

# ***The consequences of elevated CO<sub>2</sub> and land use in alpine ecosystems***

**Inauguraldissertation**

zur

Erlangung der Würde eines Doktors der Philosophie

vorgelegt der

Philosophisch-Naturwissenschaftlichen Fakultät

der Universität Basel

von

***Nicole Inauen***

aus

Appenzell

AI

St.Gallen, 2014

Genehmigt von der  
Philosophisch-Naturwissenschaftlichen Fakultät  
auf Antrag von

Prof. Dr. Christian Körner  
Prof. Dr. Ulrike Tappeiner

Basel, den 27. März 2012

Prof. Dr. Martin Spiess  
Dekan

## *Acknowledgements*

First of all, I would like to thank Christian Körner and Erika Hiltbrunner, who gave me the opportunity to work in these interesting projects and to spend such a great time in the superb alpine landscape at the ALP-FOR research and education station near the Furka Pass. I really enjoyed the time up there. I am also very thankful for your guidance, your inspiring ideas and constructive inputs and for the very helpful proofreading.

I am grateful to Ulrike Tappeiner for agreeing to co-examine this thesis.

Special thanks go to the team at the ALP-FOR research station who kindly helped me during fieldwork (although it was sometimes really exhausting, weighing these lysimeters several times a day, and sometimes very tedious, sorting endless biomass samples for weeks): Thijs van den Bergh, Tobias Bühlmann, Kristina Ehrensam, Gérard Gisler, Thomas Gross, Franziska Hochuli, Erika Hiltbrunner, Andreas Huder, David Preiswerk, Linda Reißig, Daniel Scherrer, Marc Studer, Oliver Wirz and Tobias Zehnder. I further thank those people, who helped to analyse and weigh samples in Basel, Olivier Bignucolo, Veronika Preite, Susanna Riedl, Gabrielle Schär, Martin Bader for his help with the gas exchange system LI-6400, Franco Miglietta for his assistance with the MiniFACE system, Matthias

Saurer and Rolf Siegwolf for their advice and for the analyses of stable isotopes.

I really appreciated the cooperation with my project partners of the Bio-CATCH network, and I am especially grateful to Nikolaus Obojes who answered countless e-mails with questions during the last weeks.

Further thanks go to my office mates and all people at the Institute of Botany for helpful discussions and for contributing to the pleasant working ambiance.

Special thanks go to my best friends Andrea, Elina, Julia and my dear colleague Riccarda for their highly valuable encouragement and motivating lunchtime discussions.

I am deeply thankful to my family for their great support and motivation and especially to Timon for his patience, for listening to my worries and for his precious assistance with so many things, amongst others with the layout of this thesis.

This project received funding by FOAG (the Swiss Federal Office for Agriculture, project Bio-CATCH, Urs Gantner).





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## General Introduction

This PhD thesis covers two major aspects of ‘Global Change’ and their impacts on alpine ecosystems. The first topic is the steadily increasing concentration of carbon dioxide (CO<sub>2</sub>) in the atmosphere and its direct biological effects on plant growth and water consumption of alpine vegetation. The second topic addresses the consequences of land use and its continuing decline across the Alps on the water balance of alpine grassland and finally, on water yield of alpine catchments.

### *Elevated atmospheric CO<sub>2</sub> in alpine ecosystems*

Atmospheric CO<sub>2</sub> concentration has reached levels that have been unprecedented for plant life for at least 20 million years (Petit *et al.* 1999; Pearson and Palmer 2000; Siegenthaler *et al.* 2005). By the end of this century, global atmospheric CO<sub>2</sub> concentration is likely to have more than doubled compared to pre-industrial concentrations, thus reaching levels of 730 to 1020 ppm (Meehl *et al.* 2007). Photosynthesis, especially of C<sub>3</sub> plants, is not saturated at the current CO<sub>2</sub> concentration of around 392 ppm (Mauna Loa Observatory, Hawaii, US, 2012). Rising atmospheric CO<sub>2</sub> may thus directly stimulate plant growth via increased photosynthetic rates or, especially in water-limited ecosystems, by plant water savings due to CO<sub>2</sub>-induced reductions of the aperture of leaf pores (stomata). Numerous CO<sub>2</sub> enrichment experiments have investigated these direct biological effects of higher concentrations of atmospheric CO<sub>2</sub> on plant growth and productivity in natural and agricultural ecosystems (reviews by Kimball *et al.* 2002; Nowak *et al.* 2004; Ainsworth and Long 2005; Körner 2006), though relatively few experiments were conducted at high elevation. High-elevation plants were expected to exhibit particularly strong responses to rising CO<sub>2</sub> levels, as they grow at lower CO<sub>2</sub> partial pressure, which is associated with the reduction in atmospheric pressure by roughly 10% per km of altitude in the atmosphere. Alpine plants have been shown to adjust to the external (physico-chemical) changes associated with reduced atmospheric pressure by enhanced photosynthetic efficiency of carbon capture compared to

congeneric lowland plant species (measured at equal partial pressure of CO<sub>2</sub>), thus, equalizing rates at local partial pressure of contrasting elevations (Körner & Diemer 1987, 1994). This greater CO<sub>2</sub> use efficiency in high-elevation plants has been confirmed globally by a steady reduction in <sup>13</sup>CO<sub>2</sub> discrimination with increasing elevation (Körner *et al.* 1991; Zhu *et al.* 2010). Hence, it remains unclear whether life conditions at high elevation make plants more susceptible to rising CO<sub>2</sub>, or whether the adjustments to life at high elevation neutralize such an increased responsiveness compared to plants growing at low elevation.

A first *in situ* attempt at exploring this question in high-elevation plants revealed no net biomass response to an atmosphere experimentally enriched in CO<sub>2</sub> over four seasons in the Swiss Alps, despite considerable photosynthetic stimulation and irrespective of nutrient addition (Körner *et al.* 1997). Strong competition, full exploitation of the rhizosphere and a specific set of unresponsive species were assumed to have prevented a growth stimulation by elevated CO<sub>2</sub>. Another high-elevation CO<sub>2</sub> enrichment experiment applying free-air CO<sub>2</sub> enrichment (FACE) to alpine vegetation within the alpine treeline ecotone at Stillberg in the Swiss Alps (2180 m a.s.l.) ended up with one CO<sub>2</sub> responsive and two unresponsive dwarf shrub species of the late-successional dwarf shrub understorey (Dawes *et al.* 2011a). The treeline forming evergreen conifer *Pinus unicata* also showed no response to nine years of experimental application of elevated CO<sub>2</sub> at any time, while the similarly sparsely grown 30-year-old *Larix decidua* showed a cumulative increase in canopy size, stem basal area and total new shoot production under elevated CO<sub>2</sub>, although, the annual responses in tree ring increments diminished significantly in the last two treatment years (Dawes *et al.* 2011b). In both of these high-elevation tree species, photosynthetic stimulation was substantial and only slight downward adjustments were observed (Handa *et al.* 2005; Dawes *et al.* 2011b). These and other studies at low elevation have revealed that the stimulation of plant growth by elevated CO<sub>2</sub> is

limited by the availability of resources other than carbon in many natural ecosystems (Körner 2006; Reich *et al.* 2006). Late successional communities with a though nutrient cycle, intimately coupled to the carbon cycle, and slow growing dominant species may not be carbon limited at current atmospheric CO<sub>2</sub> concentration. In contrast, glacier forefield communities with pioneer species can be categorized as expanding systems, in which available resources per unit land area (light, water, nutrients and also space) have not been fully explored, i.e. plant communities have not yet reached a steady state regarding substrate or air space exploration (fine root turnover, leaf area index). Expanding systems permit plants to grow with little competition, which makes commonly limiting resources periodically highly available to the individual, suggesting an increased responsiveness of such systems to elevated CO<sub>2</sub>. **Chapter 2** offers a three-season assessment of the CO<sub>2</sub> responsiveness of glacier forefield species in the Swiss central Alps.

As a second, direct biological effect, elevated atmospheric CO<sub>2</sub> has been shown to affect the water consumption of vegetation by a reduction in stomatal opening and hence, lower leaf-level transpiration (e.g. Ainsworth and Rogers 2007). These water savings lead to temporally increased soil water content (Morgan *et al.* 2004) and may translate into greater total runoff whenever rainfall exceeds soil water holding capacity. The leaf-level effects are commonly more pronounced and get diminished at ecosystem-scale for reasons associated with aerodynamic coupling, rainfall patterns, soil water storage capacities or plant growth responses to elevated CO<sub>2</sub> (Leuzinger and Körner 2010) and thus, are hard to predict.

#### *Land use and the water balance of alpine grassland*

Large areas of mountainous high elevation terrain are agriculturally used worldwide. In the Alps, different types of agricultural land use, such as hay making or grazing, both often combined with manuring, have lead to specialised, characteristic alpine grassland communities, which are often highly diverse in plant species and exhibit beneficial features such as erosion control on steep slopes (Tasser *et al.* 2003; Fischer *et al.* 2008; Rudmann-Maurer *et al.* 2008). These

highly adapted mountain grasslands and traditional alpine landscapes are in danger of being lost due to the current trend of land use change across the European Alps. Economic and social changes have lead to the abandonment of 33% of all farms across the Alps between 1980 and 2000 only (Streifeneder *et al.* 2007). This structural economic change has been more severe in southern (France –47%, Italy –44%, Slovenia –56%) than in the northern part of the Alps (Austria –9%, Germany –24%, Switzerland –34%). Thus, about 20% and in some areas as much as 70% of the agricultural land in the Alps has been abandoned, especially the poorly accessible and less profitable areas, such as pastures and meadows at high elevation (Tappeiner *et al.* 2003, Tasser *et al.* 2009). At the same time, agricultural land use has been intensified on more easily accessible and more productive parcels (Tasser and Tappeiner 2002).

Below the climatic treeline, most abandoned mountain grassland gradually develops into forest or shrubland. Above the climatic treeline, up to a few hundred meters above the tree limit, former alpine pastures and meadows are rapidly invaded by dwarf shrubs (Tasser and Tappeiner 2002). In the upper alpine belt, alpine swards dominated by sedges and grasses with more fibrous, nitrogen-poor, longer-lived leaves are forming the natural climax vegetation in the Alps (Lavorel and Grigulis 2012). Generally, plant height, litter accumulation and often the maximum standing biomass increase significantly with reduced agricultural management (Tappeiner and Cernusca 1989; Wohlfahrt *et al.* 2003). As transpiration and interception are usually higher in tall vegetation, these land cover changes affect evapotranspiration (ET) and the water balance by depleting soil water stores faster and thus, yielding less runoff.

In the European Alps, total annual runoff (the sum of all drainage processes) per unit area rises with elevation, resulting from a general elevation-related increase in precipitation and a marked decrease in annual evapotranspiration (ET) in mountain ecosystems, which is almost fully explained by the shorter snow-free period at high elevation (Körner 2003; Wieser *et al.* 2008). Runoff originating from alpine regions feeds into rivers and thus into lower elevation fresh water

supply and hydroelectric power production (Weingartner *et al.* 2007). The water yield of an alpine catchment and its hydroelectric potential should thus become reduced when land use on alpine pastures and meadows is reduced or even abandoned. These hydrological consequences of the abandonment of alpine agriculture await quantification, given the large extent of the current transitions in land use, which is the topic of the **chapters 3 and 4**.

#### *Content of this PhD thesis*

In **Chapters 2 and 3**, free air CO<sub>2</sub> enrichment (FACE) technology is employed, which provides the opportunity to study the effect of elevated CO<sub>2</sub> on plant growth and ecosystem processes *in situ* under close to natural conditions even in remote environments such as the Alpine.

**Chapter 2** reports the results of three years of CO<sub>2</sub> enrichment (580 ppm) in glacier forefield plants at the alpine research and education station Furka (ALPFOR) in the Swiss Alps (2440 m a.s.l.). Nine characteristic, perennial glacier forefield species were assembled in microcosms and grown at a nearby experimental site, with four study species receiving a low dose of full mineral fertilizer (equivalent to 25 kg N ha<sup>-1</sup> a<sup>-1</sup>) in order to explore a potential nutrient limitation of the CO<sub>2</sub> response. Responses of growth dynamics and peak season biomass of the two graminoid species, four forbs and three cushion forming species were analysed by repeated nondestructive assessments and a final biomass harvest. As explanatory variables we measured leaf carbohydrate and nitrogen concentrations in all species as well as photosynthetic rates in two graminoid species.

In **Chapter 3**, the partly opposing effects of declining land use and elevated CO<sub>2</sub> on the water balance of alpine grassland and on catchment value were studied in a field experiment at the same experimental site in the Swiss Alps. Rates of evapotranspiration, soil water contents and deep seepage of four alpine grassland types were measured using undisturbed monoliths in weighing lysimeters. We selected four grassland types

to cover a wide spectrum of vegetation structures (regarding vegetation height, density or the proportion of horizontal vs. vertical structures) and of plant biomass or leaf area index: a high-stature sward dominated by *Agrostis schradieriana*, and three sward types of rather low stature dominated either by *Nardus stricta*, *Carex curvula* or by forbs. Part of the monoliths was clipped to simulate sheep grazing during three seasons (2008 to 2010). Another set was exposed to elevated CO<sub>2</sub> (580 ppm) using FACE during the 2009 growing season.

