ less in OTCs than in control plots, though the effect was observed in 2010 and 2011, but not in 2009. The responses of carbon fluxes to OTCs were affected by the individual and combined environmental factors that differed among the three comparisons (topography, watering and grazing), which we describe individually below.

Responses of respiration to soil temperature and moisture — For the un-manipulated plots, simple linear regressions showed positive responses of soil and ecosystem respiration to increasing soil moisture, and decreasing responses to increasing soil temperature (Supplemental Figure S2). Regressions including only soil moisture ($R^2 = 0.42$, $P < 0.001$ for soil respiration; $R^2 = 0.23$, $P < 0.001$ for ecosystem respiration) or soil moisture combined with soil temperature ($R^2 = 0.43$, $P < 0.001$ for soil respiration; $R^2 = 0.23$, $P < 0.001$ for ecosystem respiration) better fit the data than regressions with soil temperature alone ($R^2 = 0.01$, $P = 0.241$ for soil respiration; $R^2 = 0.01$, $P = 0.258$ for ecosystem respiration). Model selection also demonstrated that soil moisture was a stronger predictor of respiration, where b' partial regression coefficient values for moisture were greater (0.68 for soil respiration; 0.45 for ecosystem respiration) than for soil temperature (0.03 for soil respiration; 0.02 for ecosystem respiration). In general, more complex regression models that included the experimental treatments (slope position, watering, grazing

and OTC) demonstrated similar trends where soil moisture was the most important variable (*i.e.*, largest b'), even compared to the applied treatments. However, these models suffered from very low predictive power due to lack of data, and therefore are not reported or discussed further.

3.1. Slope position (topography) and climate manipulation

Soil temperature and moisture — Consistently across all three years, the upper slope was warmer and drier than the lower slope, as shown by soil temperature and moisture in the not watered (and not grazed) control plots and OTCs of the upper slope and the not grazed (and not watered) control plots and OTCs of the lower slope (Figs. 2 and 3; Table S1). Similarly, OTCs consistently elevated soil temperature and decreased soil moisture compared to the control plots (Figs. 2 and 3; Table S1). Only in 2010 was there a significant interaction between climate manipulation and slope position in affecting soil temperature and moisture (Table S2). For that year, OTCs elevated soil temperature more on the upper slope (Fig. 2) but decreased soil moisture more on the lower slope (Fig. 3).

Ecosystem and soil respiration — The effects of slope position and climate manipulation and their interaction on ecosystem respiration and soil respiration were not consistent among years. Ecosystem respiration was significantly less on the upper slope (in non-watered OTCs and controls) than on the lower slope (in non-grazed OTCs and controls) in 2009 and 2010 but not in 2011 (Fig. 4; Table S1). OTCs significantly reduced ecosystem respiration only in 2009 (Fig 4; Table S1). Soil respiration was lower on the upper slope in 2010 (Fig. 5; Table S1) and OTCs significantly reduced soil respiration only in 2010 (Fig. 5; Table S1 and Table S2); slope location and climate manipulation did not interact to affect soil respiration any year (Table S2).

3.2. Watering and climate manipulation

Soil temperature and moisture — In the watering and climate manipulation experiment on the upper slope, watering produced the expected increase in soil moisture (Fig. 3; Table S1) and OTCs decreased soil moisture. These treatment effects were apparent across all three years. At the same time OTCs increased soil temperature as expected, watering decreased soil temperature (by less than 1.0 °C, Fig. 2; Table S3). Climate manipulation and watering interacted to affect soil moisture all three years (Table S3), as there was a greater difference in moisture between watered and not watered control plots than between watered and not watered OTC plots. Climate manipulation and watering did interact to affect soil temperature except in 2009 (Table S3).

Ecosystem and soil respiration — Ecosystem respiration did not respond to watering (Fig. 4; Table S1, S3). For the upper slope, OTCs significantly decreased ecosystem respiration only in 2009 (Table S3). Watering increased soil respiration in 2010, and OTCs decreased soil respiration in 2010 (Fig. 5; Table S1). Watering and climate manipulation did not interact to affect either ecosystem respiration or soil respiration any year (Table S3).

