The Response of Soil CO_2 Fluxes to Progressively Excluding Vertebrate and Invertebrate Herbivores Depends on Ecosystem Type

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ABSTRACT

Grasslands support large populations of herbivores and store up to 30% of the world's soil carbon (C). Thus, herbivores likely play an important role in the global C cycle. However, most studies on how herbivory impacts the largest source of C released from grassland soils—soil carbon dioxide (CO_2) emissions—only considered the role of large ungulates. This ignores all other vertebrate and invertebrate herbivores and their collective effects on ecosystem properties. We progressively excluded large, medium, and small vertebrates and invertebrates from two subalpine grasslands (productive, heavily grazed short-grass; less productive, lightly grazed tall-grass) using size-selective fences, assessed the impact on soil CO_2 emissions and related biotic and abiotic variables. Exclusion resulted in significant changes in soil CO_2 emissions in both vegetation types. Short-grass soil CO_2 emissions progressively increased when large and medium mammals were excluded. However, no difference was detected among plots were all or no herbivores grazed. In contrast, tall-grass soil CO_2 emissions were not affected by mammal exclusion, but excluding all herbivores lead to reduced emissions. Soil micro-climatic parameters best predicted the patterns of soil CO_2 emissions in short-grass vegetation, whereas root biomass was the best predictor of CO_2 release in tall-grass vegetation. Our results showed that diverse herbivore communities affect soil respiration differently than assumed from previous studies that only excluded large ungulates. Such information is important if we are to understand how changes in herbivore species composition—as could happen through altered management practices, extinction or invasion—impact grassland C storage and release.

Key words: vertebrate herbivores; invertebrate herbivores; consumption; soil temperature; soil moisture; root biomass; soil microbial biomass C; Q_{10}; exclusion; soil respiration.

INTRODUCTION

Grasslands cover approximately one-third of the earth's terrestrial landscape (Lieth 1978; Foley and others 2005), and support large populations of vertebrate and invertebrate herbivores (White and others 2000). Consuming potentially more than

50% of the aboveground biomass (Detling 1988), these animal communities can have strong effects on various ecosystem properties. Physiological responses of plants to herbivory (top-down effects) include changes in (1) biomass, nutrient content, or litter quality of shoots and roots (Wardle and others 2002; Frank and others 2002b; Chapman and others 2003), and (2) the flow of carbon (C) from roots to the soil (Bardgett and Wardle 2003; Harrison and Bardgett 2008). As a result of these plant responses, the availability of soil C and nutrients may change, which can lead to an alteration of the abundance or community composition of soil microbes or invertebrates (Bardgett and others 1998, 2001; Bardgett and Wardle 2003; Wardle and others 2004a, b). These changes, in turn, can affect decomposition and nutrient mineralization processes of soil organic matter (Aho and others 1998; Belovsky and Slade 2000; Harrison and Bardgett 2008), which alters plant nutrient availability (McNaughton and others 1997; Johnson and Matchett 2001) and growth (bottom-up effects; Alward and Joern 1993; McNaughton and others 1998; Frank and others 2002b).

As grassland soils store between 10 and 30% of the world's soil carbon (Anderson 1991; Eswaran and others 1993), herbivore-induced changes in ecosystem processes likely play an important role in the global C cycle. Soil CO$_2$ respiration is the largest source of released C from terrestrial ecosystems (Raich and Schlesinger 1992) and is a function of soil (1) organic matter quality and quantity, (2) micro-climate (temperature, moisture), (3) physical properties (texture, bulk density), (4) chemical properties (pH, nutrient concentrations), and (5) biological properties (microbial biomass, composition, diversity, activity; Kirschbaum 1995). Thus, any herbivore-induced alteration of one or several of these parameters could alter soil respiration and total C stored in grassland soils.

The net impact of herbivores on grassland processes depends on the productivity of the system and the grazing intensity it receives (Bardgett and Wardle 2003; Wardle and others 2004a). Generally, increases in soil biota biomass and richness and accelerated soil C processes were reported when ecosystem productivity and grazing intensity were high, whereas the opposite was found for unproductive systems with low plant biomass consumption (see also Bakker and others 2004, 2006). Total consumption is directly related to the proportional body size and abundance of the herbivores community. Thus, herbivory represents the combined impact of several herbivore species or guilds that differ in their functional behavior, feeding habits

