Massive warming-induced carbon loss from subalpine grassland soils in an altitudinal transplantation experiment

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Abstract

Climate change is associated with a change in soil organic carbon (SOC) stocks, implying a feedback mechanism on global warming. Grassland soils represent 28% of the global soil C sink and are therefore important for the atmospheric greenhouse gas concentration.

In a field experiment in the Swiss Alps we recorded changes in the ecosystem organic carbon stock under climate change conditions, while quantifying the ecosystem C fluxes at the same time (ecosystem respiration, gross primary productivity, C export in plant material and leachate water). We exposed 216 grassland monoliths to six different climate scenarios (CS) in an altitudinal transplantation experiment. In addition, we applied an irrigation treatment (+12-21% annual precipitation) and an N deposition treatment (+3 and +15 kg N ha^{-1} a^{-1}) in a factorial design, simulating summer-drought mitigation and atmospheric N pollution.

In five years the ecosystem C stock, consisting of plant C and SOC, dropped dramatically by about -14% (-1034 ±610 g C m^{-2}) with the CS treatment representing a +3.0°C seasonal (Apr.-Oct.) warming. N deposition and the irrigation treatment caused no significant effects. Measurements of C fluxes revealed that ecosystem respiration increased by 10% at the +1.5°C warmer CS site and by 38% at the +3°C warmer CS site ($P \leq 0.001$ each), compared to the CS reference site with no warming. However, gross primary productivity was unaffected by warming, as were the amounts of exported C in harvested plant material and leachate water (dissolved organic C). As a result, the five year C flux balance resulted in a climate scenario effect of -936 ±138 g C m^{-2} at the +3.0°C CS, similar to the C stock climate scenario effect. It is likely that this dramatic C loss of the grassland is a transient effect before a new, climate adjusted steady state is reached.

1 Introduction

The organic C stock contained in soils has long been recognized both as a substantial sink for anthropogenic CO_2 emissions, but also as particularly sensitive to global warming (Schlesinger, 1977; Post et al., 1982). Indeed, today grassland soils are one of the largest terrestrial CO_2 sinks, because they contain a pool of 661 Pg C (ca. 28% of total global soil C; Jobbágy and Jackson, 2000) or >80% of C contained in the atmosphere. For Europe, this huge soil organic carbon (SOC) stock was predicted to decrease by 6-10% during the 21st century as a response to climate change (Smith et al., 2005). Thus, a shrinking sink for atmospheric CO_2 would create a positive feedback loop with globally rising temperatures, which makes research on C cycle responses vital for improving projections of how fast the climate will change (Hoeppner and Dukes, 2012).

Storage of organic C (OC) is positively related to plant growth. Thus, increased plant growth may be expected to have a similarly positive effect on ecosystem C sequestration (Vitousek et al., 1997). For example, Ammann et al. (2009) found higher C sequestration in an intensively managed compared to an extensively managed grassland. In forests productivity increases following atmospheric N deposition, revealing a strong positive correlation with C sequestration (Magnani et al., 2007). Beyond edaphic factors, the grassland OC turnover is driven to a large degree by temperature, so that warmer soils have lower SOC contents. This effect can be observed along latitudinal gradients (Jones et al., 2005), as well as along altitudinal gradients.

This leads to the apparently paradox situation that less productive ecosystems support larger soil C sinks. In Swiss grasslands for example, more than 58% of SOC is stored at 1000-2000 m a.s.l. (37% of the total area), and despite the very shallow and cold soils 24% of SOC are found above 2000 m altitude (21% of the total area; Leifeld et al., 2005; Leifeld et al. 2009). As a result the 1000-2000 m a.s.l. region stores 3.6 times more SOC per unit land area~~,~~ compared to the < 1000 m a.s.l. region, and the > 2000 m a.s.l. region stores 2.7 times more SOC, respectively.

Under current global warming, the cold regions of high altitude and high latitude are most strongly affected (Core writing team, IPCC, 2014), and predicting the fate of the large biological ~~GHG~~ CO_2 sink of low productivity grasslands in a changing climate is of highest relevance. In these environments of largely temperature limited plant growth, rising temperatures have two antagonistic effects on the soil C sequestration process: First, warming favors productivity, resulting in increased availability of organic matter. This effect is strongest at intermediate warming levels and becomes smaller at warming levels that cause seasonal water shortage (Volk et al., 2021). Although grassland may respond differently to warming depending on soil moisture availability (Liu et al., 2018) and species composition (Van der Wal and Stien, 2014), in cold environments the warming response on productivity is generally positive (Rustad et al., 2001). Second, warming favors heterotrophic soil life, thus accelerating the decomposition of plant residues (Zhou et al., 2009). If the change of the rate of productivity and the rate of decomposition are not equal, the grassland soil will behave either as a C sink or a C source for the atmosphere until a new equilibrium is reached.

In addition, air pollution in the form of atmospheric N deposition may constitute a~~n undesired~~ fertilization effect. The N deposition rate is commonly very low at sites far away from agriculture and fossil fuel burning (<5 kg N ha^{-1} a^{-1}, Rihm and Kurz, 2001), but can reach >40 kg N ha^{-1} a^{-1} elsewhere in Switzerland (Rihm and Achermann, 2016). As a consequence, fast-growing species are favored and plant growth is promoted (Vitousek et al., 1997; Bobbink et al., 2010; Phoenix et al., 2012; Volk et al., 2014). Alone and in interaction, warming and N deposition therefore increase the ecosystem plant productivity potential and ~~support the~~lead to a larger input of organic carbon to the terrestrial carbon sink~~. Increased atmospheric CO_2 concentration, an obvious candidate among drivers of increased plant productivity, was not considered here because strong evidence suggests that in low productivity grasslands increased CO_2 acts via mitigation of soil moisture depletion only (Volk et al., 2000)~~.

However, the highly complex interactions of climate parameters (e.g. water availability and temperature) and pollution factors (e.g. N) have led to assume that the C sink of terrestrial ecosystems may also turn into a substantial source of atmospheric CO_2 (Lu et al., 2011; Heimann and Reichstein, 2008). Evidence from a subalpine grassland experiment shows that changes in aboveground plant productivity are not an appropriate predictor for changes in SOC content: Yield increases caused by a N-fertilization of 14 kg N ha^{-1} a^{-1} resulted in SOC gains, but already a fertilization of 54 kg N ha^{-1} a^{-1} resulted in net SOC losses, relative to control (Volk et al., 2018). Such effects were driven by a strongly increased ecosystem respiration (ER) that overcompensated for the increased substrate input (Volk et al., 2011). In agreement, a recent interannual comparison of subalpine grassland based on different annual mean temperatures has also shown that plant productivity was positively correlated to temperature, while the ecosystem CO_2 balance, namely net ecosystem productivity (NEP), was negatively correlated (Volk et al., 2016).

In this paper, we quantify the response of a subalpine grassland ecosystem C budget in the face of multiple climate change factors that may favor plant productivity. We present a comprehensive set of data related to relevant C flux pathways to illuminate mechanisms controlling the ecosystem C sink / source properties. In a five year~~s~~ field experiment in the central Swiss Alps, a climate scenario treatment was established that ~~consist~~resulted in~~ing of~~ warming~~ and~~, ~~atmospheric N deposition~~ ~~and irrigation~~. In addition, to uncouple ~~the testing of~~potential temperature effects from temperature-driven soil moisture effects and to consider effects of atmospheric N deposition, a two-level irrigation treatment and a three-level N treatment were~~was~~ set up in a factorial design. Using a transplantation approach along an altitudinal gradient to accomplish the climate scenario treatment, we affected not only temperatures, but also the length of snow cover and the growing period. The long duration of the experiment provided a large between-year weather variability. Because the investigated grasslands had developed under a low intensity management that was unaltered for decades if not centuries, we considered the SOC stock to be in a steady state on a mid- and long-term perspective. We hypothesized that

1) Under a climate scenario (CS) similar to the present climate, changes in productivity and decomposition will compensate each other and result in small or no changes in the SOC stock over five years.

2) CS with strong temperature increases significantly alter the SOC stock towards a sink or a source, depending on whether plant productivity or SOC decomposition is affected more by~~from~~ climate change effects.

3) Irrigation mitigates effects of ~~drought~~ water shortage due to warming, and N deposition reduces possible N limitation of microbial activity; both factors thus exhibiting a favorable effect on decomposition and reducing the SOC stock.

2 Materials and Methods

This study on ecosystem C fluxes is part of the AlpGrass experiment and theis Materials section refers only to those aspects relevant to the study of the C fluxes. ~~Further details of the gas exchange measurement and parameterization are presented in the Appendix.~~ We ~~also~~ refer to Volk et al. (2021) for more details on the experimental design. Details on the gas exchange measurement and parameterization are provided in Appendix A.

The experiment used grassland monoliths to investigate climate change effects on the soil carbon stock of subalpine grassland ecosystems in the central Alps. At six sites with summer livestock grazing (within ≤ 55 km distance) in the Canton Graubünden, Switzerland, areas of 1 ha on southerly exposed, moderate slopes at an altitude of ca. 2150 m a.s.l. were selected. These sites of origin shared very similar climatic conditions, but represented a wide range of soil properties and plant communities. Detailed information on soil properties and species composition of the different origins can be found in Wüst-Galley et al. (2020).