3.3. Grazing and climate manipulation

Soil temperature and moisture — In the grazing and climate manipulation experiment on the lower slope, for 2011, the single year when we made soil temperature and moisture measurements in grazed plots, grazing increased soil temperature (Fig. 2, Table S1 and S4) but had no effect on soil moisture (Fig. 3, Table S1). As elsewhere, OTCs increased soil temperature and decreased soil moisture (Figs. 2, 3; Table S1). There was no interaction between grazing and climate manipulation in affecting either soil temperature or soil moisture in 2011 (Table S4).

Ecosystem and soil respiration — Grazing affected ecosystem respiration and soil respiration in opposing ways. In 2011, grazing decreased ecosystem respiration (Fig. 4, Table S1 and S3) but increased soil respiration (Fig. 5; Table S1). These responses to grazing resulted in a substantial difference in the contribution of soil respiration to ecosystem respiration between grazed (mean of 72%) and ungrazed plots (52%) in 2011. In 2010, there was no effect of grazing on either ecosystem or soil respiration (Figs. 4, 5; Table S1). OTCs reduced ecosystem respiration in 2010, and soil respiration in both 2010 and 2011 (Figs. 4, 5). Grazing and OTCs did not interact to affect either ecosystem or soil respiration either year (Table S4).

4. Discussion

We aimed to understand how microclimate manipulation, grazing and their interactions would affect soil and ecosystem respiration, and how these effects would vary along topographical gradients by conducting a multi-factor experiment for three years in the semi-arid steppe of northern Mongolia.

Previous experiments in the same region demonstrated that OTC chamber treatments altered soil temperature, moisture and respiration, but the effects were variable across the boreal forest, riparian and steppe ecosystems (Sharkhuu et al., 2013). The current results demonstrated that soil moisture is a more important driving factor than temperature change for carbon fluxes within this semi-arid steppe environment. This conclusion is supported by several lines of evidence. First, regression models using soil moisture better predicted soil and ecosystem respiration data than did models with soil temperature alone. A model selection analysis also showed greater partial regression coefficient values (b') values for soil moisture than for soil temperature. Second, ecosystem and soil respiration were less in OTCs in general, although

OTCs increased soil temperature. The drier conditions in OTCs are likely to have caused the decrease in ecosystem and soil respiration. Third, watering caused an increase in ecosystem and soil respiration in watered control plots compared with non-watered control plots, even though watering decreased soil temperature. This result is consistent with previous studies where water addition resulted in increased ecosystem respiration (Niu et al., 2008) and soil respiration (Liu et al., 2009). Watering induced greater soil respiration in OTCs, thus negating drying effect of OTCs on soil respiration. Watered OTCs and non-watered control plots had similar soil respiration amount. Finally, the drier upper slope had less ecosystem and soil respiration compared to the lower slope, even though the upper slope is warmer. Further, seasonal average ecosystem and soil respiration decreased over three summers (by 25-42%) as temperature increased, rainfall amount decreased, and timing of rainfall shifted in 2010 and 2011.

Our finding that soil and ecosystem respiration varies with soil moisture is consistent with previously observed reductions in soil and ecosystem respiration due to decreases in rainfall or change in rainfall timing in semi-arid grasslands (Chou et al., 2008; Hao et al., 2010; Liu et al., 2009). That soil respiration decreases in response to moisture limitation may be attributable to decreased microbial activity due to moisture limitation (Allison and Treseder, 2008; Manzoni et al., 2012), but it is not possible to discern the mechanism involved from our experiments. It is also possible that the decline in soil respiration over the three years was caused not only by changes in rainfall timing and amount, but also by gradual root decomposition from vegetation removal and trenching (Díaz-Pinés et al., 2010; Parton et al., 2007). Nevertheless, our results showed that soil respiration was highly sensitive to soil moisture, and suggest that increasing evapotranspiration due to predicted warming may reduce, rather than stimulate, CO₂ fluxes in this semi-arid steppe.

In a previous study using multiple valleys as a proxy for grazing intensity, Otgonsuren et al (2008) observed decreased bare soil respiration with increased grazing pressure and no effect on total soil CO₂ flux. However, they also observed that inter-annual variability and topographical position (across the valleys) generated greater variability in respiration than did grazing. In the current study, the grazing treatment had contrasting effects on soil and ecosystem respiration. Removal of aboveground part of plants, resulting in substantially less litter biomass accumulation in our plots (see Spence et al., 2014), is likely to have caused the decrease in ecosystem respiration in grazed plots. Similarly, other studies have shown that grazing reduced ecosystem respiration (Owensby et al., 2006; Susiluoto et al., 2008). While grazing typically reduces aboveground plant biomass, it can also increase belowground biomass (Sjögersten et al., 2012) or carbon allocation to roots (Hafner et al., 2012), and thus increase labile carbon input into soil (Gao et al., 2009; Hafner et al., 2012). These could explain the greater soil respiration observed in the grazed plots in 2011. While a soil temperature increase due to grazing would be expected to stimulate soil respiration, we found little evidence to support this.