**Chapter 4** provides a synthesis of the Bio-CATCH network, a tri-national team exploring eco-hydrological processes in alpine vegetation in three regions of the Alps. I was in charge of the synthesis on the effects of continued sustainable land use *versus* land abandonment on the water balance of alpine grassland. The three field sites in the western, central and eastern part of the Alps differed in climate, past and present land use and consequently, in the species composition of the grassland communities (near and above treeline). To resolve the water balance of different communities we adopted a standard protocol and employed a high number of stainless steel deep seepage collectors with undisturbed grassland monoliths. Different sward types were selected representing different land use practices or management intensities and a clipping treatment was applied to half of the monoliths in order to simulate mowing or grazing on three consecutive growing seasons.

**Chapter 2** is published in *Global Change Biology*. **Chapter 3** is published in the *Journal of Ecology*. **Chapter 4** is a synthesis of data of the Bio-CATCH consortium, which is still in progress and needs further consolidation before publication. Although data of different research teams was used for this synthesis, this chapter of the thesis was exclusively written by myself. **Chapter 5** summarizes the main findings and conclusions of the chapters 2 to 4.

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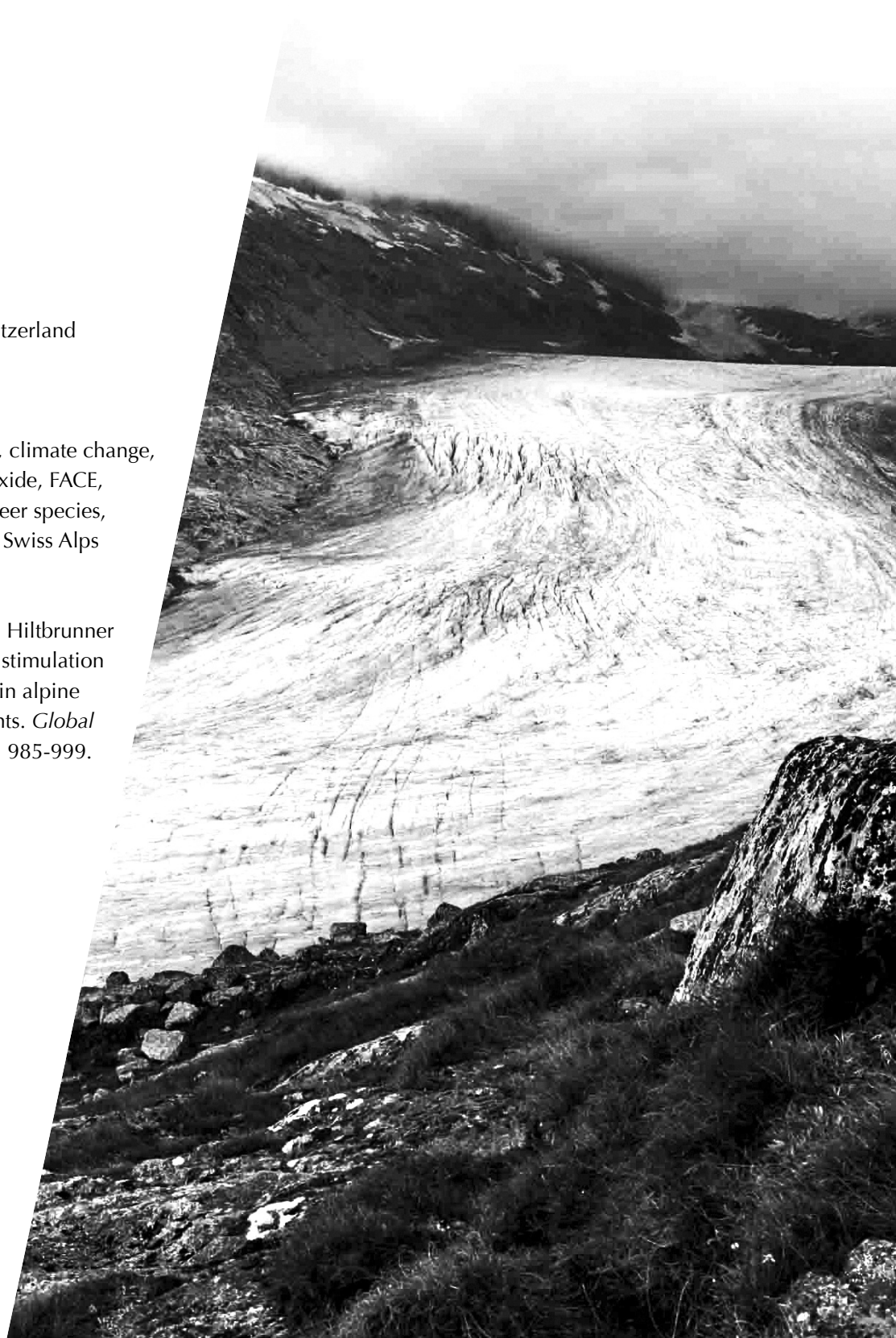
# *No growth stimulation by CO<sub>2</sub> enrichment in alpine glacier forefield plants*

Nicole Inauen  
Christian Körner  
Erika Hiltbrunner

Institute of Botany  
University of Basel  
Schönbeinstrasse 6  
CH-4056 Basel, Switzerland

Key words:  
biomass production, climate change,  
elevated carbon dioxide, FACE,  
high elevation, pioneer species,  
primary succession, Swiss Alps

Inauen N, Körner C, Hiltbrunner  
E (2012) No growth stimulation  
by CO<sub>2</sub> enrichment in alpine  
glacier forefield plants. *Global  
Change Biology*, **18**, 985-999.



### Abstract

Since 1850, glaciers in the European Alps have lost around 40% of their original area, releasing bare forefields, which are colonized by alpine pioneer species, setting the scene for later successional stages. These expanding pioneer communities are likely less restricted by resources and competition than late-successional systems. We thus hypothesized that rising atmospheric CO<sub>2</sub> concentration will enhance plant growth in these high-elevation communities. Nine characteristic, perennial glacier forefield species were assembled in microcosms and grown at a nearby experimental site in the Swiss Alps (2440 m a.s.l.). The communities were exposed to an elevated CO<sub>2</sub> concentration of 580 ppm by free-air CO<sub>2</sub> enrichment for three seasons. Four study species were additionally grown in isolation in containers, half of which received a low dose of mineral fertilizer (25 kg N ha<sup>-1</sup> a<sup>-1</sup>) in order to explore a potential nutrient limitation of the CO<sub>2</sub> response. Responses of growth dynamics and peak season biomass of the two graminoid species, four forbs and three cushion forming species were analysed by repeated non-destructive assessments and a final biomass harvest. After three seasons, none of the species were stimulated by elevated CO<sub>2</sub>, irrespective of mineral nutrient addition, which by itself enhanced growth in the fertilized plants by +34% on average. Increased CO<sub>2</sub> concentration did not affect total (above- plus below-ground) biomass but reduced aboveground biomass by -35% across all species, even in the fast growing ones. This reduced aboveground biomass was associated with higher biomass partitioning to roots. Foliar nonstructural carbohydrate concentration increased and nitrogen concentration in leaves decreased under elevated CO<sub>2</sub>. We observed downward adjustment of photosynthetic capacity by on average -26% under long-term exposure to 580 ppm CO<sub>2</sub> (assessed in graminoids only). Our results indicate that glacier forefield pioneers, growing under harsh climatic conditions are not carbon limited at current atmospheric CO<sub>2</sub> concentration.

### Introduction

Since the end of the Little Ice Age in the mid-19th century, glaciers have massively retreated and uncovered forefields of deposits of glacier sediments. In the Alps, where temperatures were found to increase more rapidly than the global mean (Rebetz & Reinhard, 2008; Brunetti *et al.*, 2009), most glaciers (90% of all Alpine glaciers <1 km<sup>2</sup>) are likely to disappear within the coming decades (Zemp *et al.*, 2006; Huss *et al.*, 2010). The bare surface of newly deglaciated glacier forefields is colonized by mosses, soil biota and vascular plants within only a few years (Cannone *et al.*, 2008; Burga *et al.*, 2010). Such pioneer species facilitate the establishment of other species through stabilization of the silty inorganic substrate, humus formation and accumulation, seed trapping and positive effects on the microclimate (Erschbamer *et al.*, 2008). The speed of this primary succession on glacier forefields might be enhanced by rising atmospheric CO<sub>2</sub>, which, by the end of this century, is likely to have more than doubled compared to pre-industrial concentrations (Meehl *et al.*, 2007). Photosynthesis, especially of C<sub>3</sub> plants, is not saturated at the current CO<sub>2</sub> concentration of around 390 ppm (Mauna Loa Observatory, Hawaii, US, 2010). The potential direct biological effects of rising CO<sub>2</sub> on photosynthesis and plant growth add to the consequences of climatic warming, much of which results from radiative forcing by CO<sub>2</sub> in the atmosphere.

High-elevation plants can be expected to exhibit particularly strong responses to rising CO<sub>2</sub> levels, as they grow at lower CO<sub>2</sub> partial pressure, which is associated with the reduction in atmospheric pressure by roughly 10% per km of elevation in the atmosphere. The mixing ratio of different gases does not change substantially with elevation. Hence, at the current CO<sub>2</sub> mixing ratio, the reduction in CO<sub>2</sub> partial pressure when ascending from sea level to 2500 m of elevation corresponds approximately to the difference between present and pre-industrial CO<sub>2</sub> partial pressure (both at sea level). As oxygen partial pressure declines in a similar proportion, the oxygenase activity of Rubisco (photorespirator) becomes reduced, thus, somewhat mitigating but not compensating the effect of CO<sub>2</sub> decline with elevation on plant carbon assimilation.

lation. Reduced atmospheric pressure also enhances diffusivity in air, facilitating faster diffusion of CO<sub>2</sub> to the mesophyll (Körner, 2003). Yet, as temperatures decline with elevation and thus slow down diffusivity, this yields again only partial compensation (Körner & Diemer, 1987; Körner *et al.*, 1991; Terashima *et al.*, 1995).

Alpine plants have been shown to adjust to the net effect of these external (physical) drivers by enhanced photosynthetic capacities (measured at equal partial pressure of CO<sub>2</sub>), thus, equalizing rates at local partial pressure of contrasting elevations (Körner & Diemer, 1987). This greater photosynthetic efficiency has been found to be maintained in alpine plants after two seasons of growth under controlled environmental conditions and various CO<sub>2</sub> regimes compared to congeneric lowland plant species (Körner & Diemer, 1994). Increased photosynthetic CO<sub>2</sub> use efficiency in alpine plants is often correlated with greater mesophyll thickness, higher nitrogen concentration per unit dry mass and higher leaf diffusive conductance (Körner, 2003). These elevational trends in leaf traits have been found globally, with the greater CO<sub>2</sub> use efficiency underpinned by a steady reduction in <sup>13</sup>CO<sub>2</sub> discrimination with increasing elevation (Körner *et al.*, 1991). The decline in atmospheric pressure, rather than temperature, has recently been confirmed as the dominant driver of these adjustments along elevational gradients (Zhu *et al.*, 2010). However, it remains unclear whether life conditions at high elevation make plants more susceptible to rising CO<sub>2</sub>, or whether the above-explained adjustments to life at high elevation prevent such an increased responsiveness compared to plants growing at low elevation.

A first *in situ* attempt at exploring this question in high-elevation plants revealed no net biomass response to an atmosphere experimentally enriched in CO<sub>2</sub> over four seasons in the Swiss Alps, despite considerable photosynthetic stimulation (Körner *et al.*, 1997). This absence of any growth response by elevated CO<sub>2</sub>—irrespective of fertilizer addition—came as a surprise, given that several *in situ* studies of lowland grassland had shown a stimulating effect of rising CO<sub>2</sub>, although these responses may have been

mediated to a large extent by soil water savings resulting from reduced stomatal conductance under elevated CO<sub>2</sub> rather than by photosynthetic stimulation (Morgan *et al.*, 2004; Dijkstra *et al.*, 2010).

The rate of photosynthesis does not translate into plant growth in a straightforward manner because of a multitude of other growth controls such as allometry, developmental stage and availability of resources other than carbon (Körner, 2006). Glacier forefields can be categorized as expanding systems, in which available resources per unit land area (light, water, nutrients) have not been fully explored, i.e. plant communities have not yet reached a steady state regarding substrate or air space exploration (fine root turnover, leaf area index). Expanding systems permit plants to grow with little competition, which makes commonly limiting resources periodically highly available to the individual. Under such conditions, elevated CO<sub>2</sub> may exert a more pronounced stimulation of plant growth than in late-successional ecosystems, in which resources other than carbon are likely to control growth (Körner, 2006).