Monoliths of 0.1 m^2 surface area (L × W × H = 37 × 27 × 22 cm) were excavated at randomly generated positions at the sites of origin and placed into precisely-fitting, well-drained plastic boxes. 216 monoliths were transported from their respective site of origin to the common AlpGrass experimental site in November 2012 and remained there until the final harvest in Oct. 2017.

2.1 Experimental site and treatment design

The AlpGrass experiment is located close to Ardez in the Lower Engadine valley (Graubünden, Switzerland). The site covers a 680 m altitudinal gradient on the south slope of Piz Cotschen (3029 m), ranging from montane forest (WGS 84 N 46.77818°, E 10.17143°) to subalpine grassland (WGS 84 N 46.79858°, E 10.17843°). We located six separate climate scenario sites (CS) at different altitudes (CS1: 2360 m, CS2: 2170 m, CS3: 2040 m, CS4: 1940 m, CS5: 1830 m, CS6: 1680 m a.s.l.). CS2 was chosen as a reference site (hereafter $CS2_{reference}$), because it had the same altitude as the sites of origin. The snow-free period lasts approximately from May to October, with a mean growing season (April to October) air temperature of 6.5 °C (Table 1).

At each of the 6 CS, 36 monoliths (six from each of six sites of origin) were installed in the ground within their drained plastic boxes, at level with the surrounding ~~grassland~~ soil surface, resulting in a total of 216 transplanted monoliths. Monoliths in their containers were set side by side without a gap. To prevent the invasion of new species or genotypes, the surroundings of the monolith-array were frequently mown.

In addition to the climate scenario treatment, an irrigation and an N deposition treatment were set up in a full-factorial design at each CS. One half of the 36 monoliths received only ambient precipitation, the other half received additional water during the growing season. Within both~~the~~ irrigation treatment levels monoliths were subjected to three levels of N deposition. At the CS sites, irrigation and N treatments were set up in a randomized complete block design (six blocks each containing all six irrigation × N treatment combinations).

2.2 Climate scenario site (CS) treatment

The climate scenario treatment was induced by the different altitudes of the CSs at the AlpGrass site, to which monoliths from the sites of origin were installed. As a result, the transplanted monoliths experienced distinctly different climatic conditions (Table 1). To describe the climate scenarios, we focused on the mean growing period temperature from April to October, instead of the annual mean temperature. The temperature under the snow cover was ca. 0 °C at all CSs. ~~We focused on the mean growing period temperature from April to October, because we assumed the consistently moderate temperature (ca. 0 °C at all CSs) under the snow cover to be of little importance for the ecosystem C budget.~~ The CS temperature treatment was defined as the deviation from $CS2_{reference}$ temperature.

		Precipitation (sum, mm)		Air temp. (Mean, °C) ±1SE		Δ T
Site	Alt. (m)	Apr. – Oct.	Annual	Apr. – Oct.	Annual	Apr. – Oct
CS1	2360	674 ±18	752 ±20	5.1 ±0.17	1.6 ±0.20	-1.4
$CS2_{ref.}$	2170	656 ±27	748 ±27	6.5 ±0.17	3.2 ±0.23	0.0
CS3	2040	629 ±26	732 ±21	7.2 ±0.17	3.7 ±0.20	0.7
CS4	1940	614 ±20	739 ±22	8.0 ±0.16	4.7 ±0.25	1.5
CS5	1830	628 ±20	780 ±17	8.3 ±0.17	4.6 ±0.21	1.8
CS6	1680	570 ±19	687 ±21	9.5 ±0.17	5.8 ±0.21	3.0

Table 1. Climate parameters at the climate scenario sites (CS) between 2012 and 2017. Precipitation sums for climate scenario sites, aggregated from April to October and annually. Mean air temperature from April to October and for the whole year. Air temperature difference (Δ T) April – Oct. for respective CS' compared to $CS2_{reference}$ ($CS2_{ref.}$).

2.3 Irrigation treatment

A two-level irrigation treatment was set up to distinguish the warming effect from the soil moisture effect, driven by warming. Precipitation equivalents of 20 mm were applied to the monoliths under the irrigation treatment in 4-6 applications throughout the growing period. Depending on the year, this treatment amounted to 80-120 mm or 12-21% of the recorded precipitation sum during the growing periods.~~A two-level irrigation treatment was set up to distinguish the warming effect from the soil moisture effect, driven by warming. Precipitation equivalents of 20 mm were applied to the monoliths under the irrigation treatment in several applications throughout the growing period. Depending on the year, this treatment amounted to 12-21 % of the recorded precipitation sum during the growing periods.~~

2.4 N deposition treatment

The N deposition treatment simulated an atmospheric N deposition from air pollution, equivalent of +3 and +15 kg N ha^{-1} a^{-1}, on top of the background deposition (3.3 kg and 4.3 kg N ha^{-1} a^{-1} at $CS2_{reference}$ and CS6, respectively). Twelve times during the growing period, a 200 ml ammonium nitrate (NH_4^{-} NO_3^{+}) in water solution was applied per monolith. Monoliths of the N deposition control group received pure water.

2.5 ~~Meteorology~~Environmental conditions

At all CSs, air temperature, relative humidity (Hygroclip 2, Rotronic, Switzerland), and precipitation were measured (ARG100, Campbell Scientific, UK). Global radiation (GR) as W m^{-2} was measured at $CS2_{reference}$ and CS6 using Hukseflux LP02-05 thermopile pyranometers. Soil temperature and SWC were measured at 8 cm depth (CS655 reflectometer, Campbell Scientific, UK). At $CS2_{reference}$ and the lowest CS6, these parameters were obtained in 18 monoliths each and at two points in the surrounding grassland per site, using time domain reflectometers (TDR) with 12 cm rods (CS655, Campbell Scientific, UK). In all other CSs, six monoliths each were equipped with such TDRs. All parameters were integrated for 10 minutes originally and later averaged for longer periods if necessary.

Ambient wet N deposition was 3.3 kg N ha^{-1} a^{-1} at $CS2_{reference}$ and 4.3 kg N ha^{-1} a^{-1} at the lowest CS6. Wet deposition was collected using bulk samplers (VDI 4320 Part 3, 2017; c.f. Thimonier et al., 2019) from April 2013 to April 2015. Nitrate (NO_3^-) was analyzed by ion chromatography (ICS-1600, Dionex, USA) and NH_4^+ was analyzed using a flow injection analyzer (FIAstar 5000, Foss, Denmark) followed by UV/VIS photometry detection (SN EN ISO 11732).

2.6 Plant productivity

Aboveground p~~P~~lant material~~ aboveground~~, including mosses and lichens, was cut annually at 2 cm above the soil at canopy maturity. Accordingly, mean harvest dates for CS1 to CS6 were 12. Aug., 26. July, 22. July, 14. July, 9. July and 5. July, respectively. Plant productivity responses to the climate scenario, N deposition and irrigation treatments were presented in Volk et al. (2021). In addition at the end of the experiment in the fall of 2017, total aboveground plant material was harvested including all stubbles, and root mass was assessed using two 5 cm diameter soil cores to 10 cm depth per monolith. For the above- and belowground fraction, C content was measured with a C/N-elemental analyzer, which allowed to calculate shoot and root C on a mass basis. Tests for effects of N deposition on mean plant C content revealed no significant differences and a common value of 47% was implied (see Bassin et al., 2015 for details on the calculation). Based on this data, shoot and root C stock in 2017 was calculated as well as the five years cumulative shoot C that was harvested 2 cm above the soil over the experimental period. In the context of this study productivity is expressed as g C m^{-2}~~.~~ per time unit.

2.7 Net ecosystem productivity (NEP)

Net ecosystem CO_2 exchange (NEE) was measured in biweekly to monthly intervals from 2013 to 2017 in day and night campaigns, covering the complete growing season. We utilized dynamic CO_2 concentration, non-flow-through, transparent acrylic chambers, fit to cover the whole grassland monolith including a fully developed canopy (Volk et al., 2011). All recorded concentration measurements were tested for linearity and omitted if $R^2 < 0.95$. Thus, reduced assimilation or respiration due to chamber effects (CO_2 substrate depletion, overheating, reduced diffusion gradient) could be safely excluded during the measurement (2 min. per monolith).

For the five year parameterization of climate scenario effects on NEE, we focused on a subset of monoliths from the control treatment group (no N deposition, no irrigation) that provided the highest measurement frequency (six control monoliths from each $CS2_{reference}$, CS4, CS6; 18 monoliths in total). Doing so, the dynamic developments of vegetation phenology and drought events were well represented. ~~For more information please refer to the Appendix.~~ We used global radiation and soil temperature at 8 cm depth to model gross primary productivity (GPP) and ecosystem respiration (ER) between measurement campaigns, in accordance with Volk et al. (2011 and 2016). The light response curve of GPP was derived at $CS2_{reference}$, and the temperature response of ER was established for $CS2_{reference}$, CS4 and CS6 separately, using an exponential function after Lloyd and Taylor (1994) and Ammann et al. (2007). NEP then resulted as GPP minus ER for a given time unit. For more information on the gas exchange measurement and parameterization please refer to ~~the~~ Appendix A.

Lacking NEE data during the snow-covered period, a potential ER substrate limitation during the winter was not accounted for, since respiration rates were on an extremely low level due to low temperatures. Accordingly, temperature normalized ER during the snow covered period was modelled to remain constant between the last fall measurement and the first measurement of the new growing period, just after snow-melt.