We acknowledge that it is difficult to extrapolate our results from a three-year grazing study into longer-term trends or predict the response of soil respiration to overgrazing since our site is lightly grazed. Previous studies have demonstrated that a decrease in substrate supply due to grazing (Rees et al., 2005; Stark et al., 2003) can reduce soil respiration (Cao et al., 2004; Johnson and Matchett, 2001; Stark et al., 2003). In addition, other studies have shown that the effects of grazing on CO₂ fluxes may also vary depending on grazing pressure and stocking density (Cao et al., 2004; Sjögersten et al., 2012). It is equally possible that high grazing pressure can eventually lead to reduced substrate supply and soil respiration. We do not, however, have

explicit data to test carbon allocation strategy and its response to climate manipulation and grazing in our system, making it difficult to generalize the result.

The interactive effects of multiple factors (*i.e.*, OTCs with topography, watering and grazing) on soil respiration were minimal. Although the system has local topographical gradients in terms of nutrient and moisture availability, and plant species and cover, the lack of statistically significant interaction terms imply that local heterogeneity may not be important for scaling up the results. Similar results were obtained in a tallgrass prairie ecosystem in the US (Zhou et al., 2006), but it is still unknown if minor interactive effects can be generalized.

However, we did observe unexpected interactive effects of treatments on ecosystem respiration. Alteration of responses of ecosystem respiration to OTCs by watering on the upper slope suggests that climate change involving multiple factors (temperature, precipitation and initial conditions) could have an unpredicted interactive effect on ecosystem processes. These interactive effects are likely due to the presence of vegetation and its direct contributions to respiration and indirect effects on soil temperature and moisture through evapotranspiration. While accounting for individual species is beyond the scope of the current study, Liancourt et al. (2013) did find that survival and biomass of a common grass *Festuca lenensis* significantly increased in OTCs. If consistent across species, such a response could explain why we observed greater ecosystem respiration in non-watered OTCs compared to non-watered control plots. Liancourt et al. (2013) also showed that species interactions and local adaptations supersede the effects of climate manipulation in this ecosystem. This suggests that ecosystem respiration may not respond to climate change as straightforwardly as we might expect.

Predicting the responses of ecosystem and soil respiration to climate change and grazing pressure is critical to ensuring the sustainability of ecosystem services. This study demonstrated

that soil moisture is the key controlling factor of carbon fluxes in this semi-arid grassland, and thus changes in precipitation may have stronger effects on the C balance of the system than temperature change. Our results also suggest that the response of heterotrophic soil respiration to multi-factored climate change may be predictable on the basis of responses to single factors, while the response of ecosystem respiration may be obscured by responses of vegetation to multiple changes.

Acknowledgements

We thank L. Spence for her contribution towards collecting data. We also thank J. Mortensen, D. Brickley, S. Undrakhbold, J. Batbaatar, N. Sandag, research camp staff, and the contributing American and Mongolian undergraduates for their support throughout the project and their help in field work. The study was conducted within the framework of PIRE-Mongolia project, supported by grant from U.S. National Science Foundation (Grant No. OISE 0729786).