This study aims at assessing the consequences of rising atmospheric CO<sub>2</sub> concentration on leaf traits and biomass production in glacier forefield plants as well as providing some indications on potentially winning or losing species under future elevated CO<sub>2</sub>. Nine characteristic species, typically colonizing glacier forefields in the Swiss central Alps, grown in microcosms under natural conditions at 2440m elevation were exposed to 580ppm CO<sub>2</sub> using free-air CO<sub>2</sub> enrichment (FACE) for three seasons. As nutrient limitation cannot be ruled out even in early successional situations and as nutrient limitation was found to decrease growth responses to CO<sub>2</sub> treatment (Poorter, 1998; Reich *et al.*, 2006a), we additionally grew four of the species in isolation and added a low dose of mineral fertilizer to half of these plants. To capture time dependent (particularly initial) CO<sub>2</sub> responses, without destructively affecting plant performance, we combined continuous monitoring of biometric traits with a final biomass harvest. As explanatory variables we measured leaf carbohydrate and nitrogen concentrations in all species as well as photosynthetic rates

in two graminoid species. We hypothesized (1) that growth in these alpine glacier forefield pioneers is stimulated by CO<sub>2</sub> enrichment, (2) that such stimulation is enhanced by nutrient addition and (3) that plants respond in a species-specific manner, with higher stimulation expected in faster growing species, compared to the slower growing, cushion forming plants.

## Materials and methods

### Study site

The experimental site was located on a SE-exposed slope near Furka Pass (46°34'N 8°25'E) in the Swiss central Alps at 2440 m a.s.l. Long-term climate data records are available from the two nearest meteorological stations 'Grimsel-Hospiz' (1980 m a.s.l.; 7 km west of the study site) and 'Gütsch ob Andermatt' (2287 m a.s.l.; 17 km northeast), both operated by the Swiss Federal Office of Meteorology and Climatology MeteoSwiss (Table 1). Interpolated annual precipitation at the study site averages around 1900 mm (Atlas

of Switzerland 3, Institute of Cartography, ETHZ) and is relatively evenly distributed over the whole year, with slightly higher monthly precipitation in winter than during the snow-free growing period. The length of the growing season varies between 2.5 and 3.5 months at this elevation. Snow melt starts in early to mid-June and the season ends with plant senescence between end of August and mid-September. Meteorological conditions during the growing season (shown in Table 1) were monitored by a weather station at the study site (Wireless Vantage Pro2 Plus™; Davis Instruments, Hayward, CA, USA). Additionally, we monitored soil temperatures 10 cm below ground (Tidbit; Onset Computer Corp., Bourne, MA, USA). The mean growing season air temperature during the experiment (Jul/Aug 2006–2008) was around 7.2 °C, which is similar to the long-term average of the weather station 'Gütsch ob Andermatt', although this station is at lower elevation (Table 1). Due to strong insolation, mean soil temperature was higher, at around 11.0 °C. Seasonal temperature variability at this altitude is high, with freezing events and snow fall possible throughout the growing season.

**Table 1** Air temperature, soil temperature (–10 cm depth) and precipitation measured at the study site during the growing seasons 2006–2008, compared to long-term averages (1961–1990) for the two closest stations of MeteoSwiss. In 2007, the season started exceptionally early

		Study site 2440 m a.s.l.			Grimsel-Hospiz*	Gütsch ob Andermatt*
		2006	2007	2008	1980 m a.s.l.	2287 m a.s.l.
T air mean (°C)	annual				1.2	–0.5
	June		5.7		6.2	4.1
	July	11.2 <sup>†</sup>	6.9	7.2	8.9	7.3
	Aug.	3.2	6.8 <sup>†</sup>	7.8	8.8	7.1
	Sept.	7.7 <sup>†</sup>	2.8	3.0	7.0	5.1
T soil mean (°C)	June		8.2	5.0		
	July	14.5	10.6	11.2		
	Aug.	8.8	9.6	11.1		
	Sept.	8.6	7.0	6.5		
Precipitation (mm)	annual				2094	1479
	June		146		160	116
	July	84	121	138	144	105
	Aug.	216	145	150	158	125
	Sept.	95	42	169	120	94

\* Long-term averages (1961–1990), provided by MeteoSwiss (The Swiss Federal Office of Meteorology and Climatology).

† Temperature series not complete (4–7 days of missing data).

The bedrock in the glacier forefield at the bottom of the 'Blauberg' (northern slope, 46°34'N 8°25'E, 2430 m a.s.l.), where the studied microcosms had been assembled and the substrate (a sandy silt) had been obtained from, belongs to the Gotthard Massive and consists mainly of granite, gneiss and siliceous schists.

#### Plant species

We studied the following nine perennial plant species, which belong to different plant families and plant functional types and are typically found in glacier forefields (nomenclature follows Lauber & Wagner, 2007): two graminoid species, *Luzula alpinopilosa* (Chaix) Breistr. (Juncaceae) and *Poa alpina* ssp. *vivipara* L. (Poaceae), and four forb species, *Leucanthemopsis alpina* (L.) Heywood (Asteraceae), *Oxyria digyna* (L.) Hill (Polygonaceae), *Ranunculus glacialis* L. (Ranunculaceae) and *Veronica alpina* L. (Scrophulariaceae), as well as three cushion forming species, *Androsace alpina* (L.) Lam. (Primulaceae), *Cerastium uniflorum* Clairv. (Caryophyllaceae) and *Saxifraga stellaris* L. (Saxifragaceae). These plant species preferentially occur on humid, siliceous substrates in alpine rock fields (Lauber & Wagner, 2007). *Poa alpina* ssp. *vivipara* has a high reproductive potential due to pseudovivipary, producing leafy plantlets instead of seeds. *Ranunculus glacialis* is one of the highest climbing flowering plants in the Alps: it was recorded at Finsteraarhorn, Swiss Alps, flowering above 4000 m a.s.l. (Werner *et al.*, 1988). In the following, we address plant species by genus only.

#### Free-air CO<sub>2</sub> enrichment system

We set up six experimental plots, of which three served as controls ('ambient'; ca. 385 ppm) and three received elevated CO<sub>2</sub> (580 ppm) by FACE, using computer-controlled pure CO<sub>2</sub> release. The technology employed (MiniFACE; Miglietta *et al.*, 1996) was designed for small experimental plots: a 1.2 m ring formed by a 12 mm diameter plastic tube was installed around each elevated plot at 15 cm above ground level. At the outer side of the rings, CO<sub>2</sub> was released under high pressure of about 3.5 bar from small holes (about 40 per ring, spaced at 10 cm in-

tervals). These CO<sub>2</sub> jets rapidly mix with the bulk air (Miglietta *et al.*, 2001), thus, a homogenous 'CO<sub>2</sub> cloud' of a target CO<sub>2</sub> concentration of 580 ppm is generated over the plots. The higher the wind speed (up to a certain threshold) the better the performance of the system, but the greater the consumption of CO<sub>2</sub>. A control unit was connected to three open-path infrared gas analysers (CARBOCAP® Carbon Dioxide Probe GMP343; Vaisala, Helsinki, Finland) set up in the centre of each ring at 15 cm above ground. CO<sub>2</sub> concentrations and wind velocity were recorded every second. The CO<sub>2</sub> enrichment system was on during daylight, when photosynthetic photon flux density (PPFD) was above the threshold of 80 μmol m<sup>-2</sup> s<sup>-1</sup> (measured by LI-190 quantum sensor; LI-COR Biosciences Inc., Lincoln, NE, USA), and CO<sub>2</sub> release was paused in the case of snow cover.

#### Experimental set-up

Study plants and soil substrate were obtained from a glacier forefield at 'Blauberg' in 2005. For logistic reasons (access and power supply), we had to transfer the plants to the nearby experimental site at the same elevation, 150 m NE of the ALPFOR research station (<http://pages.unibas.ch/botschoen/alpfor/>, accessed 4 November 2011). Of all excavated plants, we removed the two biggest and smallest individuals per species and assigned the remaining individuals randomly to treatments. The roots of all plants were rinsed and cut to a common length of ca. 5 cm, and pretreatment fresh weight of the four main species *Luzula*, *Poa*, *Ranunculus* and *Veronica* was determined to assess carry-over effects of initial plant size on treatment responses. However, no correlation was found between initial fresh weight and final plant biomass or the resultant CO<sub>2</sub> response at harvest, hence we do not present these data.

To simulate a natural glacier forefield plant community, plants were grown in a diverse species mix in microcosms with a volume of 9 L (20 cm deep) filled with original glacier forefield substrate (a sandy siliceous silt, no structured soil, i.e. no soil horizons). One individual of each cushion forming species and two individuals of each of the remaining six species were planted at random positions into each micro-

cosm. And two of these microcosms were buried side by side to ground level in the centre of the six experimental plots (12 microcosms in total).

In addition to the core experiment with microcosms, we added a fully factorial CO<sub>2</sub> x fertilizer experiment in *Poa*, *Luzula*, *Ranunculus* and *Veronica* grown in isolation in 1.5 L containers. These containers were filled with the same substrate as used for microcosms, and contained two individuals of one single species. Two containers of each species were placed around the two microcosms in each experimental plot and buried to ground level (a total of eight containers per plot). At the beginning of each growing season, ground, slowly degrading mineral fertilizer (N-P-K-Mg 17:10:13:3 with all major trace elements; H. Gilgen Optima AG, Arlesheim, Switzerland) was added to half of these 'single-species' containers. The amount of nitrogen applied was equivalent to 15 kg N ha<sup>-1</sup> a<sup>-1</sup> in 2006, 25 kg N ha<sup>-1</sup> a<sup>-1</sup> in 2007 and 2008, approximately five times the current annual wet deposition of nitrogen at the study site (max. 5 kg N ha<sup>-1</sup> a<sup>-1</sup>, Hiltbrunner *et al.*, 2005).

To test potential influences of elevated CO<sub>2</sub> on clonal offspring of *P. alpina* ssp. *vivipara*, plantlets sampled from experimental individuals were planted into separate 1.5 L containers filled with the same substrate during the course of the experiment. Two containers with plantlets taken in 2006 and two with plantlets taken in 2007 were placed in the same experimental plot as the parental plants, and exposed to the corresponding CO<sub>2</sub> treatment. The number of containers per experimental plot added up to a total of two microcosms, eight 'single-species' containers and four '*Poa* plantlet' containers, all arrayed in the centre of the experimental plots with as small as possible gaps between containers and a CO<sub>2</sub>-mixing zone of 30–40 cm between the outermost containers and the FACE rings (Fig. 2, inset photograph).

Rocky debris was placed around the microcosms and containers to assure a realistic radiation and heat balance as it occurs in glacier forefields. As melting water keeps seeping through the natural habitat throughout the season, we kept adding local, nutrient-poor glacier melting water during dry weather

periods, so that soil moisture never fell below 20% vol (TRIME-EZ; IMKO GmbH, Ettlingen, Germany), which is a high moisture in this sandy silt. CO<sub>2</sub> enrichment to 580 ppm started on 16 June 2006, right after snow melt at the study site, and was continued for three seasons, i.e. from snow melt to the end of September when plants were fully senesced in 2006 and 2007, and in the last year, 2008, until the first week of August, after the peak of standing biomass was reached, when FACE was switched off and plants were harvested.

#### *Plant biomass analyses*

For each individual, biometric growth parameters were assessed once in 2006, five times during the second growing season, 2007, and twice in 2008, to detect the temporal variation in the magnitude of potential CO<sub>2</sub> effects. This should assure that we do not miss an early season or early experiment CO<sub>2</sub> response that could disappear or propagate (accelerate) until the final harvest. The number of tillers, shoots or rosettes was assessed in all species, and the number of live and dead leaves was counted in all species, except for cushion plants. Leaf length was measured in green leaves, and in the species *Luzula*, *Poa*, *Ranunculus* and *Veronica*, leaf thickness was assessed, using a handheld micrometer with an accuracy of ±1 μm (SM 112; Teclock Corp., Nagano, Japan). For reproductive effort, the number of inflorescences or flowers, and the length of the flowering stems were censused.

Finally, all plants, including the above-mentioned clonal offspring of *Poa*, were harvested right after the peak of standing biomass, during the first week of August 2008. Leaf and total plant area were measured using the LI-3000C Portable Area Meter in combination with the LI-3050C Transparent Belt Conveyor Accessory (LI-COR Biosciences Inc.). Above-ground plant material was separated into biomass and necromass, and for biomass we separated leaves and stems (i.e. culms and peduncles, including inflorescences). As alpine forbs are generally rosette forming, there are no stems other than the flower-bearing ones. Belowground plant material was washed and separated into roots and nonroot belowground parts

(rhizome, root stock), which were added to stems. In the microcosms root biomass could not be separated by species, but was treated as a bulk sample. All plant material was oven dried for at least 48 hours at 80 °C and cooled in vacuum over silica gel before weighing.