2.8 Soil organic carbon stock

In October 2012, 0-10 cm soil cores (5 cm diameter) were obtained in the grassland immediately beside the monolith's excavation site. Again in October 2017, two soil cores within each monolith were sampled to 10 cm depth to study the change of SOC stock and belowground biomass during the five year experimental phase. All samples were dried and sieved (2 mm).

We measured soil organic C and N contents by elemental analysis (oxidation of C-CO_2 and N-NO_2 in an O_2 stream and subsequent reduction of NO_2-N_2 by a copper-tungsten granule). Separation of CO_2 and N_2 was accomplished by GC-TCD and quantification using acetanilid as an external standard (Hekatech Euro EA 3000, Wegberg, Germany). Samples were free of carbonate, so total C equals organic C. This data allowed to calculate SOC stock in 2012 and 2017 as well as the SOC stock change over the five experimental years.

2.9 Dissolved organic C (DOC)

Monolith containers at $CS2_{reference}$, CS4 and CS6 were equipped as lysimeters to collect leachates. During 2014, 2015 and 2016 leachates were pumped from underground tanks. Respective volumes were recorded and combined aliquots per monolith were used for DOC analysis (NDIR detection following thermal-catalytic oxidation at 850°C; DIMATOC 2000, Essen, Germany).

2.10 Data analyses

Data were modeled for C stocks and C fluxes. SOC stock data were available for 2012 and 2017, to calculate the SOC stock *change* over the five experimental years. We used SOC stock *change* as the primary variable for the analyses of the CS treatment effect. ~~Here, we used SOC stock *change* as the primary variable for analyses as it allowed a more accurate interpretation of the CS treatment effect. In comparison, s~~Shoot and root C stock data were available only from the destructive harvest at the end of the experiment in 2017. Using linear mixed-effects models, SOC stock change and root and shoot C stock at 2017 were modeled as a function of climate scenario site (CS, factor with 6 levels), irrigation (factor with 2 levels), and N deposition (factor with 3 levels), including all interactions. Block (36 levels: 6 CS × 6 blocks) and site of origin (six sites) were modeled as random factors (random intercepts). For root and shoot C stock, no data were available for CS1 and for the intermediate N deposition treatment, and so the number of these factors' levels was reduced accordingly. The Kenward–Roger method was applied to determine the approximate denominator degrees of freedom of fixed effects (Kenward and Roger, 1997), and the marginal and conditional R^2 values of the model were computed following Nakagawa and Schielzeth (2013). Differences in the responses between single CSs and $CS2_{reference}$ were tested based on the model contrasts (post hoc Wald *t* tests without applying multiple comparisons).

Temperature effects on SOC stock change, root and shoot C stock data were also modeled directly as a function of temperature change, induced by the climate change treatment using generalized additive models (GAMs). Generalized additive models had to be used because simple linear models could not appropriately handle this relationship. The GAMs included a fixed intercept and a smooth term for temperature change. In the case of root and shoot C stock, the Gamma function with log-link was chosen as the underlying distribution; following this amendment, model validation revealed that the assumptions of GAMs were met. The GAMs to the three response variables were modeled twice: first using all monoliths, and second using only the control monoliths that received neither irrigation nor additional N. The latter was done to receive a direct comparison to the C flux data, which were measured only on control monoliths.

Regarding C fluxes, GPP, ER and NEP of $CS2_{reference}$, CS4, and CS6 at the end of the five experimental years were analysed with a multivariate linear mixed-effects model that took into account potential correlation among GPP, ER, and NEP, calculated per monolith (controls only). It turned out that any correlation between the three categories of C fluxes was close to zero. Differences in GPP, ER and NEP between each CS4 and CS6 against $CS_{reference}$ were tested based on the model contrasts (post hoc Wald *t* tests without applying multiple comparisons). Moreover, differences in the five years cumulative shoot C and leachate C between each CS4 and CS6 against $CS2_{reference}$ were assessed with *t* tests.

Finally, we calculated the net ecosystem C balance to estimate the climate change effect by comparing the ecosystem C budget of CS4 (+1.5°C) and CS6 (+3°C) against $CS2_{reference}$ using two alternative approaches: A carbon *stock* based comparison and a carbon *flux* based comparison. For both approaches, only control monoliths were used, and differences of CS4 and CS6 against $CS2_{reference}$ were evaluated with *t* tests. All data were analyzed with the statistics software R, version 4.1.0 (R Core Team, 2021) and packages lme4 for linear-mixed effect models (Bates et al., 2015) and mgcv for GAMs (Wood, 2017).

3 Results

3.1 Soil organic C stock is much lower at high temperatures

We detected significant effects of the climate scenario (CS) treatment on soil organic C (SOC) stock (Table 2, Appendix Table A1). Across all monoliths, the cooling associated with CS1 left the SOC stock largely unchanged. At $CS2_{reference}$ and the first two warming levels CS3 and CS4, SOC stock gains of +200 g m^{-2}, +453 g m^{-2}, and +164 g m^{-2}, respectively, were observed (Fig. 1a, Table 2a). Specifically tested, neither of these SOC stock changes at CS1, CS3, and CS4 were significantly different from $CS2_{reference}$ ($P > 0.1$ each). However, at the increasingly warmer CS5 and CS6, the SOC stock was dramatically reduced by -608 g m^{-2} and -447 g m^{-2} after five years (Fig. 1a, Table 2a, $P \leq 0.004$ each, against $CS2_{reference}$).

No significant effects on SOC stock changes were associated with the irrigation and the N deposition treatments (Appendix Fig. A1, Table A1). Considering only the control monoliths, that received neither irrigation nor additional N, the same patterns appeared although with larger standard errors due to smaller sample size (Fig 1b, Table 2b).

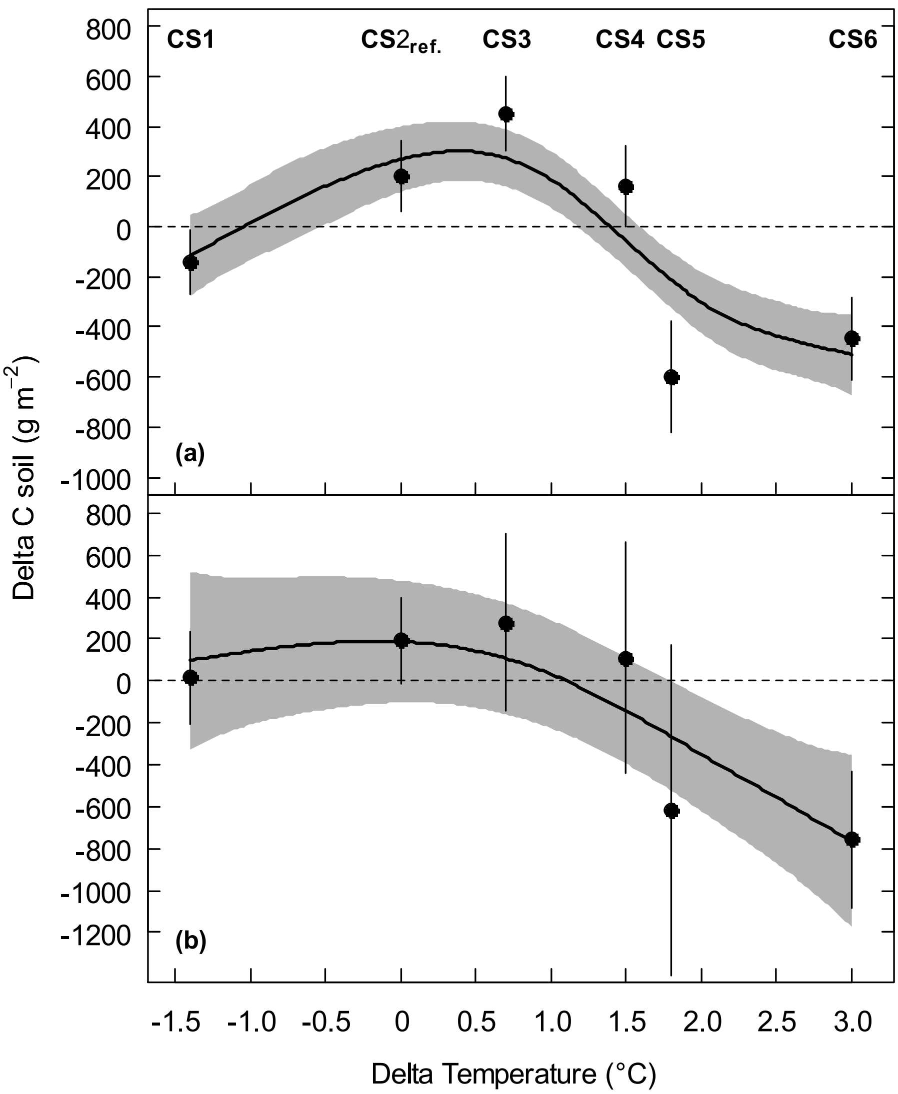

Figure 1. SOC stock change (Delta C soil) of subalpine grassland between 2012 and 2017 at six climate scenario sites (CS) as a function of the temperature change (Delta Temperature of the Apr. – Oct. mean) induced by the climate change treatment. (**a**) all monoliths, pooled across the irrigation and N treatments, and (**b**) control monoliths only that received neither irrigation nor additional N. Symbols are means ± 1 SE, and predicted lines are based on a generalized additive model (GAM) to all monoliths per group (± 1 SE, grey shaded). See Appendix Table A4 for the GAM summary.