References

- Allison, S.D., Treseder, K.K., 2008. Warming and drying suppress microbial activity and carbon cycling in boreal forest soils. *Global Change Biology* 14(12), 2898-2909.
- Bardgett, R.D., De Deyn, G.B., Ostle, N.J., 2009. Editorial: Plant-soil interactions and the carbon cycle. *Journal of Ecology* 97(5), 838-839.
- Batkishig, O., 2006. Soils of the Lake Hovsgol area and its watershed. In: C.E. Goulden, T. Sitnikova, J. Gelhaus, B. Boldgiv (Eds.), *The Geology, Biodiversity and Ecology of Lake Hovsgol (Mongolia)*. Biology of Inland Waters. Backhuys Publisher, Leiden, pp. 1-20.
- Bond-Lamberty, B., Thomson, A., 2010. Temperature-associated increases in the global soil respiration record. *Nature* 464(7288), 579-582.
- Cao, G., Tang, Y., Mo, W., Wang, Y., Li, Y., Zhao, X., 2004. Grazing intensity alters soil respiration in an alpine meadow on the Tibetan plateau. *Soil Biology and Biochemistry* 36(2), 237-243.
- Casper, B.B., Goldman, R., Lkhagva, A., Helliker, B.R., Plante, A.F., Spence, L.A., Liancourt, P., Boldgiv, B., Petraitis, P.S., 2012. Legumes mitigate ecological consequences of a topographic gradient in a northern Mongolian steppe. *Oecologia* 169(1), 85-94.
- Chapin III, F.S., McFarland, J., David McGuire, A., Euskirchen, E.S., Ruess, R.W., Kielland, K., 2009. The changing global carbon cycle: Linking plant-soil carbon dynamics to global consequences. *Journal of Ecology* 97(5), 840-850.
- Chou, W.W., Silver, W.L., Jackson, R.D., Thompson, A.W., Allen-Diaz, B., 2008. The sensitivity of annual grassland carbon cycling to the quantity and timing of rainfall. *Glob. Change Biol.* 14(6), 1382-1394.

438 Díaz-Pinés, E., Schindlbacher, A., Pfeffer, M., Jandl, R., Zechmeister-Boltenstern, S., Rubio, A.,
 439 2010. Root trenching: a useful tool to estimate autotrophic soil respiration? A case study
 440 in an Austrian mountain forest. *European Journal of Forest Research* 129(1), 101-109.
 441 Fisk, M.C., Schmidt, S.K., Seastedt, T.R., 1998. Topographic patterns of above- and
 442 belowground production and nitrogen cycling in Alpine tundra. *Ecology* 79(7), 2253-
 443 2266.
 444 Frank, A.B., Tanaka, D.L., Hofmann, L., Follett, R.F., 1995. Soil Carbon and Nitrogen of
 445 Northern Great Plains Grasslands as Influenced by Long-Term Grazing. *Journal of Range*
 446 *Management* 48(5), 470-474.
 447 Fu, B.J., Liu, S.L., Ma, K.M., Zhu, Y.G., 2004. Relationships between soil characteristics,
 448 topography and plant diversity in a heterogeneous deciduous broad-leaved forest near
 449 Beijing, China. *Plant Soil* 261(1/2), 47-54.
 450 Gao, Y., Schumann, M., Chen, H., Wu, N., Luo, P., 2009. Impacts of grazing intensity on soil
 451 carbon and nitrogen in an alpine meadow on the eastern Tibetan Plateau. *Journal of Food,*
 452 *Agriculture and Environment* 7(2), 749-754.
 453 Hafner, S., Unteregelsbacher, S., Seeber, E., Lena, B., Xu, X., Li, X., Guggenberger, G., Miede,
 454 G., Kuzyakov, Y., 2012. Effect of grazing on carbon stocks and assimilate partitioning in
 455 a Tibetan montane pasture revealed by ¹³CO₂ pulse labeling. *Global Change Biology*
 456 18(2), 528-538.
 457 Hao, Y., Wang, Y., Mei, X., Cui, X., Zhou, X., Huang, X., 2010. The sensitivity of temperate
 458 steppe CO₂ exchange to the quantity and timing of natural interannual rainfall. *Ecological*
 459 *Informatics* 5(3), 222-228.

460 Harper, C.W., Blair, J.M., Fay, P.A., Knapp, A.K., Carlisle, J.D., 2005. Increased rainfall
 461 variability and reduced rainfall amount decreases soil CO₂ flux in a grassland ecosystem.
 462 Glob. Change Biol. 11(2), 322-334.

463 Hook, P.B., Burke, I.C., 2000. Biogeochemistry in a shortgrass landscape: Control by
 464 topography, soil texture, and microclimate. Ecology 81(10), 2686-2703.

465 IPCC, 2007. Climate Change 2007: The Physical Science Basis. Contribution of Working Group
 466 1 to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change.
 467 Cambridge University Press, Cambridge, UK.

468 Johnson, L.C., Matchett, J.R., 2001. Fire and grazing regulate belowground processes in tallgrass
 469 prairie. Ecology 82(12), 3377-3389.