(Belovsky 1997; Pawar and others 2012), trampling impact, burrowing activities (Davidson and others 2012) and amount, distribution (patchy, evenly), and quality of their waste (Bakker and others 2004). This fact has been ignored in the many studies investigating the impact of grazing on soil respiration (Knapp and others 1998; Johnson and Matchett 2001; Cao and others 2004, Risch and Frank 2006; Chen and others 2008; Strebel and others 2010), as only the impact of the largest herbivore group was assessed in these studies (usually ungulates). We are not aware of any study in which the impact of a functionally diverse herbivore community on soil CO$_2$ emissions was investigated, even though differences in herbivore body size could alter vegetation-soil feedback in grassland systems (Olff and Ritchie 1998; Ritchie and Olff 1999a, b). More knowledge is needed on how functionally diverse herbivore communities, rather than just large ungulates, affect soil CO$_2$ emissions in grassland ecosystems.

For this purpose we established an exclosure experiment in the Swiss Alps. Our main objective was to assess how soil CO$_2$ emissions were affected when four groups of functionally different herbivores—large, medium, and small mammals, and invertebrates—were progressively excluded with size-selective fences from two subalpine vegetation types: short-grass and tall-grass. The nutrient-rich and fairly productive short-grass vegetation was characterized by high herbivore consumption (over 60%), whereas much less biomass was consumed (<20%) in the less productive, comparatively low forage quality tall-grass vegetation (Schütz and others 2006). We hypothesized that the progressive exclusion of our four herbivore groups would lead to: (1) Decreased soil CO$_2$ emissions in short-grass vegetation as positive feedbacks between grazing and ecosystem processes (nutrient cycling) decelerate when grazing intensity ceases (Bardgett and Wardle 2003; Wardle and others 2004a). Proportionally larger changes were expected when smaller herbivores were excluded (selective feeding, evenly distributed waste). (2) Increased soil CO$_2$ emissions in the tall-grass vegetation, as the negative effects of grazing cease and ecosystem process rates accelerate. The largest effect in this vegetation type was expected when large mammals were excluded. To assess potential mechanisms responsible for herbivory-induced changes in soil CO$_2$ emissions, we measured abiotic and biotic variables that are often affected by changes in grazing regime: soil temperature, soil moisture, aboveground biomass consumption, root biomass, and soil microbial biomass C (MBC).

MATERIALS AND METHODS

Study Area

The Swiss National Park (SNP) is located in the southeastern part of Switzerland, and covers an area of 170 km^2, 50 km^2 of which is forested, 33 km^2 is occupied by alpine and 3 km^2 by subalpine grasslands. Elevations range from 1,350 to 3,170 m a.s.l., and mean annual precipitation and temperature are 871 ± 156 mm and $0.6 \pm 0.6°C$ (average $\pm$ standard deviation) measured at the Park's weather station in Buffalora (1,980 m a.s.l.) between 1960 and 2009 (MeteoSchweiz 2011). Founded in 1914, the SNP received minimal human disturbance for almost 100 years (no hunting, fishing, or camping, visitors are not allowed to leave the trails). Large (>1 ha) homogeneous patches of short- and tall-grass vegetation characterize the subalpine grasslands. The average vegetation height of short-grass vegetation is 2–5 cm. Red fescue (*Festuca rubra* L.), quaking grass (*Briza media* L.), and common bent grass (*Agrostis tenuis* Sipthrob) are the predominating plant species in this vegetation type. Tussocks of evergreen sedge (*Carex sempervirens* Vill.) and mat grass (*Nardus stricta* L.) are predominant in the tall-grass vegetation, which averages 20 cm in vegetation height (Schütz and others 2006). Short-grass vegetation developed in areas where cattle and sheep rested (high nutrient input) during agricultural land-use (from 14th century until 1914); tall-grass vegetation developed in areas where cattle and sheep used to graze, but did not rest (Schütz and others 2003, 2006). Herbivores were shown to consume more than 60% of the biomass in short-grass compared to less than 20% in tall-grass vegetation (Schütz and others 2006). The herbivore community present in the SNP can be divided into four groups based on body size/weight: large [red deer (*Cervus elaphus* L.) and chamois (*Rupicapra rupicapra* L.); 30–150 kg], medium [marmot (*Marmota marmota* L.) and snow hare (*Lepus timidus* L.); 3–6 kg], and small vertebrate herbivores (small rodents: for example, *Clethrionomys* spp., *Microtus* spp., *Apodemus* spp.; 30–100 g) as well as invertebrates (for example, grasshoppers, caterpillars, cicadas, <5 g). Large ungulates consume the most biomass (for example, Schütz and others 2006), although the other three groups also consume considerable plant biomass in alpine ecosystems (for example, Blumer and Diemer 1996). The diet composition of large ungulates often reflects the composition of grassland vegetation (mostly graminoids; Schröder 1977), whereas smaller-sized vertebrate herbivores usually prefer to selectively graze on forbs or seeds (for example, Eskelinen 2002). The amount, distribution, and quality of waste is dependent on body size (see Hobbs 1996). Ungulates deposit the largest amounts of low quality waste in a patchy distribution, whereas small mammals and invertebrates deposit smaller amounts of higher quality waste in a more evenly distributed way (see for example, Bakker and others 2004).