CS site	2012 SOC 0-10cm		2017 SOC 0-10cm		2012-2017
(**a**) All monoliths	g C m^{-2}	SE	g C m^{-2}	SE	% change
CS1	6124	136.0	5986	149.0	-2.2
$CS2_{ref.}$	5983	150.2	6183	190.9	3.3
CS3	5973	112.4	6426	172.4	7.6
CS4	6109	171.5	6273	204.7	2.7
CS5	6313	159.7	5705	192.7	-9.6
CS6	6053	125.6	5606	192.7	-7.4
(**b**) Control monoliths	g C m^{-2}	SE	g C m^{-2}	SE	% change
CS1	6139	262.2	6154	261.6	0.2
$CS2_{ref.}$	6183	153.1	6375	247.2	3.1
CS3	6067	310.1	6345	285.4	4.6
CS4	5835	481.0	5944	711.9	1.9
CS5	5970	317.7	5350	579.4	-10.4
CS6	6238	339.8	5482	405.1	-12.1

Table 2. SOC stock (g C m^{-2}) at the beginning of the experiment and after five years of climate scenario treatment at six climate scenario (CS) sites. Data refer to (**a**) all monoliths, pooled across the irrigation and N treatments, and (**b**) control treatment that received neither irrigation nor additional N. SOC in 2012 did not significantly differ among the six CS sites (ANOVA: all monoliths: $F_{5,203} = 2.0$, $P = 0.082$; control monoliths: $F_{5,25} = 0.6$, $P = 0.676$)

3.2 Plant C stock belowground parallels soil organic C

In the final 2017 harvest, across all monoliths moderate warming at CS3 resulted in an increased root C stock of +166 g m^{-2} ($P = 0.021$, against $CS2_{reference}$), while root C stock at CS4 equaled that of $CS2_{reference}$ ($P = 0.998$, Fig. 2a). By contrast, root C stock was significantly reduced in the warmer CS5 and CS6 sites ($P < 0.001$ each, against $CS2_{reference}$), without an equivalent decrease in shoot C stock ($P > 0.2$ for all single CSs against $CS2_{reference}$, Fig. 2a). The root/shoot ratios of plant C stocks were (from $CS2_{reference}$ to CS6) 3.4, 3.5, 3.7, 1.8, and 2.1. Thus, compared to the $CS2_{reference}$ site, the relative allocation of C to roots was reduced by about half in CS5 (-48%) and CS6 (-39%), indicating that intensive warming has strongly changed the root/shoot ratio in favor of the shoots (Fig. 2a). Neither the irrigation nor the N deposition treatment had an effect on root and shoot C stock in 2017 after five years of treatment (Appendix Table A2 & A3).

Regarding the control monoliths group, the CS treatments revealed similar effects on each root and shoot C as compared to all monoliths, although the reduction of root C stock at CS6 was somewhat less pronounced (Fig. 2b).

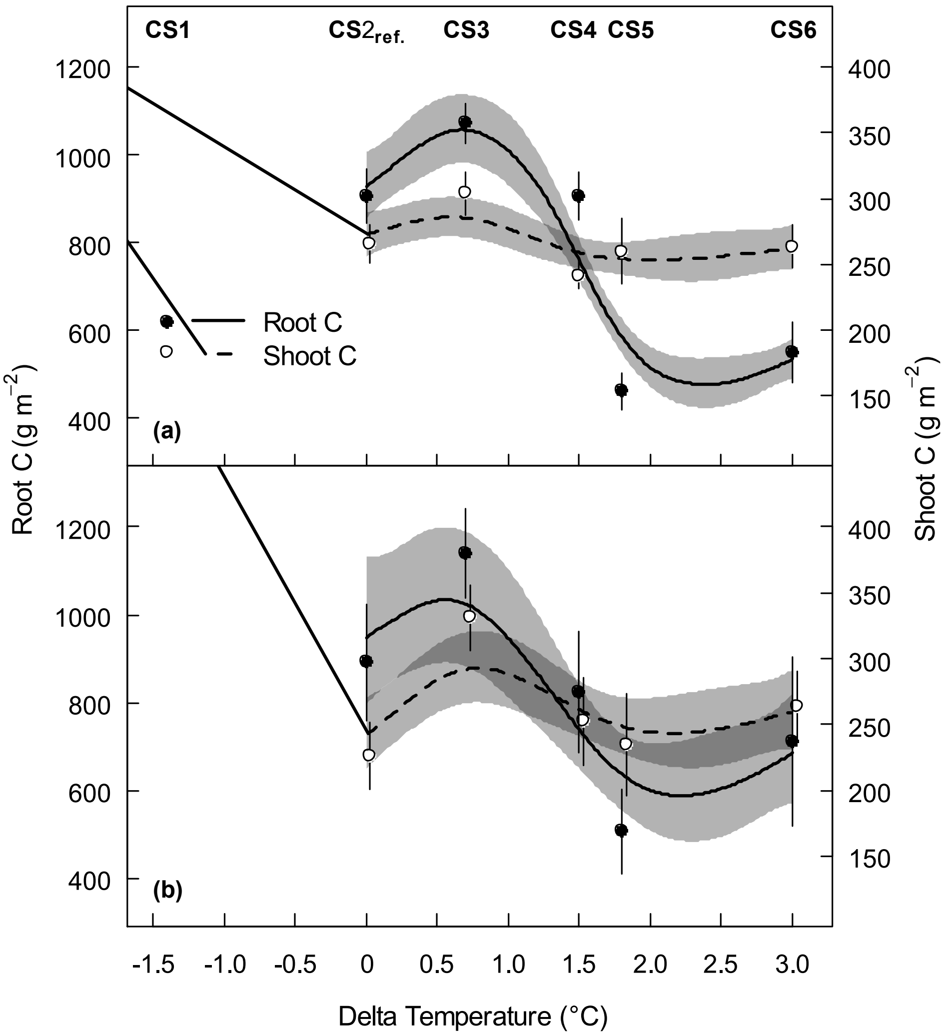

Figure 2. Root and shoot carbon stock of subalpine grassland at five climate scenario sites (CS) as a function of the temperature change (Delta Temperature of the Apr. – Oct. mean) induced by the climate scenario treatment. Data are from 2017, after five years of experimental duration. (**a**) all monoliths, pooled across the irrigation and N treatments, and (**b**) control monoliths only that received neither irrigation nor additional N. Symbols are means ± 1 SE, and predicted lines are based on a generalized additive model to all monoliths per group (± 1 SE, grey shaded; dark grey indicates the cross section of the two SE bands). See Appendix Tables A5 and A6 for the GAM summaries. No data were available for the CS1 site. Overlapping means and SEs are shifted horizontally to improve their visibility, and note the different y-axes for root and shoot C.

3.3 Increased ecosystem respiration draws down net ecosystem productivity C-balance

Seasonal temperature, soil moisture and canopy development determined the magnitude of gross primary productivity (GPP) and ecosystem respiration (ER) during five years at the three climate scenario sites $CS2_{reference}$, CS4 and CS6, where NEE was measured and parameterized (Appendix Fig. A2). Cumulative GPP CO_2 gains were not affected by the climate scenario treatment, but over time trajectories of cumulative ER CO_2

losses were significantly different from $CS2_{reference}$ in the warmest climate scenario CS6 (+38%) (Fig. 3). As a result, we found an ER driven change of the NEP balance with climate scenario. While NEP was consistently positive in $CS2_{reference}$ and CS4 (season +1.5°C), there was a critical climate step between CS4 and CS6 (season +3.0°C) resulting in a negative five -year NEP of -586 g C m^{-2} at CS6 (Fig. 4).

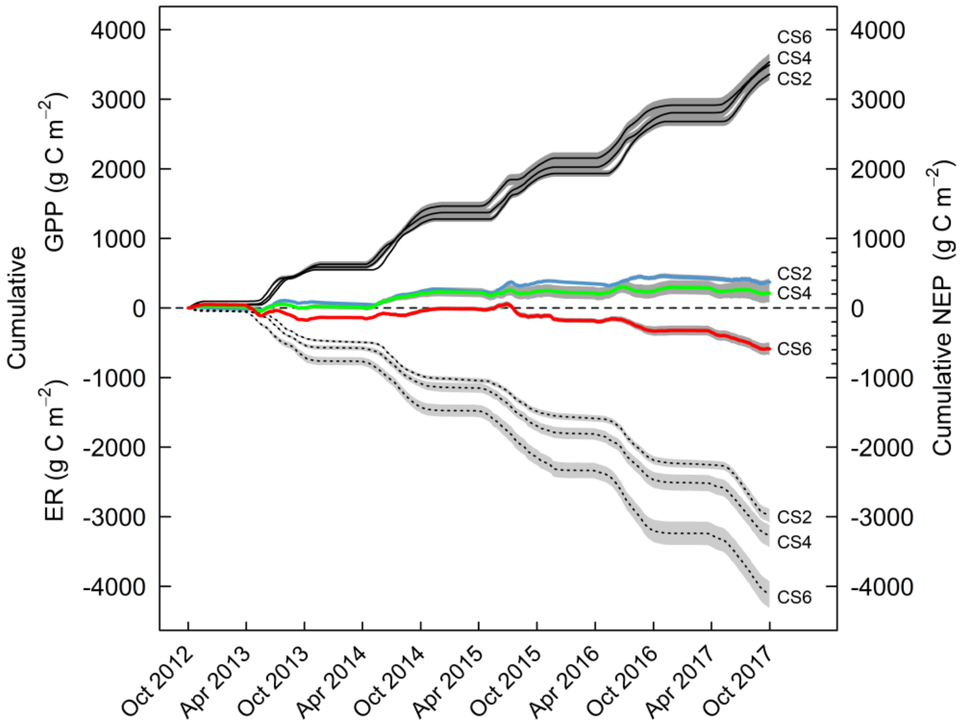

Figure 3. Cumulative trajectory of gross primary productivity (GPP, solid lines), ecosystem respiration (ER, dashed lines), and net ecosystem productivity (NEP, colored lines) at three climate scenario sites from October 2012 to September 2017. Displayed are means ± 1 SE (shaded grey) of the control treatment that received neither irrigation nor additional N.