#### Leaf trait analyses

Using leaf area and dry weight of green leaves, we calculated the specific leaf area (SLA) of all species, except for cushion plants and *Leucanthemopsis*. Nonstructural carbohydrates (NSC, starch plus soluble sugars), total carbon and nitrogen concentration and  $\delta^{13}\text{C}$  analyses were performed using oven-dried plant material (taken in August 2008), which was ground using a ball mill (Retsch MM 2000; Retsch GmbH, Haan, Germany). For the NSC analysis, invertase- and isomerase-treated subsamples were tested spectrophotometrically for glucose following the procedure described in Schappi and Korner (1997). The remaining sample was undertaken an enzymatic starch digestion followed by another glucose assay. Carbon and nitrogen concentrations were measured by a CHN-analyser (Vario EL III; Elementar GmbH, Hanau, Germany) and NSC-free leaf N was calculated by subtracting NSC concentration from total dry weight.

As the tank CO<sub>2</sub> used for CO<sub>2</sub> enrichment is produced from fossil sources, it is depleted in <sup>13</sup>C content ( $\delta^{13}\text{C}$  ca. -29‰).  $\delta^{13}\text{C}$  in plant material thus permits verification of uptake and incorporation of CO<sub>2</sub> provided by FACE. Leaf samples taken at the end of the growing season 2006 and in August 2008 were analysed for  $\delta^{13}\text{C}$  using mass spectrometry with a precision of <0.1‰ (EA-1110; Carlo Erba Thermoquest, Milan, Italy; Conflo II and Delta S; Thermo Finnigan Mat, Bremen, Germany).

Leaf-level net photosynthesis ( $A_{\text{net}}$ ) was measured in *Luzula* and *Poa* using a portable gas exchange analyser (LI-6400; LI-COR Biosciences Inc.) with a small leaf chamber (6400-15 'Arabidopsis Chamber'; LI-COR) on nine warm and sunny days at peak season in 2007. An external LED-light source (peak wavelength:  $680 \pm 10$  nm, PPFD:  $1900 \pm 100$   $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  at leaf level) was fixed above the leaf

chamber to maintain constant, saturating light conditions. Leaf temperature was set to 25 °C, close to the known (high) temperature optimum of these plants (Korner & Diemer, 1987), and vapour pressure deficit in the reference air was equal to ambient air. Plants were watered the day before and on the day of measurements to assure that water was amply available. Light-saturated  $A_{\text{net}}$  was measured at a leaf chamber CO<sub>2</sub> concentration ( $c_a$ ) of 385 ppm and subsequently at 580 ppm on two to three fully developed but not senescent leaves per plant. Single measurements did not take more than 5 minutes, and data was recorded as soon as  $A_{\text{net}}$  and stomatal conductance ( $g$ ) remained constant. Leaf area correction was performed for each leaf, calculating the true leaf area enclosed in the chamber from photographs of leaves using software for image analysis (IMAGE J 1.38x, Rasband, 2007).

#### Statistical analyses

All data were averaged per species growing in the same growth unit (microcosm or container) and analysed separately for plants grown in microcosms and plants grown in isolation in 'single-species' containers by using different models. Differences in biomass and growth parameters and leaf traits were tested by linear mixed effects modelling using the package 'nlme' (Pinheiro *et al.*, 2009) of the open-source program R, version R 2.10.1 (R Development Core Team, 2009). We applied a split-plot model with 'CO<sub>2</sub> treatment', 'species', 'fertilizer addition' (only in plants grown in isolation) and their interactions as fixed factors and 'experimental plot' (FACE or ambient rings) as random factor. The experimental plots were thus used as replicates for the whole-plot effect 'CO<sub>2</sub> treatment', while all other effects were tested using within-plot errors. For the analysis of net photosynthesis a model with the fixed factors 'growth CO<sub>2</sub> concentration', 'species' and 'leaf chamber CO<sub>2</sub> concentration' and the random factor 'experimental plot' was fit. The 'experimental plot' random effect was not significant in any model ( $P > 0.1$ , using log likelihood ratio tests). Power or log transformations were applied to meet normal distribution and homogeneity of variance. Restricted maximum likelihood estimation (REML) was used to choose the variance structure (log-likelihood ratio tests). Optimal fixed structure selection was

performed by backward selection comparing nested models using log-likelihood ratio tests with maximum likelihood estimation (ML). The final models were then refit by REML.  $P$ -values  $<0.05$  were considered statistically significant and  $P$ -values  $<0.1$  were considered marginally significant, as such trends still have an ecological meaning, especially in ecological experiments with limited power. Values in the text are given as mean  $\pm$  standard deviation and all diagrams show means and standard errors of the mean of the three ambient and the three elevated plots ( $n = 3$ ).

## Results

### *FACE performance*

In all three experimental years, FACE was started at the very beginning of the growing season, just after the snow disappeared from the plots (Table 2). So, depending on season length, the system was operational during 98, 122 and 57 days in 2006, 2007 and 2008 (the year of peak season harvest), respectively. The growing season 2007 was exceptionally long, due to very low snowfall during winter and early snow melt. Of this total treatment period, CO<sub>2</sub> enrichment was effective during 90%, 87% and 93% of the possible time. Most of the interruptions were caused by snow cover or extremely bad weather conditions (photon flux density  $<80 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) and  $<1\%$  of the missing hours was due to technical failure.

CO<sub>2</sub> concentration records continuously taken in all elevated plots indicated that the FACE system maintained concentrations close to the target level of 580 ppm (Table 2). Seasonal mean CO<sub>2</sub> concentrations at the three elevated plots calculated from one second data were (for 2006, 2007 and 2008)  $584 \pm 7$ ,  $585 \pm 6$  and  $601 \pm 11$  ppm at daytime. Night values were  $391 \pm 3$ ,  $393 \pm 1$  and  $395 \pm 2$  ppm in the three years. During daytime CO<sub>2</sub> enrichment, 77%, 81% and 60% of the one second CO<sub>2</sub> concentration records lay within  $\pm 100$  ppm of the target concentration, with only 11%, 9%, 18% of the values below 480 ppm (largely during storms) and 12%, 10%, 22% above 680 ppm (mostly during morning calms). The CO<sub>2</sub> concentration in the three elevated CO<sub>2</sub> plots did not differ significantly from each other (Table 2). The frequency distribution of CO<sub>2</sub> concentrations during a representative period of fine weather (25–31 July 2007, Supporting Information, Fig. S1) reveals that concentrations above 500 ppm were measured on 88% of the time and only few one second records (1.6%) reached values above 800 ppm.

### *$\delta^{13}\text{C}$ isotopic label*

The  $\delta^{13}\text{C}$  isotopic label of tank CO<sub>2</sub> was  $-28.8 \pm 0.2\%$  in all 3 years, as the gas delivered was produced from the same fossil source throughout the experimental period. The analysis of stable carbon isotopes of leaf material taken in 2006 showed that all species had incorporated the <sup>13</sup>C-depleted CO<sub>2</sub>, and almost completely reflected the new mixing ratio provided by FACE already after one season of CO<sub>2</sub> enrichment

**Table 2** FACE performance during the growing seasons 2006, 2007 and 2008. Note, in the last year, CO<sub>2</sub> enrichment was stopped at final harvest (first week of August 2008). CO<sub>2</sub> concentrations are means of one second readings  $\pm$  SD

	2006	2007	2008
Date of snowmelt at FACE site	14 June	25 May	12 June
FACE start date	16 June	25 May	12 June
FACE end date	22 September	24 September	7 August
CO <sub>2</sub> enrichment *	90%	87%	93%
Seasonal mean [CO <sub>2</sub> ] across all plots (ppm)	$584 \pm 7$	$585 \pm 6$	$601 \pm 11$
Seasonal mean [CO <sub>2</sub> ] plot E1 (ppm)	$579 \pm 7$	$586 \pm 10$	$598 \pm 20$
Seasonal mean [CO <sub>2</sub> ] plot E2 (ppm)	$593 \pm 16$	$583 \pm 11$	$598 \pm 20$
Seasonal mean [CO <sub>2</sub> ] plot E3 (ppm)	$581 \pm 11$	$586 \pm 10$	$605 \pm 18$
[CO <sub>2</sub> ] readings within $580 \pm 100$ ppm	77%	81%	60%

\*% realized enrichment time (relative to total treatment period) largely as influenced by snow cover during the growing season.



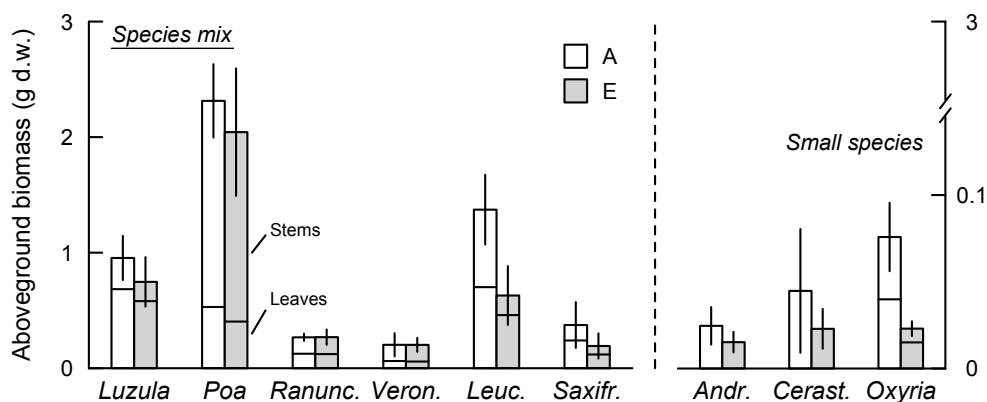
(data not shown). Leaf material collected in 2008 was significantly depleted in the heavy carbon isotope under high CO<sub>2</sub> by a mean of  $-5.5 \pm 0.6\%$  across all species (Tables 4 and 5), corresponding to an integrated CO<sub>2</sub> mixing ratio of 584 ppm (calculated for *Luzula*, exhibiting the greatest difference in  $\delta^{13}\text{C}$  between ambient and elevated CO<sub>2</sub> and assuming that these plants completely renewed their carbon in leaves after 3 years). The slowly growing, low-stature cushions of *Androsace* and *Cerastium* showed a lower depletion in leaf material ( $-4.4\%$  and  $-4.8\%$ ) than the other species, especially if compared to the faster growing species *Leucanthemopsis* ( $-6.5\%$ ), *Luzula* ( $-6.2\%$ ) and *Poa* ( $-5.7\%$ ). Root material, which has a slower turnover, was only depleted by an average of  $-4.4 \pm 1.2\%$  across all species, with a less pronounced <sup>13</sup>C depletion in *Androsace*, *Cerastium*, *Oxyria* and *Saxifraga*, indicating that these slowly growing species did not completely replace their belowground structures and stores during the three years of treatment.

#### Plant biomass response

For plants grown in a close to natural species mix (microcosms), aboveground biomass per individual at the peak of the third season was not stimulated by exposure to elevated CO<sub>2</sub> in any of the nine species studied (Fig. 1, Table 3). On the contrary, we found a decrease in aboveground biomass in some species under high CO<sub>2</sub> (by  $-32\%$  across all species grown

in the microcosms), but no significant effect of CO<sub>2</sub> treatment on root biomass (bulk sample of all plants per container) nor on total (above- plus belowground) biomass was observed. Both, leaf biomass and stem biomass (including inflorescences) as well as inflorescence weight alone ( $F_{1,4} = 8.28$ ,  $P = 0.045$ ) were slightly reduced under CO<sub>2</sub> enrichment. These results were underpinned by the non-destructive biometric measurements continuously conducted from 2006 to 2008 (not for cushion forming species): the number of leaves was slightly reduced under high compared to ambient CO<sub>2</sub> in all species, except *Veronica*, shortly prior to harvest in 2008 (on average by  $-18\%$  over all species,  $F_{1,4} = 5.50$ ,  $P = 0.079$ ), and leaf length was reduced in *Oxyria* ( $-25\%$ ) and *Poa* ( $-17\%$ ), but not in the other species in 2008 (species  $\times$  CO<sub>2</sub> interaction:  $F_{4,44} = 5.38$ ,  $P = 0.001$ ). These traits were not significantly altered by CO<sub>2</sub> enrichment in the previous years 2006 and 2007. Growth conditions for the cushion forming species *Androsace*, *Cerastium* and *Saxifraga* as well as for *Oxyria* were not favourable in the microcosms, possibly due to intense competition by non-cushion species, particularly graminoids. Thus, this group of small-sized species showed random mortality and high variability within treatment groups.