Experimental Design

We selected 18 subalpine grassland sites (9 short-grass, 9 tall-grass vegetation). The sites were spread across the entire park on dolomite parent material at altitudes of 1,975–2,300 m. At each site we established an exclosure network (fences) in spring 2009 (early June), immediately after snowmelt. Each exclosure network consisted of a total of five 2×3 m sized plots that progressively excluded the different herbivores listed above (further labeled according to the herbivore guilds that had access to the respective plots ''All'', ''Marmot/Mice/Invertebrates'', ''Mice/Invertebrates'', ''Invertebrates'', ''None''). The ''All'' treatment was thus accessible to all herbivores, was not fenced and was located at least 5 m away from a 2.1-m tall and 7×9 m main fence that enclosed the other four treatments. This fence was constructed of 10×10 cm wooden posts and electrical equestrian tape (AGRARO ECO, Landi, Bern, Switzerland; 20 mm width) mounted at 0.7, 0.95, 1.2, 1.5, and 2.1 m above the ground that were connected to a solar charged battery (AGRARO Sunpower S250, Landi, Bern, Switzerland). We also mounted non-electrically charged equestrian tape at 0.5 m to help exclude deer and chamois, yet allow marmots and hares to enter safely. Within each main fenced area we randomly established four 2×3 m plots: (1) The ''Marmot/Mice/Invertebrates'' plot remained unfenced, thus, with the exception of red deer and chamois, all herbivores were able to access the plot. (2) The ''Mice/Invertebrates'' plot consisted of a 90-cm-high electric sheep fence (AGRARO Weidezaun-netz ECO, Landi, Bern, Switzerland; mesh size 10×10 cm) connected to the solar panel and excluded all medium-sized mammals (marmots, hares), but provided access for small mammals and invertebrates. (3) The ''Invertebrates'' plot provided access for invertebrates only and was surrounded by 1 m high metal mesh (Hortima AG, Hausen, Schweiz; mesh size 2×2 cm). (4) The ''None'' plot was surrounded by a 1-m tall mosquito

net (Sala Ferramenta AG, Biasca, Switzerland; mesh size 1.5 × 2 mm) to exclude all herbivores. This plot was covered with a roof constructed of a wooden frame lined with mosquito mesh that was mounted on the wooden corner posts. We also treated this plot with a biocompatible insecticide (Clean kill original, Eco Belle GmbH, Waldshut-Tiengen, Germany) when needed to remove insects that might have entered during data collection or that hatched from the soil.

To assess whether the design of the ''None'' exclosure (mesh and roof) affected the micro-climatic condition associated with soil CO_2 emissions, we erected ''micro-climate control'' exclosures at six of the 18 sites. These exclosures were built as the ''None'' exclosures, but were open at the bottom (20 cm) of the 3 m side of the fence facing away from the prevailing wind to allow invertebrates to enter. A 20-cm high and 3-m long strip of metal mesh was used to block access to small mammals. Thus, this construction allowed a comparable micro-climate to the ''None'' plots, but also a comparable grazing pressure to the ''Invertebrates'' plots. We compared various properties within these exclosures against one another to assess if our construction altered the conditions in the ''None'' plots (Online Appendix Table A1). We were able to show that the exclusion of invertebrates lead to significantly higher aboveground biomass and vegetation height. As a consequence, soil temperature decreased (reduction in solar heating due to higher and denser canopy), which lead to an increase in soil moisture (less soil evapotranspiration due to lower temperatures). The only parameter that was directly altered by the roof construction was the total amount of UV light, yet this change did not reflect the amount of biomass produced. Consequently, the exclusion of herbivores rather than the construction of our exclosures was responsible for potential differences in soil CO_2 emissions.