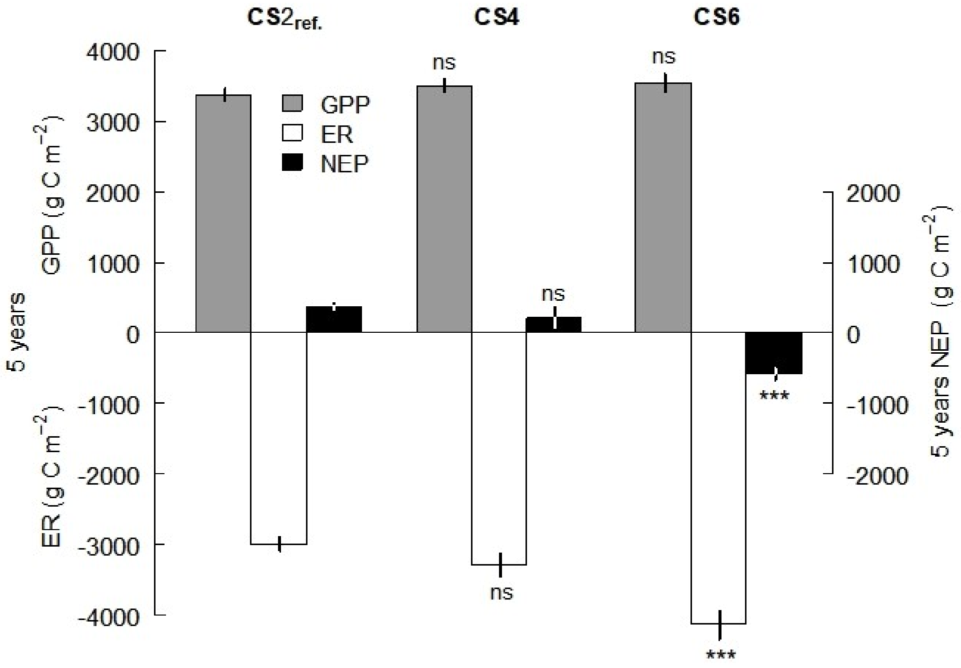

Figure 4. C flux balance of five year totals of gross primary productivity (GPP), ecosystem respiration (ER), and net ecosystem productivity (NEP) at three climate scenario sites. Displayed are means ± 1 SE of the control treatment that received neither irrigation nor additional N. Significance tests are against $CS2_{reference}$ within each C flux category; moreover, all three means to NEP were significantly different from zero ($P < 0.05$).
*** $P < 0.001$. ^{ns} $P > 0.1$

3.4 Cumulative shoot C harvested and leachate C lost

Cumulative shoot C harvested over the five experimental years and cumulative losses of leachate C were small relative to the cumulative ER losses: cumulative shoot C was about one tenth of ER, and leachate C less than one hundredth (Table 3b). Cumulative shoot C harvested at CS4 and CS6 was each not significantly different from that at $CS2_{reference}$ ($P > 0.5$ each), and the same held true for cumulative losses of leachate C ($P > 0.5$ for CS4 and CS6).

3.5 C stock changes matched cumulated C fluxes in net ecosystem C balance

The net ecosystem C balance largely agreed between the two approaches (Table 3). Compared to $CS2_{reference}$, the C stock method assigned a -473 g C m^{-2} balance to the CS4 site ($t_{10} = 0.57$, $P = 0.581$) and a -1034 g C m^{-2} balance to the CS6 site ($t = 1.70$, $P = 0.12$). In comparison, the C flux based method revealed a -120 g C m^{-2} balance to CS4 site ($t_{10} = 0.65$, $P = 0.530$) and a -936 g C m^{-2} balance to CS6 site ($t = 6.81$, $P < 0.001$). Taken together, while some, but not significant, C loss was associated with a seasonal warming of +1.5° C, both approaches demonstrated a massive C loss with a seasonal warming of +3.0° C.

(a) C stock based climate scenario effect (warming) on ecosystem C balance

	CS2$_{reference}$		**CS4 (+1.5°C)**				**CS6 (+3.0°C)**			
	C stock (g C m^{-2})		C stock (g C m^{-2})				C stock (g C m^{-2})			
	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
SOC 2017	6375	247.2	5944	711.9			5482	405.1		
Root C 2017	891	132.9	822	138.0			712	193.7		
Shoot C 2017	226	25.5	253	33.3			264	25.5		
Total	7492	366.1	7019	746.2			6458	487.3		
Climate scenario effect (Difference to CS2$_{reference}$)					**-473**	**831.1^{ns}**			**-1034**	**609.5°**

(b) C flux based climate scenario effect (warming) on ecosystem C balance

	CS2$_{reference}$				**CS4 (+1.5°C)**				**CS6 (+3.0°C)**			
	C gain (g C m^{-2})		C loss (g C m^{-2})		C gain (g C m^{-2})		C loss (g C m^{-2})		C gain (g C m^{-2})		C loss (g C m^{-2})	
	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
GPP 5a cum.	3358	81.8			3493	79.7			3536	121.1		
ER 5a cum.			-2986	97.1			-3280	159.1			-4122	193.8
Shoot C harvested 5a cum.			-420	59.1			-383	57.5			-398	32.3
Leachate C 5a cum.			-21	2.5			-19	2.7			-21	2.9
Total	3358	81.8	-3427	129.3	3493	79.7	-3682	185.5	3536	121.1	-4541	218.9
Balance			-69	79.4			-189	167.8			-1005	112.4
Climate scenario effect (Difference to CS2$_{reference}$)							**-120**	**185.7^{ns}**			**-936**	**137.7*** **

cum.: cumulative. *** $P < 0.001$. ° $P = 0.12$. ns $P > 0.2$.

Table 3. Net ecosystem C balance for CS2$_{reference}$, CS4 and CS6, alternatively based on C stocks (a) and C fluxes (b). Data for all stocks and fluxes are means ± 1 SE from the same six control monoliths per CS that received neither irrigation nor additional N.

4 Discussion

Physical and chemical soil properties limit the potential maximum size of the SOC stock. While belowground biomass turnover rate, root exudates and aboveground litter production rate determine the major C input rate, the C output rate is determined by decomposition of OC through soil microbiota. Both C-input and -output strongly depend on temperature and water availability. As a consequence of the altitudinal transplantation, the climatic conditions at the climate change CS sites were radically different compared to $CS2_{reference}$. Thus depending on the climate scenario, the parameters that drove C-input and -output have changed alongside and the grassland has either acted as a marginal C sink or a strong C source. Only during the long winter period the warming treatment was effectively suspended and climatic conditions under the snow cover were very similar. For a discussion of the importance of winter- vs. summer warming please compare Kreyling et al. (2019).

It is important to note, that our description of the C balance temperature response is not based on soil temperature, but based on air temperature change, because it is the reference to describe climate change effects on ecosystems. Also, under field conditions there is no single soil temperature, but an extremely dynamic, diurnal soil depth temperature gradient, that drives the CO_2 evolution from various organic matter fractions with different temperature sensitivities (Conant et al., 2011; Subke and Bahn, 2010).

4.1 The C stock of soil and plants

By integrating the C stock changes of a grassland ecosystem with intact C input pathways, our study avoids many of the shortfalls that impair the prediction of the fate of the terrestrial soil C sink, such as monitoring the temperature sensitivity of SOC decomposition in incubated soils (Crowther et al., 2015).

At the moderately warmer CS3 and CS4 and at the colder CS1, SOC stocks were not significantly different from $CS2_{reference}$ that was used as the reference site. The quite substantial, yet not significant SOC stock gain at CS3 may suggest the chance for a net soil C sink at increased seasonal mean temperatures up to 0.7°C. This hypothesis is supported by the increased root C stock found there ($P = 0.021$, Fig. 2a). We suggest that mitigation of the thermal growth limitation has increased plant productivity (Volk et al., 2021) and created a larger potential for plant OC input. Assuming that the root turnover rate is not reduced, this means that the input of OC to the soil has increased.