470 Klein, J.A., Harte, J., Zhao, X.Q., 2005. Dynamic and complex microclimate responses to
 471 warming and grazing manipulations. Glob. Change Biol. 11(9), 1440-1451.

472 Kölbl, A., Steffens, M., Wiesmeier, M., Hoffmann, C., Funk, R., Krümmelbein, J., Reszkowska,
 473 A., Zhao, Y., Peth, S., Horn, R., Giese, M., Kögel-Knabner, I., 2011. Grazing changes
 474 topography-controlled topsoil properties and their interaction on different spatial scales in
 475 a semi-arid grassland of Inner Mongolia, P.R. China. Plant Soil 340(1-2), 35-58.

476 Liancourt, P., Sharkhuu, A., Ariuntsetseg, L., Boldgiv, B., Helliker, B., Plante, A., Petraitis, P.,
 477 Casper, B., 2012. Temporal and spatial variation in how vegetation alters the soil
 478 moisture response to climate manipulation. Plant and Soil 351(1), 249-261.

479 Liancourt, P., Spence, L.A., Song, D.S., Lkhagva, A., Sharkhuu, A., Boldgiv, B., Helliker, B.R.,
 480 Petraitis, P.S., Casper, B.B., 2013. Plant response to climate change varies with
 481 topography, interactions with neighbors, and ecotype. Ecology 94(2), 444-453.

482 Liu, W.X., Zhang, Z., Wan, S.Q., 2009. Predominant role of water in regulating soil and
 483 microbial respiration and their responses to climate change in a semiarid grassland. *Glob.*
 484 *Change Biol.* 15(1), 184-195.

485 Liu, X., Wan, S., Su, B., Hui, D., Luo, Y., 2002. Response of soil CO₂ efflux to water
 486 manipulation in a tallgrass prairie ecosystem. *Plant and Soil* 240(2), 213-223.

487 Lu, Y., Zhuang, Q., Zhou, G., Sirin, A., Melillo, J., Kicklighter, D., 2009. Possible decline of the
 488 carbon sink in the Mongolian Plateau during the 21st century. *Environmental Research*
 489 *Letters* (4), 045023.

490 Luo, Y., 2007. Terrestrial Carbon-Cycle Feedback to Climate Warming. *Annual Review of*
 491 *Ecology, Evolution, and Systematics* 38(1), 683-712.

492 Luo, Y., Gerten, D., Maire, G.L., Parton, W.J., Weng, E., Zhou, X., Keough, C., Beier, C., Ciais,
 493 P., Cramer, W., Dukes, J.S., Emmett, B., Hanson, P.J., Knapp, A., Linder, S., Nepstad,
 494 D., Rustad, L., 2008. Modeled interactive effects of precipitation, temperature, and [CO₂]
 495 on ecosystem carbon and water dynamics in different climatic zones. *Global Change*
 496 *Biology* 14(9), 1986-1999.

497 Luo, Y., Zhou, X., 2006. *Soil respiration and the environment*. Academic Press.

498 Manzoni, S., Schimel, J.P., Porporato, A., 2012. Responses of soil microbial communities to
 499 water stress: results from a meta-analysis. *Ecology* 93(4), 930-938.

500 Marion, G.M., Henry, G.H.R., Freckman, D.W., Johnstone, J., Jones, G., Jones, M.H., Lévesque,
 501 E., Molau, U., Mølgaard, P., Parsons, A.N., Svoboda, J., Virginia, R.A., 1997. Open-top
 502 designs for manipulating field temperature in high-latitude ecosystems. *Glob. Change*
 503 *Biol.* 3(S1), 20-32.

504 Mazerolle, M.J., 2012. AICcmodavg. R package version 1.24. In: R Foundation for Statistical
505 Computing. Vienna, Austria.

506 Namkhajantsan, G., 2006. Climate and climate change of the Hovsgol Region. In: E.C.
507 Goulden, Sitnikova, T., Gelhaus, J. and Boldgiv, B. (Ed.), The Geology, Biodiversity and
508 Ecology of Lake Hovsgol (Mongolia). Backhuys Publisher, pp. 63-76.

509 Nandintsetseg, B., Greene, J.S., Goulden, C.E., 2007. Trends in extreme daily precipitation and
510 temperature near lake Hövsgöl, Mongolia. *International Journal of Climatology* 27(3),
511 341-347.