The fences were dismantled in late October 2009 to protect them from snow pressure and avalanches and remounted in early May 2010 immediately after spring snowmelt. The two years differed in climatic conditions. The winter (September–March) preceding the 2009 growing season was considerably wetter (463 mm) compared to winter 2010 (297 mm; measured at the nearby weather station at Buffalora; MeteoSchweiz 2011). In contrast, our study area received considerably more precipitation in the 2010 growing season (April–August: 432 mm) compared to the year before (317 mm). The average growing season temperature in 2010 was 1°C colder (7.1°C) compared to the same period in 2009 (8.1°C).

Bi-weekly ungulate pellet counts (on two 4 wide × 25 m long plots per site; adapted from Neff 1968) and grasshopper counts (on ten 0.5 × 0.5 m plots per site; method adapted from Gardiner and others 2002; for details see Spalinger and others 2012) showed that all sites were grazed by large ungulates and invertebrates during both years (Online Appendix Table A2). In addition, marmot populations were counted twice during both summers (observation counts) indicating that marmots were present at all sites (Online Appendix Table A2). Small mammal populations were not assessed at the individual sites and no attempt was made to quantify herbivore numbers and composition within the individual exclosure networks. However, using game cameras (Moultrie 6MP Game Spy I-60 Infrared Digital Game Camera, Moultrie Feeders, Alabaster, AL, USA), we did observe that mice were present in some fences and that the medium- and small-sized mammals (marmot/hares and mice) were not afraid to enter the fences and graze on their ''designated'' plots.

Measuring Soil CO_2 Emission, Soil Temperature, and Soil Moisture

In situ soil CO_2 emissions were measured with a PP-Systems SRC-1 soil respiration chamber (closed circuit) attached to a PP-Systems EGM-4 infrared gas analyzer (PP-Systems, Amesbury, MA, USA) on two randomly selected locations on one subplot within each of the 90 plots. For each measurement the soil chamber (15 cm high; 10 cm diameter) was placed on a permanently installed PVC collar (10 cm diameter) driven 5 cm into the soil at the beginning of the study (June 2009). The measurements were conducted between 0900 and 1700 h every 2 weeks from late-June to late-August 2009 ($n = 5$) and late-May to late-August 2010 ($n = 7$); until snow fell. Freshly germinated plants growing within the PVC collars were removed prior to each measurement to avoid measuring plant respiration/photosynthesis. The two measurements collected per plot every 2 weeks were averaged. Soil temperature and soil moisture were measured at a depth of 0–10 cm mineral soil at five random locations per plot during each soil CO_2 sampling date. Soil temperature was measured with a waterproof digital pocket thermometer (Barnstead International, Dubuque IA, USA) and soil moisture by time domain reflectometry with a Field-Scout TDR-100 (Spectrum Technologies, Plainfield IL, USA).

Aboveground Biomass Consumption by Herbivores

We estimated aboveground biomass on one randomly located 1×1 m subplot at peak biomass using the non-destructive canopy intercept method (Frank and McNaughton 1992). Briefly, this method estimates aboveground biomass based on hits of a pin that was passed through the vegetation. Prior to the study a reference system (dry biomass vs. hits) was established for the entire area. Consumption was calculated for each plot, using the ''None'' plot as baseline for each network cluster (0% consumption) and the ''All'' treatment as baseline for maximum consumption of all herbivore groups.

Root Biomass and Microbial Biomass Carbon Sampling

Soil samples were collected in early September 2009 and 2010 on the 90 subplots assigned for destructive sampling. Prior to sampling the soils, we removed the vegetation from two 10×100 cm strips within the subplot. Thereafter we randomly selected five spots within the two strips and collected five 2.2 cm (diameter) $\times$ 10 cm soil samples with a soil corer (Giddings Machine Company, Windsor, CO, USA), resulting in a total of 450 cores each year. The samples were dried at 30°C and roots were manually separated from the soil material. We hand picked each sample for 1 h, allowing retrieval of over 90% of all roots present in the sample. The roots were then dried at 65°C for 48 h and weighed to the nearest mg. The average root biomass of the five cores was used to derive root biomass per 1 m^2 plot.

Another three soil samples were randomly collected on the strips where the vegetation was removed to determine mineral soil MBC. For this purpose, we first removed the dense root layer generally present within the top 1–5 cm of our soils and then collected a 5 cm (diameter) $\times$ 10 cm mineral soil core (AMS Samplers, American Falls, ID, USA). The three samples were combined (90 samples for each sampling year), immediately put on ice, taken to the laboratory, passed through a 2-mm sieve and stored at 4°C. MBC was then determined using the substrate-induced method of Anderson and Domsch (1978).