In plants grown in isolation ('single-species' containers), we found a similar, significant reduction of aboveground biomass (on average by  $-19\%$ ) in



**Fig. 1.** Aboveground biomass (mean  $\pm$  SE;  $n = 3$ ) consisting of leaves and stems (including inflorescences; except for the cushion plants *Androsace* and *Cerastium*) of nine glacier forefield species (*Luzula alpinopilosa*, *Poa alpina*, *Ranunculus glacialis*, *Veronica alpina*, *Leucanthemopsis alpina*, *Saxifraga stellaris*, *Androsace alpina*, *Cerastium uniflorum* and *Oxyria digyna*) growing under ambient (A) and elevated CO<sub>2</sub> (E) in a close to natural species mix (microcosms). Note the different scale of the y-axis for the small species on the right of the dashed line.

response to CO<sub>2</sub> enrichment across all four species (Fig. 2, Table 3). This reduction was mainly caused by lower stem biomass, as leaf biomass did not differ significantly between ambient and elevated CO<sub>2</sub>. The nondestructive monitoring of biometric traits, however, revealed reduced numbers of leaves under 580 ppm CO<sub>2</sub> in the last year of CO<sub>2</sub> treatment in *Luzula* (−16%) and *Poa* (−29%), but not in *Ranunculus* and even opposite in *Veronica* (+25%; species × CO<sub>2</sub> interaction:  $F_{3,31} = 3.23$ ,  $P = 0.036$ ), associated with

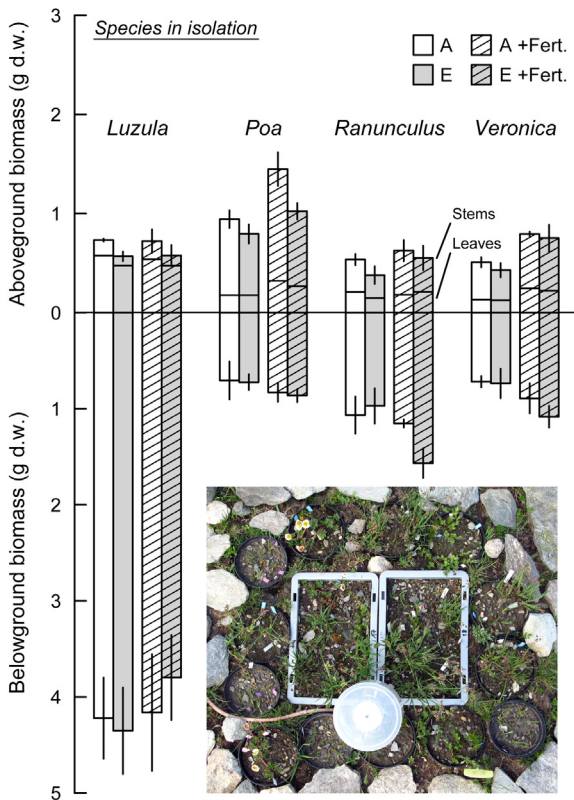
small, proportional changes in shoot number in these species (data not shown). Under CO<sub>2</sub> enrichment, inflorescence weight alone was significantly reduced ( $F_{1,4} = 11.78$ ,  $P = 0.027$ ), which was caused by a slightly smaller number of inflorescences per individual plant ( $F_{1,4} = 4.79$ ,  $P = 0.094$ ; data not shown). In *Poa*, the number of asexually formed plantlets per inflorescence was increased under elevated CO<sub>2</sub> in the years 2006 and 2007, but not at harvest in 2008 (data not shown). Root biomass, on the other hand, did not

**Table 3** Linear mixed effects models testing for biomass differences between CO<sub>2</sub> treatments, species, fertilizer addition (fert.) and their interactions in plants grown in a species mix (microcosms) or in isolation. In the species mix, root biomass and total (above- plus belowground) biomass are bulk samples of all species per microcosm

Species mix	df	F	P-values		Species in isolation	df	F	P-values	
<i>Aboveground biomass (g d.w.)</i>									
CO <sub>2</sub>	1, 4	6.16	0.068	(*)	CO <sub>2</sub>	1, 4	11.85	0.026	*
Species	8, 75	50.39	<0.001	***	Species	3, 28	23.22	<0.001	***
CO <sub>2</sub> × species	8, 75	1.01	0.436		CO <sub>2</sub> × species	3, 28	0.53	0.663	
					Fert.	1, 28	17.78	<0.001	***
					CO <sub>2</sub> × fert.	1, 28	0.02	0.904	
					Species × fert.	3, 28	2.55	0.076	(*)
<i>Leaf biomass (g d.w.)</i>									
CO <sub>2</sub>	1, 4	4.59	0.099	(*)	CO <sub>2</sub>	1, 4	0.71	0.448	
Species	8, 75	33.32	<0.001	***	Species	3, 28	47.29	<0.001	***
CO <sub>2</sub> × species	8, 75	0.62	0.759		CO <sub>2</sub> × species	3, 28	0.27	0.849	
					Fert.	1, 28	9.85	0.004	**
					CO <sub>2</sub> × fert.	1, 28	0.16	0.690	
					Species × fert.	3, 28	2.91	0.052	(*)
<i>Stem biomass (g d.w.)</i>									
CO <sub>2</sub>	1, 4	7.14	0.056	(*)	CO <sub>2</sub>	1, 4	14.06	0.020	*
Species	6, 58	28.76	<0.001	***	Species	3, 28	67.23	<0.001	***
CO <sub>2</sub> × species	6, 58	1.48	0.200		CO <sub>2</sub> × species	3, 28	1.86	0.160	
					Fert.	1, 28	18.09	<0.001	***
					CO <sub>2</sub> × fert.	1, 28	0.66	0.424	
					Species × fert.	3, 28	2.04	0.131	
<i>Root biomass (g d.w.)</i>									
CO <sub>2</sub> (bulk sample)	1, 4	0.04	0.858		CO <sub>2</sub>	1, 4	1.17	0.341	
					Species	3, 28	157.35	<0.001	***
					CO <sub>2</sub> × species	3, 28	0.27	0.844	
					Fert.	1, 28	3.49	0.072	(*)
					CO <sub>2</sub> × fert.	1, 28	0.26	0.617	
					Species × fert.	3, 28	1.74	0.182	
<i>Total (above- and belowground) biomass (g d.w.)</i>									
CO <sub>2</sub> (bulk sample)	1, 4	0.62	0.474		CO <sub>2</sub>	1, 4	0.22	0.665	
					Species	3, 28	92.00	<0.001	***
					CO <sub>2</sub> × species	3, 28	0.31	0.821	
					Fert.	1, 28	8.81	0.006	**
					CO <sub>2</sub> × fert.	1, 28	0.06	0.812	
					Species × fert.	3, 28	2.32	0.097	(*)

(\*) $P < 0.1$ ; \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

show a significant response to elevated CO<sub>2</sub> in any of the individually grown species, and neither did total (above- plus belowground) biomass. Thus, as a result of reduced aboveground biomass, biomass partitioning between roots, stems and leaves was altered in favour of roots under CO<sub>2</sub> enrichment in unfertilized plants and even more so in fertilized plants (10% higher root mass fraction,  $F_{1,4} = 9.02$ ,  $P = 0.040$ , and 24% lower stem mass fraction,  $F_{1,4} = 8.27$ ,  $P = 0.045$ ; data not shown).

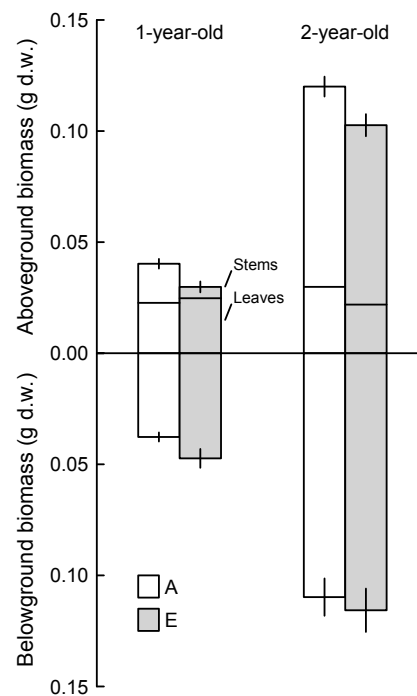


**Fig. 2** Above- and belowground biomass (mean ± SE;  $n = 3$ ) consisting of leaves, stems (including inflorescences) and roots of four glacier forefield species (*Luzula alpinopilosa*, *Poa alpina*, *Ranunculus glacialis* and *Veronica alpina*) grown in isolation, in response to ambient (A) or elevated CO<sub>2</sub> (E) and to fertilizer addition (+Fert.). The inset photograph of the core of one of the MiniFACE rings was taken early in the third season of treatment ('multi-species' microcosms in the centre, 'single-species' containers for the fertilizer experiment at the periphery; the white cap hides the open-path infrared gas analyser).

The rather moderate fertilizer addition to half of the plants grown in isolation led to a large and highly significant stimulation of aboveground growth in *Poa* (+42%), *Ranunculus* (+29%) and *Veronica* (+65%), but not in *Luzula* (Fig. 2, Table 3). This growth enhancement was caused by both, leaf and stem biomass, as the total amount of shoots was increased.

Nevertheless, aboveground biomass was reduced if fertilizer was added together with elevated CO<sub>2</sub>, as compared to fertilizer addition only. Fertilizer addition enhanced root growth and total plant biomass to a lesser extent than aboveground biomass (by 20% and 26% across the four separately grown species), however there was again no further stimulation by elevated CO<sub>2</sub>. Importantly, necromass and therefore senescence were not affected by the CO<sub>2</sub> and fertilizer treatments in either growth unit (microcosm or 'single-species' container), revealing that the treatments did not enhance biomass turnover (data not shown).

Similar to the results in adult *Poa*, the test in clonal offspring of *Poa* grown under elevated CO<sub>2</sub> showed a reduction in aboveground biomass of 1-year-old (−26%) and 2-year-old (−14%) second-generation plants grown from plantlets (Fig. 3;  $F_{1,4} = 10.04$ ,  $P = 0.034$ ). Like in adult *Poa* plants grown in isolation, this was mainly caused by lower stem biomass ( $F_{1,4} = 33.53$ ,  $P = 0.004$ ), especially in 1-year-old plants, as leaf biomass was not significantly affected. However, total (above- plus belowground) biomass was not significantly altered, and root biomass increased slightly under CO<sub>2</sub> enrichment (not statistically significant), which, together



**Fig. 3** Clonal offspring biomass (mean ± SE;  $n = 3$ ) of 1- and 2-year-old *Poa alpina* individuals, consisting of leaves, stems (including inflorescences) and roots, under ambient (A) or elevated CO<sub>2</sub> (E).

with the reduced aboveground biomass, again led to a significantly higher root to total biomass fraction ( $F_{1,4}=10.48$ ,  $P=0.032$ ) and thus, a lower stem mass fraction ( $F_{1,4}=10.64$ ,  $P=0.031$ ; data on fractions not shown).

Comparing the results for plants jointly grown in microcosms and those grown in isolation (unfertilized plants only), the species *Ranunculus* (+71%,  $F_{1,10}=13.80$ ,  $P=0.004$ ) and *Veronica* (+131%,  $F_{1,10}=21.34$ ,  $P=0.001$ ) showed increased plant size when grown alone, as compared to plants grown in microcosms, where they most likely experienced higher competition from the more competitive graminoids (Figs 1 and 2). In contrast, the graminoids grew more vigorously in the microcosms, compared to those grown in isolation (*Luzula* +31%, not significant; *Poa* +151%,  $F_{1,10}=23.22$ ,  $P<0.001$ ), showing increased leaf and stem biomass. In other words, provided with larger space, *Poa* in particular became a strong competitor that reduced growth in the other taxa. However, this did not affect the  $\text{CO}_2$  response of *Poa* nor of the other species and in general, the effect of elevated  $\text{CO}_2$  was not significantly different between plants grown in a natural assemblage in microcosms or in isolation.

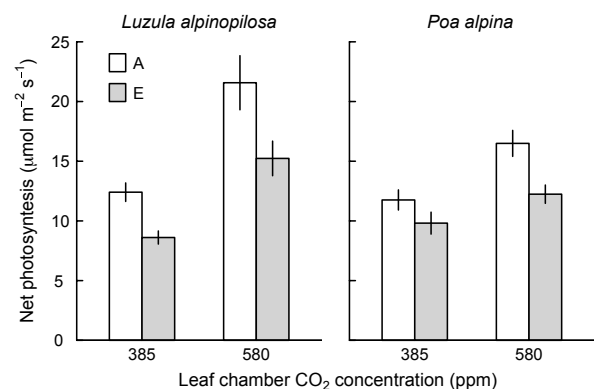
#### Leaf trait responses

In all species grown in the microcosms, foliar NSC concentration was increased by 26% on average under high  $\text{CO}_2$  due to both, sugars and starch (Tables 4a and 5). The increase was especially high in the species with the lowest growth rates, such as *Oxyria* (+65%), *Cerastium* (+59%) and *Saxifraga* (+39%). Accordingly, the C/N ratio was on average 7% higher under elevated  $\text{CO}_2$ , with the highest increase observed in *Oxyria* (+20%), *Luzula* (+14%) and *Ranunculus* (+13%). The enhanced C/N ratio under  $\text{CO}_2$  enrichment was mainly caused by an overall -10% lower nitrogen concentration (N) in leaves, which was also evident when expressed on a NSC-free dry matter basis. The N reduction was most pronounced in the species *Oxyria* (-18%), *Ranunculus* (-16%), *Luzula* (-14%) and *Leucanthemopsis* (-13%). Accumulation of NSC in leaves led to a lower SLA in plants grown in microcosms under elevated  $\text{CO}_2$  (by -6% on average), except for *Poa*, the only species benefit-

ing from growing in 'multi-species' mixtures, which showed no difference in SLA. However, no  $\text{CO}_2$  effect on leaf thickness was observed in any species, so the SLA effect was largely due to NSC accumulation.