At extreme warming climate scenarios, the dynamics of root OC stock were strikingly similar to SOC stock change, and both were substantially reduced at CS5 and CS6 (compare Figs. 1 & 2). This indicates that under these climatic conditions a reduced supply of organic material from belowground plant fractions is one likely reason for the shrinking SOC stock at CS5 and CS6. Importantly though, because SOC derives from dead plant material, OC supply to the soil does not depend directly on the plant standing C stock, but on the turnover rate of this C stock. We suggest that in our study the allocation pattern at the control site $CS2_{reference}$ is representative for the high R/S ratio commonly found at high altitudes (e.g. Leifeld et al., 2013). It is thus likely that the reduced belowground biomass at the warmer CS reflects an increased turnover rate of belowground plant material and the associated C stock and a transition towards a new functional root/shoot equilibrium (Poorter et al., 2012) with a lower R/S ratio, typical for low altitudes. Although there can be compensatory root growth and C storage in the subsoil below 10 cm depth (Jia et al., 2019), we assume that in our grassland there is only a small compensation potential for topsoil SOC stock losses in these depths, because the soils are only ca. 20 cm deep in total. In agreement, a similar response pattern, but much larger in scale, was reported from a large, natural geothermal warming grassland experiment in Iceland (Poeplau et al., 2017): 0.7°C warming increased topsoil SOC by 22%, while further warming led to dramatic SOC stock decreases.

The N resource is of great importance for plant productivity and microbial decomposition of SOC. For example in a similar subalpine grassland (Alp Flix Experiment), a 10 and 50 kg N ha^{-1} a^{-1} deposition treatment led to a 9% and 31% increase in plant productivity, respectively (Volk et al., 2011). In the same experiment, there was a strong N-related increase in SOC stock at low deposition rates up to 10 kg N ha^{-1} a^{-1} and smaller increases at high deposition rates up to 50 kg N ha^{-1} a^{-1} (Volk et al., 2016). Yet, in the present study with a maximum deposition treatment of 15 kg N ha^{-1} a^{-1}, we observed neither a plant response (Volk et al. 2021) nor a SOC response (Table A1), suggesting no mitigation of a (presumed) N limitation of plant productivity or microbial activity. As a result, also the 15 kg N treatment appears to be below the critical load for a change of the SOC stock. Still, this conclusion needs to be viewed with caution because N effects on SOC stock could change over longer time-scales. For example, in the Alp Flix Experiment it was shown that, after seven years of exposure, most of the added N was taken up by plants and did not reach soil N pools (Bassin et al., 2015). This implies that N availability for soil microorganisms may not have changed with our 15 kg N ha^{-1} a^{-1} treatment after five years, but may do so after a longer lag phase.

Water availability is an essential factor for the ecosystem response to warming (compare below), but the irrigation treatment in our experiment yielded no effect. We assume that the applied amount was insufficient to make a difference, in particular at the warmer CSs, because we deem it likely that water was a limiting factor there. For details on water availability at the climate scenarios and the effect of irrigation on aboveground plant productivity please refer to ~~(~~Volk et al.~~,~~ (2021; Table 2). Thus, results from the current experiment must leave it open whether mitigation of water shortage due to warming would change SOC stocks.

Warming, nitrogen and water must also be expected to affect plant species composition, which in turn may affect ecosystem C fluxes. In a very similar environment Bassin et al. (2009) studied eleven key plant species of a subalpine pasture and found only very small responses of growth to N deposition, except for the cyperaceous *Carex sempervirens*. Within the experiment described here, Wüst-Galley et al. (2020) predicted an increased grass cover at the expense of forbs and legumes with rising temperatures and N deposition, while they found increased sedge cover with cooler temperatures and N deposition. In consequence, changes in plant species composition in response to the applied climate change scenarios can be assumed, but attempting to predict effects on the ecosystem C stock would be highly speculative.

4.2 CO_2 fluxes (GPP, ER, NEP)

Lacking other pathways of OC input, such as manure applications for fertilization, the single source for all OC contained in our grassland ecosystem are photosynthetic assimilates (GPP). Despite a positive effect of warming on aboveground plant productivity (Volk et al., 2021), the five years GPP flux – quantifying the total amount of assimilated C – was not significantly different between climate scenario treatments $CS2_{reference}$ and each of CS4 and CS6 (Fig. 4). This result is in well agreement with a meta-analysis of C flux of 70 grassland sites (Wang et al., 2019).

The annual mean ER observed at CS4 was very similar (656 g C m^{-2}) compared to the soil respiration of 729 g C m^{-2} that Bahn et al. (2008) reported from a grassland site that had the same altitude. However, ER at CS6 developed quite differently: the ecosystem respiration metabolized 1136 g C m^{-2} more in five years compared to

$CS2_{reference}$ (Tab. 3B). Since soil respiration at in situ measurements is mostly driven by young OM (≥ 90%; Giardina et al., 2004), we assume that except for autotrophic respiration mostly all of the substrate for the ER observed here originated from the topsoil. For a small part, the substrate for a higher ER at higher temperatures must also derive from decaying belowground plant material that became obsolete with the new, temperature adjusted allocation patterns. Assuming a similar biomass turnover rate at the different CSs and lacking other sources, we argue that only previously protected SOC may have supplied the remaining substrate for the C loss via ER.

The asymmetric response of GPP and ER to warming in our experiment resulted in a substantially negative CO_2 balance, i.e. a negative NEP. By contrast, GPP and ER responded equivalently to warming in a mixed-grass prairie (C_3 forbs and C_4 grasses), yielding no change in NEP (Xu et al., 2016). Further, in the Alp Flix Experiment on subalpine grassland Volk et al. (2016) reported that the lowest NEPs were found in warm and dry years, while NEP was highest in a cool and moist year. Also the warming of a tallgrass prairie suggested ecosystem C losses in dry years, but C gains in wet years (Jung et al., 2019; but see also Reynolds et al. (2014) for a situation when warming and drought lead to reduced ER). Analogously, in experiments containing an elevated CO_2 fumigation treatment that led to water saving effects, warming stimulated ER only under elevated CO_2 (Ryan et al., 2015). We thus conclude that the wide range of possible NEP responses to warming depends on the warming benefit vs. water limitation trade-off when the temperature is rising.

4.3 Consistency of C stock changes vs. cumulated C fluxes

Because the C balance for $CS2_{reference}$ represents the situation without a climate scenario effect, comparison with the C balance at CS4 and CS6 reflects the effect of five years of climate scenario treatment, alternatively based on the 2012-2017 C stocks changes (Table 3a) and on the five year cumulated C fluxes (Table 3b). All three CS were evenly affected by potential management- or inter-annual weather-effects, so that the climate scenario effect alone is estimated. Theory demands that the climate scenario effect, calculated from SOC plus plant C stock, must match the respective effect based on C flux balances, given that all relevant pathways of C input and output were successfully covered. Our data impressively demonstrate such a congruence (Tab. 3). In absolute terms the ecosystem five year C loss due to the climate effect was ca. 1 kg C m^{-2} at CS6 (+3°C) in agreement of both methods. This means, that 14% of the previously stored greenhouse gas CO_2 has now been returned to the atmosphere.

In contrast, other mountain grassland studies that present annual C balances often report C sinks. These studies mostly use Eddy Covariance measurements and have no multi-level treatments or replications that would allow to test a mechanistic hypothesis against the ecosystem response or assign a between subject error to the reported fluxes.

For example, a recent analysis of ~~14 managed grassland sites reports~~ net GHG balances (including N_2O and CH_4) at 14 managed grassland sites~~, that~~ resulted in C sinks between 70 and 4671 g CO_2–eq. m^{-2} $year^{-1}$. Fluxes of non-gaseous C~~,~~ like manure or harvested biomass~~, are~~were, however, not ~~reported~~provided (Hörtnagel et al., 2018). Rogger et al. (2022) reported a mean net biome production (NBP) gain ~~(including e.g. harvest and fertilization related C fluxes)~~ of 154 (± 80) g C m^{-2} $year^{-1}$ ~~the,~~ from a 15 -year data set on a site at 1000 m a.s.l. ~~site~~ in Switzerland (including e.g. harvest- and fertilization-related C fluxes), which~~. This~~ represents a C sequestration of ca. 2.3 kg m^{-2} during the entire experiment. Also ~~from~~in the European GREENGRASS network (9 sites, including very intensively managed grassland), ~~Soussana et al. (2007) the authors report that on average~~

the annual C storage NBP in the grassland plots was on average 104 (±73) g C m^{-2} $year^{-1}$ (Soussana et al., 2007). Finally, i~~I~~n a~~n exceptionally~~ comprehensive 10 -year comparison ~~, Ammann et al. (2022) report from~~between an extensive ~~grassland~~ (ecosystem was a C source) and an intensive grassland (ecosystem was a C sink), ~~that~~ effects of management and weather on annual NBP were of similar magnitude (Ammann et al., 2022). Yet, in n~~N~~one of these ~~reports~~studies ~~above is accompanied by~~ SOC stocks were measured~~ments~~.

~~Only~~In comparison, in an overview of the all-European CARBOMONT project Berninger et al. (2015) ~~don't~~did not identify substantial C sink properties, but find that '~~E~~especially~~,~~ the natural mountain grasslands in our study were quite close to carbon neutrality'. ~~By comparison~~In our five year experiment, the equivalent value based on C flux measurements at ~~our~~ $CS2_{reference}$ indicated a mean C source of 14 g C m^{-2} $year^{-1}$, essentially undistinguishable from zero (Table 3~~ (b)~~).

Taken together, w~~W~~e suspect that substantial C sequestration situations cannot be considered typical in permanent mountain grasslands, but that a deviation from a zero balance indicates either a weather driven year-~~-~~to-~~-~~year variability~~,~~ or an unaccounted agricultural management effect. This implies~~,~~ that ~~often~~ annual C budgets often represent a spotlight on a highly dynamic transition phase of the ecosystem OC stock.