Statistical Analyses

We used the linear mixed model approach to investigate how herbivore exclusion affected soil CO_2 emissions in each vegetation type. Soil CO_2 emission was the dependent variable, modeled as a function of the fixed-factors year, treatment, year × treatment and sampling date. Site was included as a random factor. We adjusted this model for temporal autocorrelation using a first-order autocorrelation structure (AR[1]). Pairwise comparisons were made for the main effect treatment using the Bonferroni confidence interval adjustment. The same modeling approach was used to assess treatment effects on soil temperature and soil moisture. In addition, we calculated linear mixed effects models to assess treatment differences in consumption, root biomass, and MBC as a function of the fixed-factors year, treatment, and year × treatment, with site as a random factor. We transformed all the data for these analyses using Box-Cox transformation to meet the normality criteria.

We assessed the temporal relationship between soil CO_2 emissions and soil temperature/soil moisture during the two growing seasons using linear regression analysis. Further, we assessed which of the abiotic and biotic parameters (soil temperature, soil moisture, consumption, root biomass, and soil MBC) were the best predictors of soil CO_2 emissions for each vegetation type. To do this we averaged all plot measurements of soil CO_2 emission, soil temperature (Box-Cox transformed), soil moisture, consumption (ln-transformed), root biomass (ln-transformed), and soil MBC (ln-transformed) and then calculated stepwise multiple regression models (backward selection) separately by vegetation type. In addition, we calculated the apparent temperature sensitivity of soil CO_2 emission (Q_{10})—defined as the relationship of field measured soil respiration against seasonal temperatures (see Smith and others 2008)—for each treatment of each vegetation type (see Lloyd and Taylor 1994 for equations) to assess how grazing removal affected the temperature sensitivity of soil CO_2 emissions. All statistical analyses were performed with the PASW Statistics 19.0 statistical package (IBM SPSS, Chicago, IL, USA).

RESULTS

Soil CO_2 emissions varied considerably during the two growing seasons (Online Appendix Figure 1A). The seasonal and inter-annual variance was explained by differences in soil temperature, whereas soil moisture had no explanatory power (Online Appendix Figure 1B, C). Overall, the average emissions were 23% lower in 2010 (0.61 ± 0.011 g CO_2 m^{-2} h^{-1}; mean $\pm$ standard error) compared to 2009 (0.81 ± 0.016 g CO_2 m^{-2} h^{-1}, for statistics see Online Appendix Table A3).

Progressively excluding vertebrate and invertebrate herbivores resulted in significant changes in soil CO_2 emissions in both vegetation types (short-grass: $F = 5.276$, $p < 0.001$; tall-grass: $F = 7.261$, $p < 0.001$, Online Appendix Table A3). Yet, the response of soil CO_2 flux to herbivore exclusion differed between the two vegetation types (Figure 1A). For short-grass vegetation, excluding large as well as medium sized herbivores enhanced soil CO_2 flux, with the peak value found for the "Mice/Invertebrates" treatment. Interestingly, soil CO_2 flux did not differ between the "All" and "None" treatments. Even though significant differences in biomass consumption were found between our treatments ($F = 19.819$, $p < 0.001$; Figure 1B), they did not correspond with the observed soil CO_2 flux pattern (Figure 1B, C). For tall-grass vegetation, significantly less soil CO_2 was released where all herbivores were removed ("None") compared to all other treatments (Figure 1A). In addition, significantly lower emissions were measured when only invertebrates were present compared to when the large ungulates were removed. Changes in soil CO_2 emissions were, again, not associated with changes in biomass consumption, as consumption was consistent between treatments due to high between-plot variability (Figure 1B, C; $F = 2.089$, $p = 0.091$).

Soil temperature differed between treatments in both vegetation types, whereas soil moisture varied by treatment only in short-grass vegetation (see Online Appendix Figure A2, A, B). No herbivore removal response was found for root biomass or MBC in either vegetation type (see Online Appendix Figure 2A, C, D). However, root biomass patterns best explained the treatment-caused variability in soil CO_2 in tall-grass vegetation ($r^2 = 0.202$, $F_{1,43} = 10.873$, $p = 0.002$, Table 1). Soil temperature and soil moisture were, in contrast, the best predictors of soil CO_2 flux in the short-grass vegetation ($r^2 = 0.356$, $F_{2,42} = 11.618$, $p < 0.001$, Table 1).