Plants grown in isolation showed less pronounced leaf trait responses to  $\text{CO}_2$  enrichment than the same species grown in the 'multi-species' community (Tables 4b and 5). NSC concentration was only significantly increased by elevated  $\text{CO}_2$  in fertilized *Poa* individuals grown in isolation (+21%). C/N ratios showed slight but not statistically significant increases under high  $\text{CO}_2$  (+5%) in *Luzula*, *Ranunculus* and *Veronica*, related to slight reductions in leaf N concentration by -7% in all species, apart from *Poa*. Fertilizer addition did not lead to significantly lower C/N ratios or higher N concentration in leaves; hence, N was most likely diluted by growth. Yet, leaf thickness responded positively to nutrient addition (+10% across all species), especially in *Poa* (+19%).

Light saturated leaf-level photosynthesis ( $A_{\text{net}}$ ) measured in the second season of  $\text{CO}_2$  enrichment in plants grown at ambient  $\text{CO}_2$  was significantly stimulated by short-term exposure to a leaf chamber  $\text{CO}_2$  concentration ( $c_a$ ) of 580 ppm in both graminoid species tested: in *Luzula*,  $A_{\text{net}}$  increased by 74% and in *Poa* by 40% (Fig. 4, Supporting Information, Table S1,  $F_{1,39}=40.16$ ,  $P<0.001$ ;  $A_{\text{net}}$  was pooled for plants grown in microcosms or in isolation and for nutrient treatments, as these factors had no significant effect). This  $\text{CO}_2$  stimulation of  $A_{\text{net}}$  was retained



**Fig. 4** Net photosynthesis ( $A_{\text{net}}$ , mean  $\pm$  SE;  $n=3$ ) of *Luzula* and *Poa* under full illumination ( $1900 \pm 100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ), at leaf chamber  $\text{CO}_2$  concentrations ( $c_a$ ) of 385 ppm and 580 ppm, in plants grown under ambient (A) or elevated  $\text{CO}_2$  (E).

**Table 4** Leaf traits: specific leaf area (SLA), leaf thickness, nonstructural carbohydrate concentration (NSC), carbon to nitrogen ratio, nitrogen concentration and <sup>13</sup>C isotopic signature in leaves (mean ± SE; n = 3) of glacier forefield plants exposed to ambient (A) or elevated (E) CO<sub>2</sub>. (a) Plants grown in a species mix (microcosms), (b) plants grown in isolation without or with an additional fertilizer treatment (+Fert.)

Species	Treatment	SLA (cm <sup>2</sup> g <sup>-1</sup> )	Leaf thickness (µm)	NSC (mg g <sup>-1</sup> d.w.)	C/N ratio	N (mg g <sup>-1</sup> d.w.)	δ <sup>13</sup> C (‰)
<b>(a) Species mix</b>							
<i>Luzula</i>	A	10.3 ± 0.6	320 ± 23	170 ± 13	23.0 ± 0.5	21.0 ± 0.4	-29.3 ± 0.1
	E	9.5 ± 0.4	288 ± 6	172 ± 18	26.1 ± 1.4	18.1 ± 1.1	-35.5 ± 0.5
<i>Poa</i>	A	6.2 ± 0.1	319 ± 6	259 ± 4	33.0 ± 0.4	13.9 ± 0.1	-28.6 ± 0.2
	E	6.2 ± 0.2	313 ± 4	290 ± 23	34.4 ± 2.0	13.5 ± 0.8	-34.3 ± 0.7
<i>Ranunculus</i>	A	9.0 ± 0.5	645 ± 38	259 ± 19	23.0 ± 0.1	20.4 ± 0.3	-27.2 ± 0.1
	E	8.2 ± 0.4	660 ± 14	268 ± 13	25.9 ± 0.6	17.3 ± 0.2	-32.7 ± 0.4
<i>Veronica</i>	A	19.8 ± 0.5	283 ± 15	116 ± 5	24.4 ± 0.6	19.5 ± 0.3	-28.5 ± 0.2
	E	18.0 ± 1.5	295 ± 19	131 ± 4	26.0 ± 0.4	17.8 ± 0.2	-33.8 ± 0.6
<i>Androsace</i>	A				34.1 ± 1.1	14.1 ± 0.4	-29.0 ± 0.1
	E				34.2 ± 2.1	12.5 ± 1.1	-33.5 ± 1.2
<i>Cerastium</i>	A			65 ± 10	28.3 ± 2.6	15.4 ± 0.9	-27.8 ± 0.7
	E			104 ± 3	28.5 ± 0.6	15.0 ± 0.3	-32.5 ± 1.1
<i>Leucanthem.</i>	A			127 ± 9	22.7 ± 1.0	21.4 ± 0.9	-28.8 ± 0.4
	E			147 ± 8	24.8 ± 1.5	18.6 ± 1.1	-35.3 ± 0.9
<i>Oxyria</i>	A	20.6 ± 0.4		43 ± 1	15.5 ± 1.0	30.5 ± 2.5	-28.2 ± 0.1
	E	19.1 ± 0.4		72 ± 7	18.6 ± 0.6	25.2 ± 0.5	-33.7 ± 0.6
<i>Saxifraga</i>	A			218 ± 9	50.8 ± 2.0	8.6 ± 0.4	-28.2 ± 0.7
	E			303 ± 62	50.3 ± 3.0	8.6 ± 0.6	-33.6 ± 0.5
<b>(b) Species in isolation</b>							
<i>Luzula</i>	A	9.6 ± 0.1	287 ± 3	189 ± 13	25.0 ± 1.0	19.4 ± 0.8	-29.8 ± 0.2
	E	9.1 ± 0.3	295 ± 13	177 ± 20	25.5 ± 0.2	18.4 ± 0.3	-35.4 ± 0.5
	A (+Fert.)	9.6 ± 0.0	323 ± 18	197 ± 25	22.6 ± 1.0	21.5 ± 1.0	-28.9 ± 0.3
	E (+Fert.)	9.3 ± 0.2	298 ± 2	202 ± 10	24.9 ± 1.2	19.0 ± 0.8	-35.7 ± 0.8
<i>Poa</i>	A	6.3 ± 0.3	305 ± 21	246 ± 4	39.4 ± 2.5	11.7 ± 0.8	-28.5 ± 0.3
	E	6.2 ± 0.6	278 ± 16	254 ± 25	39.3 ± 3.0	11.8 ± 0.9	-34.1 ± 1.2
	A (+Fert.)	5.5 ± 0.2	352 ± 4	244 ± 3	38.9 ± 2.1	11.8 ± 0.7	-28.0 ± 0.3
	E (+Fert.)	5.5 ± 0.4	340 ± 5	295 ± 19	37.5 ± 1.9	12.4 ± 0.7	-33.6 ± 0.8
<i>Ranunculus</i>	A	9.0 ± 1.1	748 ± 29	265 ± 34	23.3 ± 1.1	20.1 ± 0.8	-27.0 ± 0.5
	E	8.7 ± 0.5	588 ± 36	243 ± 37	24.8 ± 2.3	18.3 ± 1.8	-32.2 ± 0.2
	A (+Fert.)	8.6 ± 0.4	715 ± 105	249 ± 12	22.6 ± 1.9	20.6 ± 2.1	-27.1 ± 0.2
	E (+Fert.)	8.3 ± 0.2	723 ± 26	232 ± 22	22.7 ± 0.9	19.7 ± 0.9	-32.2 ± 0.5
<i>Veronica</i>	A	16.8 ± 0.6	340 ± 15	141 ± 10	26.4 ± 2.1	18.7 ± 1.6	-28.3 ± 0.4
	E	17.3 ± 0.2	330 ± 10	142 ± 28	28.2 ± 2.4	17.0 ± 1.4	-33.1 ± 1.2
	A (+Fert.)	15.8 ± 1.6	358 ± 3	136 ± 32	26.4 ± 0.5	18.4 ± 0.8	-27.6 ± 0.5
	E (+Fert.)	16.2 ± 0.4	358 ± 31	121 ± 16	27.5 ± 1.1	17.3 ± 0.6	-32.8 ± 0.5

**Table 5** Linear mixed effects models testing for differences in leaf traits between CO<sub>2</sub> treatment, species and fertilizer addition (Fert.) and the interaction between CO<sub>2</sub> treatment and species for plants grown in a species mix (microcosms) or in isolation (other interactions not shown, since they were not significant at the 0.1 level)

Species mix					Species in isolation				
	df	F	P-values			F	P-values		
<i>SLA (cm<sup>2</sup> g<sup>-1</sup>)</i>									
CO <sub>2</sub>	1, 4	9.71	0.036	*	CO <sub>2</sub>	1, 4	1.34	0.311	
Species	4, 44	291.18	< 0.001	***	Species	3, 28	298.57	<0.001	***
CO <sub>2</sub> x species	4, 44	0.71	0.591		CO <sub>2</sub> x species	3, 28	0.60	0.622	
					Fert.	1, 28	0.78	0.385	
<i>Leaf thickness (μm)</i>									
CO <sub>2</sub>	1, 4	0.19	0.688		CO <sub>2</sub>	1, 4	1.97	0.233	
Species	3, 34	160.55	< 0.001	***	Species	3, 25	238.25	<0.001	***
CO <sub>2</sub> x species	3, 34	1.09	0.365		CO <sub>2</sub> x species	3, 25	0.97	0.421	
					Fert.	1, 25	15.65	<0.001	***
<i>NSC (mg g<sup>-1</sup> d.w.)</i>									
CO <sub>2</sub>	1, 4	11.46	0.028	*	CO <sub>2</sub>	1, 4	0.02	0.884	
Species	7, 55	77.67	< 0.001	***	Species	3, 30	28.30	<0.001	***
CO <sub>2</sub> x species	7, 55	1.29	0.275		CO <sub>2</sub> x species	3, 30	0.99	0.412	
					Fert.	1, 30	0.05	0.828	
<i>C/N ratio</i>									
CO <sub>2</sub>	1, 4	14.35	0.019	*	CO <sub>2</sub>	1, 4	0.05	0.829	
Species	8, 66	75.56	< 0.001	***	Species	3, 28	72.70	<0.001	***
CO <sub>2</sub> x species	8, 66	0.44	0.893		CO <sub>2</sub> x species	3, 28	0.45	0.720	
					Fert.	1, 28	1.95	0.174	
<i>Nitrogen (mg g<sup>-1</sup> d.w.)</i>									
CO <sub>2</sub>	1, 4	26.93	0.007	**	CO <sub>2</sub>	1, 4	1.08	0.357	
Species	8, 66	67.17	< 0.001	***	Species	3, 28	65.52	<0.001	***
CO <sub>2</sub> x species	8, 66	0.69	0.697		CO <sub>2</sub> x species	3, 28	0.79	0.508	
					Fert.	1, 28	1.65	0.210	
<i>δ<sup>13</sup>C (‰)</i>									
CO <sub>2</sub>	1, 4	188.23	< 0.001	***	CO <sub>2</sub>	1, 4	219.33	<0.001	***
Species	8, 78	38.14	< 0.001	***	Species	3, 30	24.87	<0.001	***
CO <sub>2</sub> x species	8, 78	16.13	< 0.001	***	CO <sub>2</sub> x species	3, 30	1.37	0.271	
					Fert.	1, 30	0.1	0.828	

\**P*<0.05; \*\**P*<0.01; \*\*\**P*<0.001.

in the long run, though at a much reduced rate of only +23% stimulation in *Luzula* and +4% stimulation in *Poa* ( $F_{1,4} = 4.73$ ,  $P = 0.095$ , ambient compared to elevated grown plants measured at their respective growth CO<sub>2</sub> concentration). So, plants grown under elevated CO<sub>2</sub> exhibited a significantly lower A<sub>net</sub> than individuals grown under ambient when measured at the same, high or low, leaf chamber CO<sub>2</sub> concentration (*Luzula* -30% and *Poa* -22%,  $F_{1,4} = 15.96$ ,  $P = 0.016$ ), suggesting strong downward adjustment of photosynthetic capacity after long-term exposure to elevated CO<sub>2</sub> treatment in both graminoid species tested. The graminoids grown under elevated CO<sub>2</sub> operated at slightly increased leaf internal CO<sub>2</sub> concen-

trations ( $c_i$ , +18 ppm across both species and both leaf chamber CO<sub>2</sub> concentrations,  $F_{1,4} = 7.47$ ,  $P = 0.052$ ), reflecting their down-regulated biochemical CO<sub>2</sub> uptake efficiency (Supporting Information, Table S1). Stomatal conductance (g) was reduced by the high leaf chamber CO<sub>2</sub> concentration ( $c_a$ ) of 580 ppm compared to 385 ppm CO<sub>2</sub> in both ambient and elevated grown individuals (*Luzula* -11%, *Poa* -29%,  $F_{1,59} = 8.70$ ,  $P = 0.005$ ). These stomatal responses to elevated CO<sub>2</sub> will likely reduce water loss also in the long run, as we found no significant long-term adjustment of stomatal conductance in plants grown under elevated CO<sub>2</sub> (when compared with ambient grown individuals measured at the same  $c_a$ ).