Short-term grassland warming studies like our experiment must be regarded with caution when used to make long-term predictions, but analyses from the Icelandic ForHot experiment rated the parameter 'SOC stock' to be a stable and consequently a useful predictor for the future state of the ecosystem already after 5-8 years of warming treatment (Walker et al., 2020). Because temperature sensitivity does not increase with soil depth (Pries et al., 2017) or varying recalcitrance of organic matter (Conen et al., 2006), topsoil temperature responses are representative also for subsoil responses. Thus, we assume that we missed no pathway of additional C input to supply the substrate consumed by increased ER and present a valid balance here.

Consequently, with respect to stocks and fluxes, we expect three alternative developments under sustained warming:

A) The remaining SOC stock is sufficiently protected to resist further decomposition at high rates and ER will soon decrease.

B) Despite a very recalcitrant remaining SOC stock, the positive biomass response at intermediate climate scenarios not covered in this three level comparison may supply sufficient new, labile OC from plants and ER may remain high, with no further decline of the SOC stock.

C) The more active microbial community succeeds in accessing even more of the previously protected SOC stock for decomposition and ER will remain high, leading to a further decline of the SOC stock.

5 Conclusion

The small change in the SOC stock at the $CS2_{reference}$ site after five years supports our initial assumption that the grassland was in (or close to) a steady state situation. The warming climate scenario treatments led to up to 14% reduced C stocks of the grassland ecosystem in five years, with a critical level between 1.5 and 3.0° C seasonal warming. Independent ecosystem C flux measurements confirmed this result and showed that there was no equivalent productivity increase to compensate for the strongly increased ER, itself an indicator of accelerated decomposition. In the view of resource limitation, we suggest that the dramatic C loss of the grassland is a transient effect before a new, climate-adjusted steady state is reached.

Author contribution

MV and SB designed the experiment, MV, ALW and SB conducted field work. MV and MS analyzed the data. MV led the writing of the manuscript, with significant contribution from MS. All authors contributed critically to the drafts and gave final approval for publication.

Data availability

The data analyzed for the current study will be made available at the CERN Zenodo data repository https://doi.org/ … /zenodo. …

Competing interests

The authors declare that they have no conflict of interest

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Appendix A

Supplementary information on the gas exchange measurement and parameterization

A 2.1 CO_2 flux sign convention

Throughout this study we adopt an ecosystem perspective when stating gas fluxes. This implies that gross primary productivity (GPP) has a positive value, while ecosystem respiration (ER) has a negative value. Net ecosystem exchange (NEE) is positive when GPP > ER. Analogously, net ecosystem productivity (NEP) for a given time is positive, if the ecosystem is accumulating C.

A 2.2 NEE

On 7 – 12 days per year, on snow free days between April and December, NEE was measured for five years. NEE_{day} measurements were done in full sunlight, between ca. 2 h before and after solar noon (clear sky, midday conditions). NEE_{night} measurements were started no earlier than 1 h after sunset.

To measure NEE, we used a dynamic CO_2 concentration, non flow-through cuvette made of transparent polyacrylics (30 × 40 × 35 cm). An infrared CO_2 probe (GMP343 diffusion model, Vaisala, Vantaa, Finland) connected to a handheld control and logger unit (MI70 Indicator, Vaisala) was mounted inside the cuvette to directly measure the chamber [CO_2]. A small fan created moderate turbulence inside the cuvette (0.5–0.8 m s^{-1}) to facilitate air mixing. During the measurement, the cuvette was tightly sealed to the rim of the box containing the monolith using a cell foam band.

After placement of the chamber we waited a few moments for a continuous CO_2 concentration trend to develop, then data recording was started. CO_2 concentration changes were measured at 5 s intervals during a 2 min measurement period per monolith. The first 10 s of data were omitted in subsequent measurements to allow for initial adjustment of chamber [CO_2]. The quality of the measurement was considered acceptable if a linear regression of [CO_2] vs. time during the following 110 s yielded R^2 of 0.95 or better, indicating strictly linear changes in chamber [CO_2]. The short measurement period was chosen to minimize changes in environmental conditions inside the chamber and avoid fogging of the cuvette at high evapotranspiration rates. CO_2 concentration did not drop below 340 ppm or rise above 500 ppm. For each flux measurement, soil temperature of the respective monolith was recorded at 8 cm depth, using a handheld electric thermometer.

A 2.3 Ecosystem respiration (ER)

Measured NEE_{night} was considered to represent ER for the entire day:

$$\mathrm{ER} = \mathrm{NEE_{night}}$$

For the days between measurements (bi-weekly to monthly during the snow-free period), ER was parameterized. First, ER for each monolith was normalized for temperature (10 °C at 8 cm soil depth) using the exponential function for NEE_{night}/soil temperature established earlier. Second, on the basis of the ER gained during measurement nights, a normalized daily ER between measurement nights was linearly interpolated. These normalized values integrate the effects of seasonal changes of substrate availability, heterotrophic and autotrophic biomass, and soil moisture availability. Ultimately, ER on hourly basis was calculated using normalized ER values for the respective day and hourly soil temperature values.

A 2.4 GPP

NEE_{day} data were used to estimate GPP according to:

$$GPP = NEE_{day} - ER$$

GPP estimates from mid-day, clear sky NEE_{day} measurements reflect a situation without radiation limitation for assimilation. Therefore, GPP estimates reflect potential GPP at maximum radiation (GPP_{pot}) at seasonal solar altitude. At the beginning of the season GPP_{pot} was interpolated to rise exponentially between snow-melt and the first measurement of the season. Between the measurement days, GPP_{pot} was linearly interpolated for every day. This way, effects of canopy development and soil moisture availability are reflected in the model.

A 2.5 GPP light response

From clear sky NEE_{day} measurements at fully developed canopy stages, we parameterized light response curves of GPP. Between photosynthetic compensation points in the morning and evening, NEE data were collected at a frequency of 50 min or higher. Maximum GPP was observed at GR of ≥ 900 W m^{-2}. No significant differences between treatments were found, and light use efficiency α was subsequently derived from data pooled across treatments. Light response was described by a nonlinear, least squares fit of flux data to a rectangular hyperbolic light response model (Michaelis–Menten model):

$$GPP = \frac{\alpha \times \beta \times GR}{\alpha \times GR + \beta}$$

where GPP is in µmol CO_2 m^{-2} s^{-1}, α is the initial slope of the light response curve (the light use efficiency factor in µmol CO_2 J^{-1}), β is the asymptote of GPP_{pot}, and GR is in W m^{-2}. We calculated actual GPP for each hour based on interpolated GPP_{pot} for every day of the growing season, together with hourly means of GR and previously established light use efficiency α:

$$GPP = \frac{\alpha \times GR}{1 - \frac{GR}{900\ W\ m^{-2}} + \frac{\alpha \times GR}{GPP_{pot}}}$$

A 2.6 NEP

NEP was used in the sense of describing the balance between GPP and ER, equivalent of an hourly, daily, or annual CO_2 balance for the ecosystem, neglecting other potential C imports or exports. Hourly ER flux rates were used to calculate hourly sums of C loss. Hourly GPP flux rates were used to calculate hourly sums of C gain for each monolith. NEP was then derived by subtracting hourly losses of respired carbon (ER) from hourly C gains (GPP) for a given period for each individual monolith:

$$NEP = GPP - ER$$

Appendix B~~A~~

Table A1 Summary of analyses for the effects of climate scenario (CS), irrigation, and N deposition on *SOC stock change* of subalpine grassland between 2012 and 2017. *F* tests refer to the fixed effects of a linear mixed-effects model; the marginal and conditional R^2 values were 0.19 and 0.33, respectively. The random block variance was estimated to be zero and was therefore removed from the model.

Variable	df_{num}	df_{den}	*F* value	*P*
Climate scenario (CS)	5	173.0	7.1	< 0.001
Irrigation	1	173.0	< 0.1	0.886
N	2	173.0	< 0.1	0.978
CS × Irrigation	5	173.0	1.3	0.276
CS × N	10	173.0	0.5	0.881
Irrigation × N	2	173.0	0.7	0.522
CS × Irrigation × N	10	173.0	1.1	0.382

df_{num}: degrees of freedom of term; df_{den}: degrees of freedom of error

Table A2 Summary of analyses for the effects of climate scenario (CS), irrigation, and N deposition on *root carbon stock* of subalpine grassland in 2017, after five years of experimental treatment. *F* tests refer to the fixed effects of a linear mixed-effects model; the marginal and conditional R^2 values were 0.47 and 0.63, respectively. No data were available for CS1 and the intermediate N-deposition treatment N3.

Variable	df_{num}	df_{den}	*F* value	*P*
Climate scenario (CS)	4	22.9	30.6	< 0.001
Irrigation	1	69.9	0.4	0.522
N	1	69.9	< 0.1	0.862
CS × Irrigation	4	69.9	1.1	0.371
CS × N	4	69.9	3.5	0.011
Irrigation × N	1	69.9	1.0	0.330
CS × Irrigation × N	4	70.0	0.6	0.637

df_{num}: degrees of freedom of term; df_{den}: degrees of freedom of error

Table A3 Summary of analyses for the effects of climate scenario (CS), irrigation, and N deposition on *shoot carbon stock* of subalpine grassland in 2017, after five years of experimental treatment. *F* tests refer to the fixed effects of a linear mixed-effects model; the marginal and conditional R^2 values were 0.16 and 0.32, respectively. No data were available for CS1 and the intermediate N-deposition treatment N3.