The apparent temperature sensitivity of soil respiration (Q_{10}) was generally higher for tall-grass (average for all treatments: 2.65) compared to short-grass vegetation (average 1.94; Figure 2, inset). In addition, we found that exclusion of herbivores had a strong effect on Q_{10} as we detected the lowest values in the "All" plots in both vegetation types (Figure 2). In the short-grass vegetation, mainly large mammalian herbivores influenced temperature sensitivity of soil respiration (ΔQ_{10} between
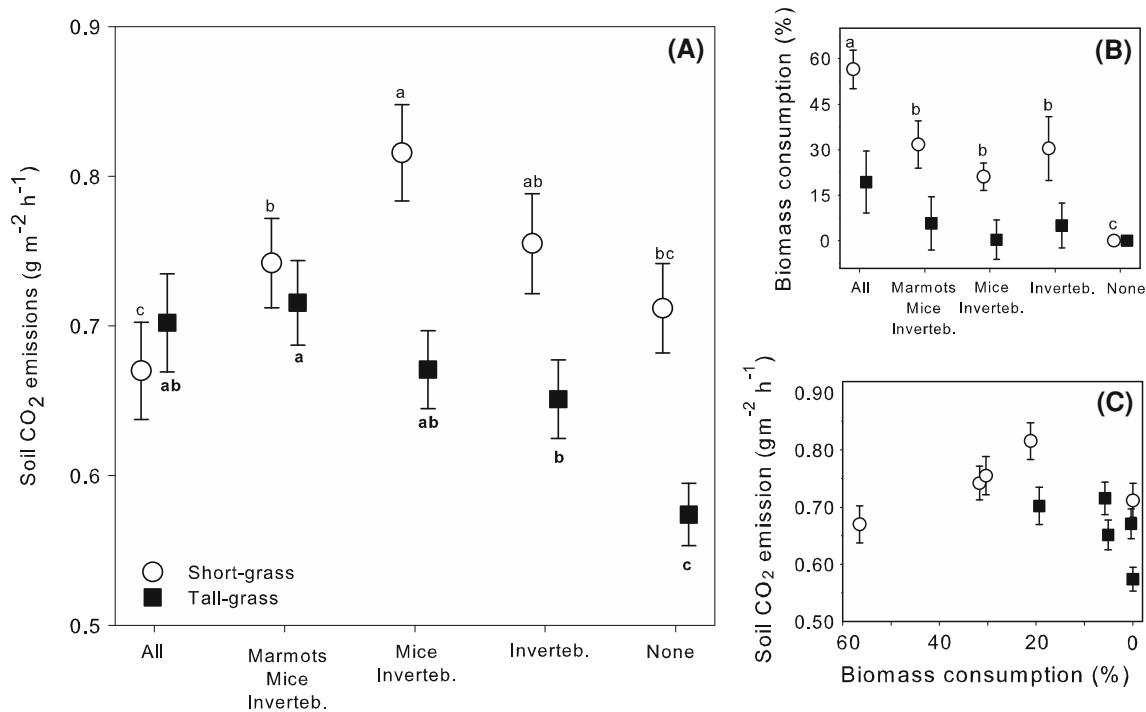

Figure 1. Treatment effects on soil CO_2 emissions and biomass consumption for the two different vegetation types separately. Values represent means ± standard error. *Different* normal *font letters* indicate significant differences between the short-grass treatments ($\alpha = 0.05$); *bold letters* for the tall-grass treatments. **A** soil CO_2 emissions, **B** biomass consumption, **C** soil CO_2 emissions related to changes in biomass consumption. *Interveb.* invertebrates.

Table 1. Final Model Coefficients for the Stepwise Linear Regression Models (After Backward Selection) Explaining Soil CO_2 Emissions for Short- and Tall-Grass Vegetation Separately

Model	B	SEB	β	t	p
Short-grass vegetation					
Intercept	−3.806	1.855		−2.052	0.046
Soil moisture	0.160	0.003	0.861	4.490	<0.001
Soil temperature	2.932	1.276	0.441	2.297	0.027
Tall-grass vegetation					
Intercept	−0.645	0.397		−1.625	0.111
Root biomass	0.192	0.058	0.449	3.297	0.002