512 National Statistical Office of Mongolia, 2015. Number of Livestock. <http://en.nso.mn>. Accessed
513 May 25, 2015.

514 Nippert, J.B., Ocheltree, T.W., Skibbe, A.M., Kangas, L.C., Ham, J.M., Arnold, K.B.S.,
515 Brunsell, N.A., 2011. Linking plant growth responses across topographic gradients in
516 tallgrass prairie. *Oecologia* 166(4), 1131-1142.

517 Niu, S., Wu, M., Han, Y., Xia, J., Li, L., Wan, S., 2008. Water-mediated responses of ecosystem
518 carbon fluxes to climatic change in a temperate steppe. *New Phytologist* 177(1), 209-219.

519 Otgonsuren, A., Goulden, C.E., Burke, I.C., Bulgan, B., 2008. Soil CO₂ flux in Hövsgöl National
520 Park, Northern Mongolia. *Mongolian Journal of Biological Science* 6(1-2), 31-38.

521 Owensby, C.E., Ham, J.M., Auen, L.M., 2006. Fluxes of CO₂ from grazed and ungrazed tallgrass
522 prairie. *Rangeland Ecology and Management* 59(2), 111-127.

523 Pacific, V., McGlynn, B., Riveros-Iregui, D., Welsch, D., Epstein, H., 2008. Variability in soil
524 respiration across riparian-hillslope transitions. *Biogeochemistry* 91(1), 51-70.

525 Parton, W., Silver, W.L., Burke, I.C., Grassens, L., Harmon, M.E., Currie, W.S., King, J.Y.,
526 Adair, E.C., Brandt, L.A., Hart, S.C., Fasth, B., 2007. Global-Scale Similarities in

527 Nitrogen Release Patterns During Long-Term Decomposition. *Science* 315(5810), 361-
528 364.

529 R Development Core Team, 2011. R: A language and environment for statistical computing,
530 reference index version 2.13. In: R Foundation for Statistical Computing. Vienna,
531 Austria.

532 Rees, R.M., Bingham, I.J., Baddeley, J.A., Watson, C.A., 2005. The role of plants and land
533 management in sequestering soil carbon in temperate arable and grassland ecosystems.
534 *Geoderma* 128(1-2), 130-154.

535 Rustad, L.E., Campbell, J.L., Marion, G.M., Norby, R.J., Mitchell, M.J., Hartley, A.E.,
536 Cornelissen, J.H.C., Gurevitch, J., Gcte, N., 2001. A meta-analysis of the response of soil
537 respiration, net nitrogen mineralization, and aboveground plant growth to experimental
538 ecosystem warming. *Oecologia* 126(4), 543-562.

539 Sato, T., Kimura, F., 2006. Regional Climate Simulations to Diagnose Environmental Changes
540 in Mongolia. *Bulletin of the Terrestrial Environment Research Center, University of*
541 *Tsukuba* (7), 59-69.

542 Sato, T., Kimura, F., Kitoh, A., 2007. Projection of global warming onto regional precipitation
543 over Mongolia using a regional climate model. *J. Hydrol.* 333(1), 144-154.

544 Selsted, M.B., van der Linden, L., Ibrom, A., Michelsen, A., Larsen, K.S., Pedersen, J.K.,
545 Mikkelsen, T.N., Pilegaard, K., Beier, C., Ambus, P., 2012. Soil respiration is stimulated
546 by elevated CO₂ and reduced by summer drought: Three years of measurements in a
547 multifactor ecosystem manipulation experiment in a temperate heathland (CLIMAITE).
548 *Global Change Biology* 18(4), 1216-1230.

549 Sharkhuu, A., Plante, A.F., Enkhmandal, O., Casper, B.B., Helliher, B.R., Boldgiv, B., Petraitis,
550 P.S., 2013. Effects of open-top passive warming chambers on soil respiration in the semi-
551 arid steppe to taiga forest transition zone in Northern Mongolia. *Biogeochemistry* 115(1-
552 3), 333-348.

553 Sjögersten, S., van der Wal, R., Woodin, S., 2012. Impacts of Grazing and Climate Warming on
554 C Pools and Decomposition Rates in Arctic Environments. *Ecosystems* 15(3), 349-362.