## Discussion

One would expect high-elevation plants, naturally growing under reduced partial pressure of CO<sub>2</sub>, to be particularly responsive to atmospheric CO<sub>2</sub> enrichment. When this hypothesis was first tested in late-successional alpine grassland communities, it found no support irrespective of nutrient addition of 45 kg ha<sup>-1</sup> a<sup>-1</sup> (Körner *et al.*, 1997). Strong competition, full exploitation of the rhizosphere and a specific set of unresponsive species were assumed to have prevented growth stimulation by elevated CO<sub>2</sub>. In the current experiment, we show that alpine pioneer vegetation, growing in open terrain and provided with ample space to expand is not promoted either. Three years of FACE at 2440 m elevation neither showed any stimulation of biomass production nor of reproduction, with or without additional nutrient supply. The absence of growth enhancement by elevated CO<sub>2</sub> was found in all species and there were no significant species-specific differences in the plant biomass response to CO<sub>2</sub> treatment, not even between fast- and slow-growing species (more and less competitive species). Furthermore, there was no differential CO<sub>2</sub> response between plants grown in a close to natural species mix and plants grown in isolation. Along the three-season duration of CO<sub>2</sub> enrichment, we observed that CO<sub>2</sub> responses of the monitored biometric traits became slightly more negative towards the end of the study, certainly not ramping up. At final harvest, aboveground biomass of the glacier forefield species studied here was reduced by overall –35% in plants grown under CO<sub>2</sub> enrichment (across all species grown in microcosms and in isolation), which was mainly caused by the reduced production of reproductive parts (including flower stalks).

A third high-elevation CO<sub>2</sub> enrichment experiment in low-stature alpine vegetation was conducted concurrently within the alpine treeline ecotone at Stillberg in the Swiss Alps (2180 m a.s.l.). This 9 year FACE study ended up with one CO<sub>2</sub> responsive and two unresponsive dwarf shrub species of the late-successional dwarf shrub understorey (Dawes *et al.*, 2011a): *Vaccinium myrtillus*, the species with the lowest elevation abundance optimum, i.e. in the montane belt, was stimulated by elevated CO<sub>2</sub> (+12% annual

shoot growth), whereas *Empetrum nigrum* and *Vaccinium uliginosum*, both with an alpine preference, revealed no effect. The treeline forming evergreen conifer *Pinus unicata* also showed no response to elevated CO<sub>2</sub> at any time, while the similarly sparsely grown 30-year-old *Larix decidua* showed a cumulative increase in canopy size, stem basal area and total new shoot production under elevated CO<sub>2</sub>, although, the annual responses in tree ring increments diminished significantly in the last two treatment years (Dawes *et al.*, 2011b). In both of these high-elevation tree species, photosynthetic stimulation was substantial and only slight downward adjustments were observed (Handa *et al.*, 2005; Dawes *et al.*, 2011b). Other comparable ecosystems were studied in the Arctic: CO<sub>2</sub> enrichment in sub-arctic forest-heath in North Sweden (Gwynn-Jones *et al.*, 1997) and in wet, *Eriophorum vaginatum*-dominated tussock tundra in Alaska (Tissue & Oechel, 1987; Oechel *et al.*, 1994) either showed very small, no or even slightly negative growth responses to elevated CO<sub>2</sub> that were all highly species-specific. Photosynthetic downward adjustment was also a common response in these cold and nutrient-poor arctic ecosystems.

Herbaceous plants grown under high CO<sub>2</sub> typically accumulate more starch and sugars in leaves, reflecting an imbalance between carbon sources and sinks. Supposedly, as a response to the overflow of assimilate production, leaf nitrogen concentration is reduced even when expressed on a NSC-free dry matter basis, suggesting down-regulation of Rubisco synthesis (Stitt & Krapp, 1999). In our FACE experiment, elevated CO<sub>2</sub> induced a significant increase in total NSC concentration in leaves (only in plants grown in mixture), a higher C/N ratio and reduced leaf nitrogen concentration, which otherwise is typically high in plants of cold environments (Körner, 1989). Accordingly, photosynthetic capacity, which was in the range of photosynthetic capacities determined for alpine herbaceous species by Körner & Diemer (1987), showed downward adjustment by on average –26% after long-term exposure to elevated CO<sub>2</sub> (here studied in *Luzula* and *Poa* only), as was described before (Ellsworth *et al.*, 2004; Nowak *et al.*, 2004; Ainsworth & Long, 2005).

In the previous CO<sub>2</sub> enrichment experiment in the same region in late-successional alpine grassland, NSC accumulation and N (NSC-free) depletion in leaves under high CO<sub>2</sub> was more pronounced in the forbs (*Leontodon helveticus*, *Trifolium alpinum*) than in the dominant sedge *Carex curvula* or in *P. alpina* (Schäppi & Körner, 1997). At community level, elevated CO<sub>2</sub> led to a long-term stimulation of maximum daytime net CO<sub>2</sub> uptake by 41% in the second and third year with very moderate downward adjustments of photosynthetic rates, but this stimulation disappeared by the peak of the fourth season of CO<sub>2</sub> enrichment (Diemer & Körner, 1998). Given these results in late-successional alpine communities as well as the consistent reduction of leaf N in almost all species examined in the present study (except for *Poa*), the associated down-regulation of leaf-level photosynthesis is likely to be common to alpine plant species under long-term exposure to elevated CO<sub>2</sub>.

C<sub>3</sub> species have been found to show photosynthetic downward adjustment and reduced growth responses after longer-term exposure to elevated CO<sub>2</sub> when grown in small rooting volumes, which can act as a surrogate for nutrient limitation (Arp, 1991; Pierce *et al.*, 2003). In the current microcosm experiment, plant assemblages were grown in rather large substrate volumes relative to the small plant size. In their natural habitat in the glacier forefield, these species grow in fine sandy silt that is confined to gaps among rocks and scree, hence their rooting volumes are naturally quite restricted. Four of the nine species were used in a supplementary experiment where they were grown in isolation in 1.5 L containers each (same substrate as *in situ* and in microcosms), in order to explore the interaction of nutrient addition with the CO<sub>2</sub> response. Of the four species, only *Poa* showed significantly increased growth (size) in the larger 'multi-species' microcosms (9 L) compared to the 1.5 L containers, whereas the two forbs were actually smaller, and *Luzula* grew only slightly faster (not significant) when grown in microcosms. Yet, in no case did the increased growth, either in the microcosms or in isolation, facilitate a positive CO<sub>2</sub> effect.

Negative growth responses to elevated CO<sub>2</sub> have rarely been reported, and if so, mostly in treatment combinations of increased CO<sub>2</sub> with rather low nutrient availability. Under such conditions, plants exposed to elevated CO<sub>2</sub> may allocate more biomass to roots, presumably to forage for growth-limiting nutrients and as compensatory adjustment of the carbon source/sink balance (Brouwer, 1962; Sigurdsson *et al.*, 2001; Suter *et al.*, 2002). In the present study, such a shift in biomass partitioning in favour of roots was observed as well, which might have contributed to the negative response in aboveground biomass. The reduced aboveground biomass in plants exposed to high CO<sub>2</sub> under low nutrient availability may also be linked to an enhanced microbial nutrient immobilization induced by increased plant C inputs to the rhizosphere (Diaz *et al.*, 1993; Hättenschwiler & Körner, 1998; Luo *et al.*, 2004). However, although effectively stimulating plant growth in all species except for *Luzula* in this study, nutrient addition did not lead to a positive growth response to CO<sub>2</sub> enrichment. The missing fertilizer response in *Luzula* could be due to a subcritical fertilizer dose (25 kg N ha<sup>-1</sup> a<sup>-1</sup>) for that species, as it has previously been shown that plant responses to fertilizer addition are highly species-specific (Bowman *et al.*, 2006; Bassin *et al.*, 2007; Bobbink *et al.*, 2010). With their nearly twice as high fertilizer addition, Körner *et al.* (1997) arrived at a 100% increase of aboveground biomass in late-successional alpine grassland, but still found no positive CO<sub>2</sub> effect. Hence, irrespective of the fertilizer dose, fertilizer stimulated growth did not facilitate a higher susceptibility to increased C availability in alpine plants, contrasting widely established theory (Strain & Bazzaz, 1983; Nowak *et al.*, 2004; Körner, 2006; Reich *et al.*, 2006b).

In addition to the potentially enhanced microbial competition for nutrients under elevated CO<sub>2</sub>, the absence of any CO<sub>2</sub>-driven growth stimulation may be related to soil moisture. At low elevation, positive growth responses to CO<sub>2</sub> enrichment in grassland are almost exhaustively explained by CO<sub>2</sub>-induced water savings (Morgan *et al.*, 2004). Such stomata driven water savings under high CO<sub>2</sub> (rather than photosynthesis-driven growth responses) had also been observed in a long-term CO<sub>2</sub> experiment



in a water-limited scrub-oak ecosystem (Seiler *et al.*, 2009). In contrast, our alpine glacier forefield plants, similar to the late-successional alpine grassland and the dwarf shrub heath described above, grew without water shortage and hence, water saving effects could not translate into growth responses. This seems to be the most likely explanation of the lack of CO<sub>2</sub> responsiveness in these alpine communities.

Besides high soil moisture, low temperature, at least during the night, and determinate growth in a short growing season in the alpine belt may further constrain positive CO<sub>2</sub> effects on plant growth. Low temperature inhibition of tissue formation is likely to cause these alpine plants to be carbon sink limited (C-investment) rather than C-source limited (C-uptake; Körner, 2003). However, interactions of CO<sub>2</sub> responses with seasonal differences in temperature and enhanced nutrient supply were not observed in the late-successional alpine grassland (Körner *et al.*, 1997), similar to the dwarf shrub understorey at the alpine treeline, where no interactions with experimental soil warming were found (Dawes *et al.*, 2011a). Soil warming was hypothesized to alleviate constraints by low temperature or low N availability on growth and thus, on CO<sub>2</sub> responses. In *Larix*, however, the CO<sub>2</sub> response at the treeline was clearly temperature dependent, showing stronger stimulation in years with relatively high spring temperatures and early snow melt (Dawes *et al.*, 2011b).

Finally, atmospheric CO<sub>2</sub> concentration is currently twice as high as during the last glacial maximum and nearly 40% higher than only 200 years ago, which may have removed C-constraints to alpine plant growth, adapted to far lower CO<sub>2</sub> concentrations (Körner 2006). In view of all data currently available for alpine plant responses to elevated CO<sub>2</sub>, we conclude that CO<sub>2</sub> is not a limiting resource for high-elevation plant life at the present atmospheric CO<sub>2</sub> concentration.

In conclusion, none of our three original hypotheses found support. Three seasons of FACE revealed that the studied glacier forefield pioneers are not stimulated by elevated CO<sub>2</sub> but rather appeared to become constrained by this treatment, even when combined with enhanced nutrient supply of 25 kg ha<sup>-1</sup> a<sup>-1</sup>. This result is surprising, since young, expanding systems, like this early successional community, were expected to be particularly responsive to elevated CO<sub>2</sub>. We conclude that the ineffectiveness of any stomata driven water savings under elevated CO<sub>2</sub> in such humid habitats, low temperatures, as well as inherently slow growth, cause these alpine plants to be carbon saturated at current atmospheric CO<sub>2</sub> concentration. The great robustness of our data across species and growth conditions, and the similar findings in an earlier alpine grassland experiment add weight to this conclusion. We thus do not expect major changes in species composition in alpine glacier forefield communities. Nevertheless, some uncertainties remain, as only slight changes in carbon allocation among organs or plant species in response to elevated CO<sub>2</sub> may induce gradual, long-term changes in dominance and species composition of communities not captured in the course of three seasons. In the light of other factors of 'Global Change', such as climate warming, regional changes in precipitation, snow cover and cloudiness, increased nitrogen deposition and land use changes, direct biological effects of atmospheric CO<sub>2</sub> enrichment appear to be a marginal issue in humid alpine ecosystems.

### Acknowledgements

We are indebted to Franco Miglietta for his great help with the MiniFACE system and Arthur Weber AG in Schattdorf, Switzerland for the gas transport to our site under rather harsh weather conditions. We further thank Matthias Saurer and Rolf Siegwolf for carbon isotope analyses, Martin Bader for his help with the gas exchange system LI-6400, Georges Grun for building a custom light source for LI-6400, Franziska Hochuli, Linda Reißig, Kristina Ehram, Oliver Wirz, Gabrielle Schär and Susanna Riedl for their assistance with the final biomass harvest, Olivier Bignucolo for NSC and C/N analyses and Sebastian Leuzinger for assistance with CO<sub>2</sub> concentration data analyses. We

gratefully acknowledge the Korporation Ursern for allowing us to use their land for years, the Alpine Research Station Furka, ALPFOR (University of Basel, Korporation Ursern and Swiss Army) for providing facilities and accommodation. Finally, special thanks to the Freiwillige Akademische Gesellschaft Basel (FAG) for the financial support that allowed us to buy the MiniFACE system. N.I. received funding by the Swiss Federal Office for Agriculture.