B unstandardized regression coefficient, SEB standard error of B, β standardized regression coefficient.
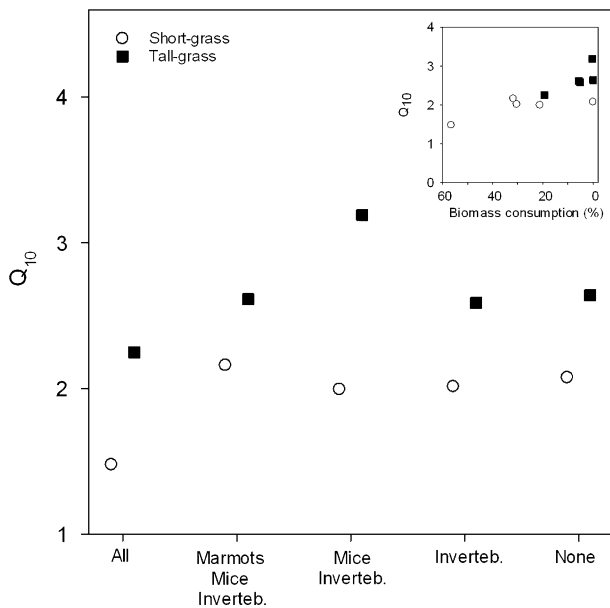
Figure 2. Temperature sensitivity of soil CO_2 emissions (Q_{10}) for the different treatments and both vegetation types. *Inset* Q_{10} of the different treatments plotted against biomass consumption. *Interveb.* invertebrates.

"All" and "Marmots/Mice/Invertebrates" = 0.68), whereas all herbivore groups seemed to contribute to Q_{10} changes in tall-grass vegetation.

DISCUSSION

Changes in Soil CO_2 Emissions Due to Herbivore Removal

Progressively excluding functionally different herbivores using size-selective fences only partially yielded the expected responses in soil CO_2 emission in the two vegetation types. Removing the large and medium herbivores led—against our expectations—to higher rather than lower CO_2 emissions in

the short-grass vegetation, whereas the system responded with the expected decline in CO_2 emissions when small mammals and invertebrates were removed. Still, there was no overall difference in CO_2 emissions between the "All" and "None" herbivory treatments. Soil CO_2 emissions did not respond to the removal of large and medium mammals in the tall-grass system, but declined as small mammals and invertebrates were excluded. Increases (for example, Knapp and others 1998; Johnson and Matchett 2001; Cao and others 2004; Chen and others 2008), decreases (Frank and others 2002a; Jun and others 2008), and no changes (Risch and Frank 2006; Strebel and others 2010) in soil CO_2 emission as a result of reducing herbivore pressure have been reported in the literature. However, these findings mostly stem from studies that only excluded domestic or wild ungulates (exception: Strebel and others 2010; geese), whereas we excluded additional herbivore groups—including invertebrates—resulting in highly variable ecosystem responses depending on the herbivore group excluded.

Potential Mechanism in the Short-Grass Vegetation

Soil micro-climatic conditions (soil moisture, soil temperature) were the best predictors of soil CO_2 emissions in short-grass vegetation. We do not have supporting data to explain how the exclusion of functionally different herbivores impacted the soil micro-climate and caused the observed pattern in soil CO_2 emissions, but we propose two pathways based on the literature: (1) Differences in canopy height and structure due to consumption and selective feeding altered the radiant heating of the soil (for example, Turner and others 1992; Chen and others 2013) and therefore the soil moisture regime (Chen and others 2013). (2) The herbivore exclusion treatments resulted in changes in trampling (ungulates) or burrowing (marmots,

mice, voles) regimes that affected soil physical properties such as bulk density, soil porosity, and water holding capacity (Binkley and others 2003; Davidson and others 2010, 2012), which thus led to alterations in the soil micro-climate.

Given the impact of our treatments on soil temperature and soil moisture it is possible that changes in soil micro-climate directly led to changes in soil CO$_2$ emissions by changing the activity of the soil community (see for example, Luo and Zhou 2006). Alternatively, exclusion-driven changes in soil micro-climate may have indirectly effected soil CO$_2$ emissions by altering plant physiological processes (Vargas and others 2011; Gomes-Casanovas and others 2012) or N mineralization rates (Bakker and others 2004) by altering the abundance and composition of the soil microbial (Patra and others 2005; Zhou and others 2010) or arthropod communities (Freckmann and others 1979; Mulder and others 2003). Although we do not have any data on how our treatments affected microbial activity or plant physiological properties (for example, root respiration, photosynthesis, microbial respiration), we recently assessed soil organic matter decay rates in our treated plots (2010; unpublished results) and detected few differences in decay between treatments. In support, we found no differences in MBC in the present study. Similarly, Hodel (2011) found no differences in the microbial community structure (assessed through T-RFLP) after the first and second growing season of our experiment. Results from our plots have shown that herbivore exclusion alters the richness, but not the size of the collembolan community without affecting the abundance and diversity of mites (Raschein 2012). It is possible that nematode abundance and biomass were altered by changes in soil moisture (Freckmann and others 1979; Chen and others 2013) and soil temperature (Mulder and others 2003; Chen and others 2013), although we have no information to support this claim. Further investigations—in particular with regard to nematodes—are necessary to fully assess the relationship between soil micro-climate, biotic activity, and CO$_2$ emissions.