555 Sotta, E.D., Veldkamp, E., Guimarães, B.R., Paixão, R.K., Ruivo, M.L.P., Almeida, S.S., 2006.
556 Landscape and climatic controls on spatial and temporal variation in soil CO₂ efflux in an
557 Eastern Amazonian Rainforest, Caxiuanã, Brazil. *Forest Ecology and Management*
558 237(1-3), 57-64.

559 Spence, L., Liancourt, P., Boldgiv, B., Petraitis, P., Casper, B., 2014. Climate change and
560 grazing interact to alter flowering patterns in the Mongolian steppe. *Oecologia* 175(1),
561 251-260.

562 Stark, S., Tuomi, J., Strommer, R., Helle, T., 2003. Non-parallel changes in soil microbial carbon
563 and nitrogen dynamics due to reindeer grazing in northern boreal forests. *Ecography*
564 26(1), 51-59.

565 Susiluoto, S., Rasilo, T., Pumpanen, J., Berninger, F., 2008. Effects of grazing on the vegetation
566 structure and carbon dioxide exchange of a fennoscandian fell ecosystem. *Arctic,*
567 *Antarctic, and Alpine Research* 40(2), 422-431.

568 Wu, Z., Dijkstra, P., Koch, G.W., Peñuelas, J., Hungate, B.A., 2011. Responses of terrestrial
569 ecosystems to temperature and precipitation change: A meta-analysis of experimental
570 manipulation. *Global Change Biology* 17(2), 927-942.

571 Zhou, X., Sherry, R.A., An, Y., Wallace, L.L., Luo, Y., 2006. Main and interactive effects of
572 warming, clipping, and doubled precipitation on soil CO₂ efflux in a grassland
573 ecosystem. *Global Biogeochemical Cycles* 20(1).

Figure legends

Figure 1. Schematic of experimental plot design in the upper and lower slope positions showing open-top chambers (solid outline hexagons), watering (shaded hexagons), grazing (dashed outline block) and control treatments (dotted outline hexagons). Bare soil areas are represented by the darkly shaded triangular areas.

Figure 2. Seasonal mean soil temperature ($^{\circ}\text{C}$, mean $\pm$ standard deviation) in open-top climate manipulation chambers (OTCs, solid bar) and control plots (open bar) in response to the watering treatment on the upper slope and the grazing treatment on the lower slope.

Figure 3. Seasonal mean soil moisture (% VWC, mean $\pm$ standard deviation) in open-top climate manipulation chambers (OTCs, solid bar) and control plots (open bar) in response to the watering treatment on the upper slope and the grazing treatment on the lower slope.

Figure 4. Seasonal mean vegetated soil (ecosystem) respiration rate ($\text{g CO}_2 \text{ m}^{-2} \text{ h}^{-1}$, mean $\pm$ standard deviation) in open-top climate manipulation chambers (OTCs, solid bar) and control plots (open bar) in response to the watering treatment on the upper slope and the grazing treatment on the lower slope.

Figure 5. Seasonal mean bare soil respiration rate ($\text{g CO}_2 \text{ m}^{-2} \text{ h}^{-1}$, mean $\pm$ standard deviation) in open-top climate manipulation chambers (OTCs, solid bar) and control plots (open bar) in response to the watering treatment on the upper slope and the grazing treatment on the lower slope.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Table 1

The mean of relative contribution (%) of bare soil respiration to vegetated soil (ecosystem) respiration (mean ± standard error).

	2009		2010		2011	
	Con	OTC	Con	OTC	Con	OTC
Non-watered	85 ± 10	92 ± 7	65 ± 6	50 ± 5	59 ± 4	54 ± 8
Watered	91 ± 10	96 ± 11	60 ± 4	57 ± 5	60 ± 3	62 ± 9
Upper slope mean	88 ± 7	94 ± 7	63 ± 4	54 ± 4	59 ± 3	58 ± 6
Non-grazed	61 ± 5	64 ± 3	65 ± 10	56 ± 5	52 ± 4	52 ± 9
Grazed	-	-	67 ± 10	60 ± 10	75 ± 10	69 ± 10
Lower slope mean	61 ± 5	64 ± 3	66 ± 8	58 ± 4	64 ± 7	60 ± 9
Upper+lower slope mean	77 ± 5	82 ± 5	65 ± 5	56 ± 3	62 ± 4	59 ± 6
Overall mean	79.5 ± 5		60.5 ± 4		60.5 ± 5	