Variable	df_{num}	df_{den}	*F* value	*P*
Climate scenario (CS)	4	24.8	1.1	0.365
Irrigation	1	70.7	2.6	0.108
N	1	70.7	0.7	0.397
CS × Irrigation	4	70.7	0.2	0.948
CS × N	4	70.7	2.4	0.056
Irrigation × N	1	70.7	3.1	0.085
CS × Irrigation × N	4	70.7	0.5	0.725

df_{num}: degrees of freedom of term; df_{den}: degrees of freedom of error

A general note to the generalized additive models: In all models, the default from the mgcv package has been used with the exception that the 'gamma' statement of the gam() function was sometimes changed to adapt the degree of smoothing of the fitted line. This, however, did not or only marginally influence the inference drawn from the model, i.e. the *P* values for smooth terms reported in Tables A4 – A6.

Table A4 Summary of analyses for the effects of temperature change (Delta Temperature) induced by the climate change treatments on *soil carbon stock change* (Delta C soil) of subalpine grassland between 2012 and 2017. *F*- and *t* values and approximate *P* values refer to a generalized additive model that used a smooth term to delta temperature.

	All monoliths			Control monoliths		
Parametric term	df	*t* value	*P*	df	*t* value	*P*
Intercept	1	0.9	0.386	1	0.7	0.486
Smooth term	edf	*F* value	*P*	edf	*F* value	*P*
s (Delta Temperature)	2.80	5.1	0.001	2.11	1.3	0.281

df: degrees of freedom; edf: effective degrees of freedom (which can be fractional in smooth terms of generalized additive models). *s*: smoothing function applied to term.

Table A5 Summary of analyses for the effects of temperature change (Delta Temperature) induced by the climate change treatments *root carbon stock* (Root C) at 2017 after five years of experimental treatment. *F*- and *t* values and approximate *P* values refer to a generalized additive model that used a smooth term to delta temperature.

	All monoliths			Control monoliths		
Parametric term	df	*t* value	*P*	df	*t* value	*P*
Intercept	1	175.6	< 0.001	1	80.5	< 0.001
Smooth term	edf	*F* value	*P*	edf	*F* value	*P*
s (Delta Temperature)	2.88	17.0	< 0.001	2.56	2.0	0.125

s: smoothing function applied to term.

Table A6 Summary of analyses for the effects of temperature change (Delta Temperature) induced by the climate change treatments *shoot carbon stock* (Shoot C) at 2017 after five years of experimental treatment. *F*- and *t* values and approximate *P* values refer to a generalized additive model that used a smooth term to delta temperature.

	All monoliths			Control monoliths		
Parametric term	df	*t* value	*P*	df	*t* value	*P*
Intercept	1	190.9	< 0.001	1	105.3	< 0.001
Smooth term	edf	*F* value	*P*	edf	*F* value	*P*
s (Delta Temperature)	2.58	0.9	0.415	2.46	1.0	0.432

s: smoothing function applied to term.

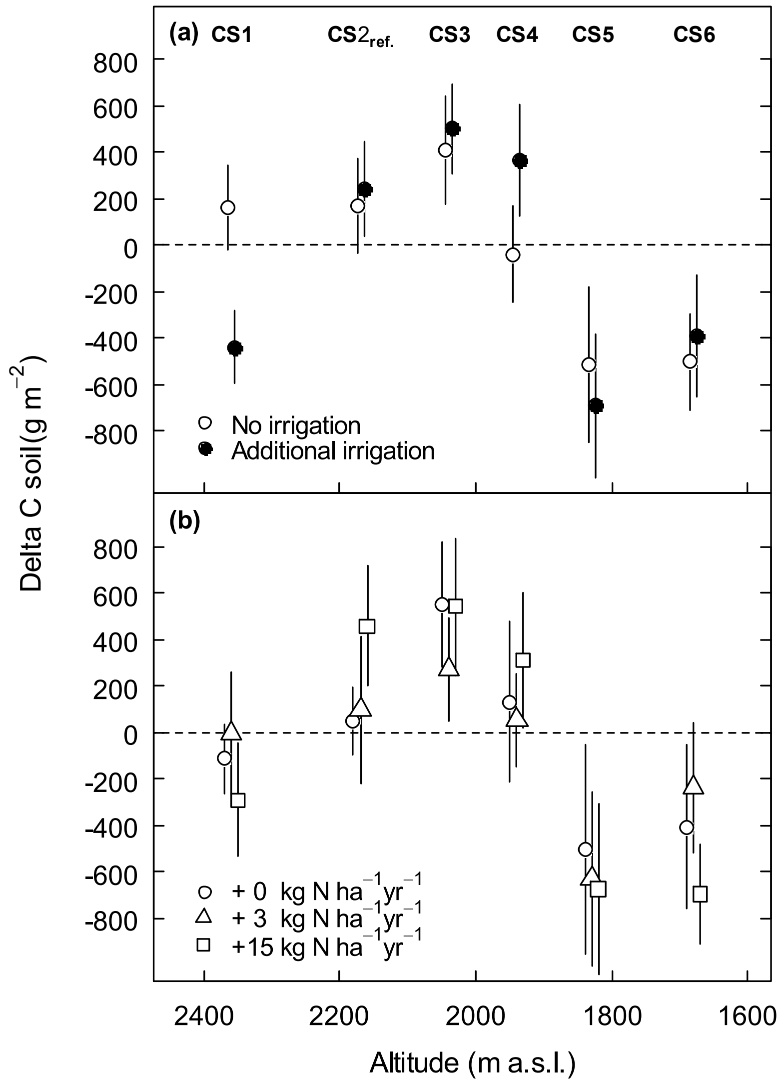

Figure A1 Soil carbon stock change (Delta C) of subalpine grassland between 2012 and 2017 as a function of the altitude of climate scenario sites (CSs) and a) the irrigation treatment, and b) the N deposition treatment (0, 3, 15 kg N ha^{-1} yr^{-1}, in addition to 4-5 kg N background deposition). Data denote means ± 1 SE, shifted horizontally to improve their visibility.

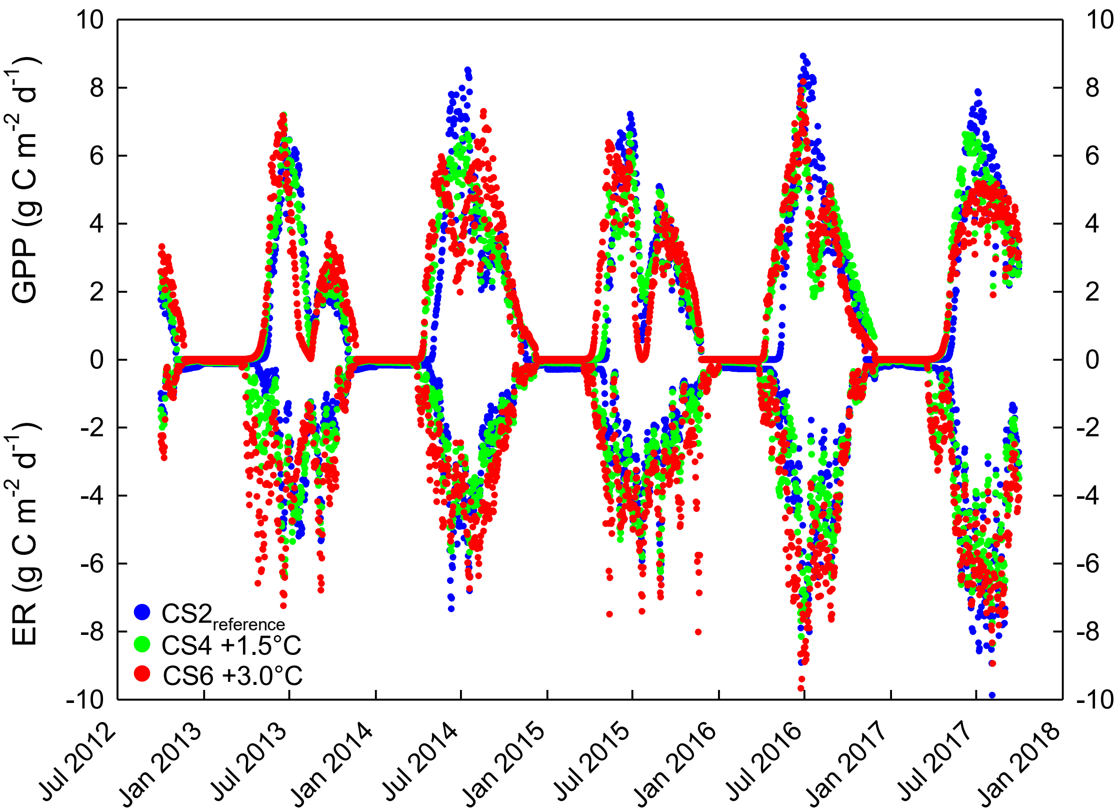

Figure A2 Daily flux sums (mean) of CO_2 gross primary productivity (GPP) and ecosystem respiration (ER). Colored dots indicate means from six control treatment monoliths (neither irrigation nor additional N) per $CS2_{reference}$ (blue), CS4 (green) and CS6 (red), respectively. The ecosystem perspective, rather than the atmosphere perspective was assumed, resulting in negative ER values (C loss) and positive GPP values (C gain).