Potential Mechanism in the Tall-Grass Vegetation

Root biomass was found to be the best predictor of soil CO$_2$ respiration in the tall-grass vegetation. Even though our treatments did not lead to significant differences in root biomass (see Online Appendix Figure A2, C), it is possible that changes in total consumption or changes in selective feeding (specific plant species/plant parts) altered the C

allocation from shoots to roots. This, in turn, could have reduced root respiration and consequently soil CO$_2$ emissions. However, results published by Thorne and Frank (2009) showed no evidence for increased mass-specific root respiration in a clipping experiment of four grass species. A much more likely pathway to explain our changes in soil CO$_2$ emissions is that herbivore exclusion affected plant physiological processes such as photosynthesis (Milchunas and Lauenroth 1993; Wilsey and others 2002), root exudation, or fine root turnover (Bardgett and Wardle 2003; Frank and others 2002b). Thus, the significantly lower soil CO$_2$ emission rates measured in the ''None'' plots could be a result of lower substrate availability and therefore lower microbial activity.

Additional Potential Mechanisms Explaining the Patterns Found

The herbivore exclusion treatments may have affected the amount, distribution, composition, and decomposability of animal waste (dung, urine), ultimately resulting in changes in substrate availability for belowground community activity (Bakker and others 2004). By progressively excluding herbivores by body size in our study, the distribution of dung likely changed from large ''pellets'' of low quality that were patchily distributed (for example, ungulates present) to small ''pellets'' or frass of high quality that were more evenly distributed (for example, small mammals and invertebrates present). More evenly distributed higher quality waste could result in increased resource availability for plants, which in turn, could alter plant physiological processes, resource allocation between shoots and roots and therefore soil CO$_2$ emissions. Further, depending on whether facilitation or competition are the dominating interactive forces between the different herbivore groups, one or several groups of herbivores might positively or negatively respond to the exclusion of others in terms of total abundance. Davidson and others (2010) has, for example, shown that the exclusion of cattle and prairie dogs favored the numbers of grasshoppers. Changes in interactive forces could therefore affect the amount of biomass that a certain herbivore group consumes. As a consequence, the total amount of waste deposited within the system by a specific herbivore group might in- or decrease. Our data on consumption indicates that the four herbivore groups competed for resources: the exclusion of large and medium vertebrates resulted in compensatory biomass consumption of the smaller remaining species (compare Ritchie and

Olff [1999b]), which potentially led to larger quantities of evenly distributed waste.

Changes in Temperature Sensitivity of Soil Respiration (Q_{10}) Due to Herbivore Removal

Our study revealed considerable increases in the apparent temperature sensitivity of soil respiration when large herbivores were removed from short- and tall-grass vegetation, as also reported by other authors (Cao and others [2004]; Chen and others [2008]). Interestingly, no further increase in sensitivity was detected with the exclusion of the other herbivores in the short-grass vegetation, whereas the exclusion of each herbivore group in the tall-grass vegetation caused changes in the temperature sensitivity of soil respiration. As the temperature sensitivity of soil respiration is also controlled by soil moisture, photosynthesis rates, and substrate supply of the soil (Davidson and others [2006]), which in turn are differentially influenced by herbivores, understanding how progressive herbivore exclusion affects Q_{10} mechanistically is even more difficult than understanding the changes in actual CO_2 fluxes. Further investigations, ideally under controlled conditions, could shed more light into this issue. Nevertheless, our findings indicated that—at least for our study area—removing functionally different grazers leads to changes in the temperature sensitivity of soil respiration.

CONCLUSIONS

As one of the first studies on the effects of both vertebrate and invertebrate herbivore exclusion on soil CO_2 emissions in grassland ecosystems, our results suggest that the controls on soil respiration are substantially more complicated than assumed from previous studies that only excluded large ungulates. Given the multiple processes that different herbivores affect in grassland ecosystems-as discussed in this study-assessing their impact on the soil CO_2 emissions remains difficult. Yet, our results provide initial evidence of how changes in competitive interactions among herbivores—as could happen through changes in management, extinction or invasion—alter the grassland soil C cycle.

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