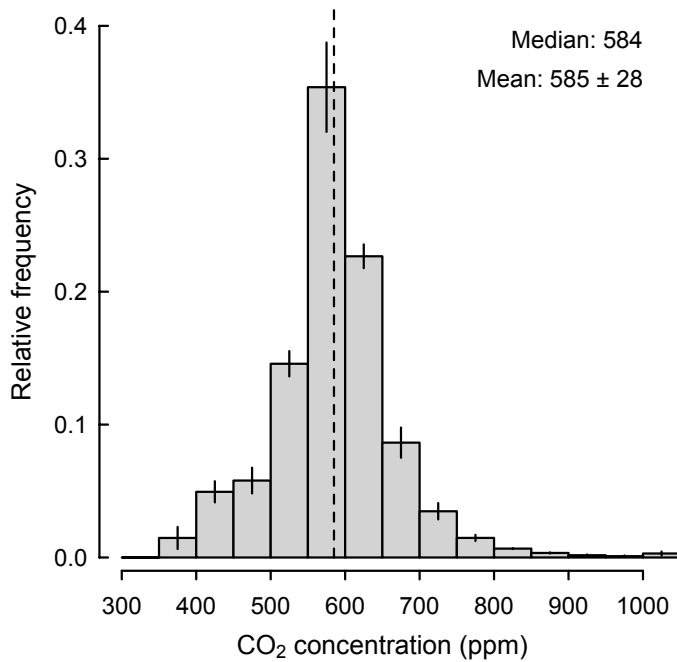
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## Supporting Information



**Figure S1.** Frequency distribution of daytime CO<sub>2</sub> concentrations (one second readings; mean ± SE of the 3 elevated plots) recorded during a representative summer week (25–31 July 2007).

**Table S1** Net photosynthesis ( $A_{\text{net}}$ ), leaf internal CO<sub>2</sub> concentration ( $c_i$ ) and leaf diffusive conductance to water vapour ( $g$ ; mean ± SE;  $n = 3$ ) of *Luzula* and *Poa* grown under ambient (A) or elevated CO<sub>2</sub> (E) and measured at leaf chamber CO<sub>2</sub> concentrations ( $c_a$ ) of 385 ppm and 580 ppm

Species	Treatment	$c_a$ (ppm)	$A_{\text{net}}$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	$c_i$ (ppm)	$g$ ( $\text{mmol m}^{-2} \text{s}^{-1}$ )
<i>Luzula</i>	A	385	12.4 ± 0.8	266 ± 12	206 ± 19
	E		8.6 ± 0.5	295 ± 4	200 ± 19
	A	580	21.6 ± 2.3	368 ± 5	194 ± 20
	E		15.2 ± 1.4	381 ± 20	167 ± 25
<i>Poa</i>	A	385	11.8 ± 0.8	294 ± 6	267 ± 42
	E		9.8 ± 0.9	301 ± 13	226 ± 33
	A	580	16.5 ± 1.1	409 ± 24	179 ± 24
	E		12.2 ± 0.8	433 ± 21	174 ± 25



# *Hydrological consequences of declining land use and elevated CO<sub>2</sub> in alpine grassland*

Nicole Inauen  
Christian Körner  
Erika Hiltbrunner

Institute of Botany  
University of Basel  
Schönbeinstrasse 6  
CH-4056 Basel, Switzerland

Key words:  
abandonment, catchment water yield,  
deep seepage, ecosystem services,  
evapotranspiration,  
free air CO<sub>2</sub> enrichment (FACE),  
land use change, leaf matter,  
Swiss Alps, weighing lysimeters,  
δ<sup>18</sup>O

Inauen N, Körner C, Hiltbrunner E  
(2013) Hydrological consequences of declining land use and elevated CO<sub>2</sub> in alpine grassland. *Journal of Ecology*, **101**, 86–96.



## Summary

1. Large areas of alpine pastures and meadows currently face declining land use or abandonment, which leads to tall-grass transition ecosystems with higher leaf area index (LAI), potentially increased evapotranspiration (ET) and thus, reduced water yield. Elevated atmospheric CO<sub>2</sub>, on the other hand, is known to reduce stomata opening and hence, leaf-level transpiration, which may translate into higher soil moisture and enhanced total runoff. Here, we quantify these opposing effects of global change on the water balance of alpine grassland in a field experiment in the Swiss Alps (2440 m a.s.l.).
2. Rates of ET and deep seepage (percolation water) of four alpine grassland types (dominated by *Agrostis*, *Nardus*, *Carex* or forbs) were measured using intact monoliths in 51 weighing lysimeters. A part of the monoliths was clipped to simulate sheep grazing during three seasons (2008–2010). Another set was exposed to elevated CO<sub>2</sub> (580 ppm) using free-air CO<sub>2</sub> enrichment (FACE) during the 2009 growing season.
3. Simulated grazing reduced bright day ET by on average –12% across all years, with the most pronounced effects in the high-stature swards. Correspondingly, the higher biomass and LAI in unclipped grassland lowered the seasonal sum of deep seepage by –13% in a drier summer (2009) and by –5% in a rather wet summer (2010) compared to clipped swards.
4. CO<sub>2</sub> enrichment reduced ET in all grassland types by –3% to –7%, increased δ<sup>18</sup>O in foliage and enhanced soil moisture, but not deep seepage. Hence, future CO<sub>2</sub> slightly counteracts the land use effects at canopy level, however, not in terms of water yield.
5. *Synthesis.* Our results indicate that both grazing and elevated CO<sub>2</sub> are mitigating the effects of dry spells on alpine vegetation. The net effect of the continuous decline in land use and of elevated CO<sub>2</sub> is negative for catchment water yield and thus, for potential hydroelectric power production. Although these economic ‘costs’ are rather moderate per hectare of alpine grassland, sums are substantial when scaled to the vast areas potentially affected in the Alps. These calculated ‘costs’ attribute economic value to the eco-hydrological benefits of land care at these high elevations.

## Introduction

Does the management of alpine grassland matter for the ecosystem water balance and catchment water yield? Large areas of mountainous high-elevation terrain are used agriculturally worldwide (mainly livestock grazing), some are maintained in a sustainable way, others are either overgrazed or face abandonment. While overgrazing and associated soil erosion have received substantial attention (e.g. Morgan 2005), the hydrological consequences of declining land use and abandonment have not. A dense, tall leaf canopy is likely to transpire more, depletes soil water stores faster and yields less deep seepage (percolation water). The water yield of a catchment should thus become reduced and likewise the hydroelectric potential, when land use on alpine pastures and meadows is reduced or even abandoned. Hence, in addition to aspects of the preservation of the available agricultural area, of soil and biodiversity conservation (Tasser, Mader & Tappeiner 2003; Fischer *et al.* 2008), there are eco-hydrological consequences of alpine land abandonment that await quantification, given the large extent of the current transitions in land use.

Across the European Alps, often referred to as the water tower of Europe (Weingartner, Viviroli & Schädler 2007), 33% of all farms were abandoned between 1980 and 2000 (Streifeneder *et al.* 2007). As a result, large areas of poorly accessible alpine grassland are currently no longer used, and traditional, labour intensive practices of land management are being abandoned (e.g. decline in shepherding, substitution of meadows by pastures) with a concurrent intensification in land use on more easily accessible and more productive parcels (MacDonald *et al.* 2000; Tasser & Tappeiner 2002; Gellrich *et al.* 2007). In Switzerland, mountain pastures and meadows currently cover almost 28% of montane and alpine terrain (> 1000 m a.s.l.), representing 35% (537 800 ha) of the total Swiss agricultural land (FSO 2005), of which 60% are at risk of being abandoned in certain regions of the Alps in future (Gotsch, Flury & Rieder 2004). These abandoned grasslands either develop into forest and/or shrubland (74%; FSO 2005; Gellrich *et al.* 2007) or – at high-elevation – end up as dwarf shrub heaths or tall swards with high leaf area



index, necromass and plant litter production (Tappeiner & Cernusca 1989; Wohlfahrt *et al.* 2003; Merz *et al.* 2009). Since tall vegetation intercepts and also transpires more water, these land cover changes are likely to affect evapotranspiration (ET) and the water balance (Körner, Wieser & Cernusca 1989; Asner *et al.* 2004; Tasser, Tappeiner & Cernusca 2005; Garcia-Ruiz & Lana-Renault 2011). Here, we quantify these catchment-wide eco-hydrological consequences of land use change, which may also help policy, landscape planners as well as stakeholders in a comprehensive economic assessment of sustainable management versus land abandonment in alpine terrain.

Atmospheric CO<sub>2</sub> has been shown to affect the water consumption of grassland as well, and is likely to reach more than twice pre-industrial concentration by the end of this century (Meehl *et al.* 2007). Elevated concentrations of CO<sub>2</sub> are known to reduce stomata opening and hence, leaf-level transpiration with water savings inevitably leading to temporally increased soil water content in grassland (Morgan *et al.* 2004) and possibly translating into greater total runoff. The leaf-level effects are commonly diminished at ecosystem-scale for reasons associated with aerodynamic coupling, rainfall patterns, soil water storage capacities or plant growth responses to elevated CO<sub>2</sub> (Leuzinger & Körner 2010) and thus, are hard to predict.

In this study, we aim to quantify these partly opposing effects of reduced land use or abandonment and elevated atmospheric CO<sub>2</sub> concentration on ET, soil moisture and growing season runoff in alpine grassland of the central Swiss Alps. We employed *in-situ* lysimeters with intact monoliths of alpine grassland subjected to grazing simulation (clipping) and to free-air CO<sub>2</sub> enrichment (FACE). This approach is novel by combining a high number of monoliths of different grassland types with FACE and applying mass balance lysimeters to assess the treatment effects on different time scales and components of the water balance. Integrated over time, effects on seasonal sums of deep seepage (percolation water from soil surface to the subsoil and groundwater) and ET are quantified. We expect abandonment of grazing to reduce water yield in alpine grassland, and a future double pre-industrial CO<sub>2</sub> atmosphere to increase it.

## Materials and methods

### Study site

In the Ursern Valley (central Swiss Alps), where the present study site is located (Furka Pass, 46°34'N 8°25'E, 2440 m a.s.l.), alpine grassland and dwarf shrub heaths cover 68% of the land surface area of the alpine zone, between 2100 m and 2700 m a.s.l. (T. van den Bergh, unpubl. data). This zone represents 58% of the upper catchment of the river Reuss down to the village of Andermatt (22 730 ha), a typical high-alpine catchment. Grassland dominated by *Carex curvula*, *Nardus stricta* (lower alpine zone) and variants of forb-rich turf cover vast areas of alpine terrain in the Alps including our study area. The grassland surrounding our study site is currently grazed at different intensities by small stationary herds of sheep and bigger migrating flocks during summer and autumn (on average every second year). Grassland occurs on relatively deeply weathered profiles of partly podzolized alpine brown earth on siliceous bedrock.

Precipitation generally increases with elevation in the Alps, and annual sums average at c. 1900 mm at the study site (Atlas of Switzerland 3, Institute of Cartography, ETHZ). The growing season lasts between 2.5 and 3.5 months at this elevation with snowmelt in June and plant senescence in September. Meteorological conditions during the growing season were monitored by a weather station (Wireless Vantage Pro2 Plus™, Davis Instruments, Hayward, CA, USA) set up at the study site at 1.5 m above ground. Precipitation was additionally measured at 40 cm above ground level by a rain gauge (RAINEW 111, RainWise Inc., Bar Harbor, ME, USA) and at 5 cm by two totalisators (funnel and bottle). Year-round climate data records are available from the meteorological station 'Gütsch' (2287 m a.s.l.; 17 km northeast) operated by the Swiss Federal Office of Meteorology and Climatology, MeteoSwiss. The 2009 growing season was warmer and drier than the other experimental seasons (2008 and 2010) with particularly low precipitation in August (see Table S1. in Supporting Information).

### *Methods of water balance measurement*

We employed weighing lysimeters, set up in summer 2008, to solve the hydrological water balance equation in monoliths of alpine grassland vegetation (Fig. 1). In its simplest form, precipitation (P) equals the sum of ET (incl. interception), total runoff (R, the sum of surface runoff and deep seepage) and differences in soil water content ( $\Delta S$ ).

$$P = ET + R + \Delta S$$

The sum of evaporation from soil surface and transpiration by foliage (ET) was measured on a daily basis by weighing the lysimeters on bright days. Interception contributes only a small fraction to the total water balance of alpine grassland (c. 7% of growing season P; Körner, Wieser & Cernusca 1989). The seepage volume, collected in reservoirs below the lysimeters, is a measure of total percolation water (R), i.e. deep seepage plus surface runoff, since the c. 2 cm collar at the upper edge of the lysimeters prevents lateral water flows into and out of the lysimeters. As soils are naturally saturated with water after snowmelt in spring, and because of repeated saturating rain events during the growing season, the  $\Delta S$  term (variation in volumetric soil water content, SWC) of the ecosystem water balance usually becomes negligible on a seasonal basis. Thus, knowing the amount of seepage water, precipitation and  $\Delta S$  over one season, seasonal ET can be calculated (including days with weather conditions unsuitable for assessing ET by weighing).

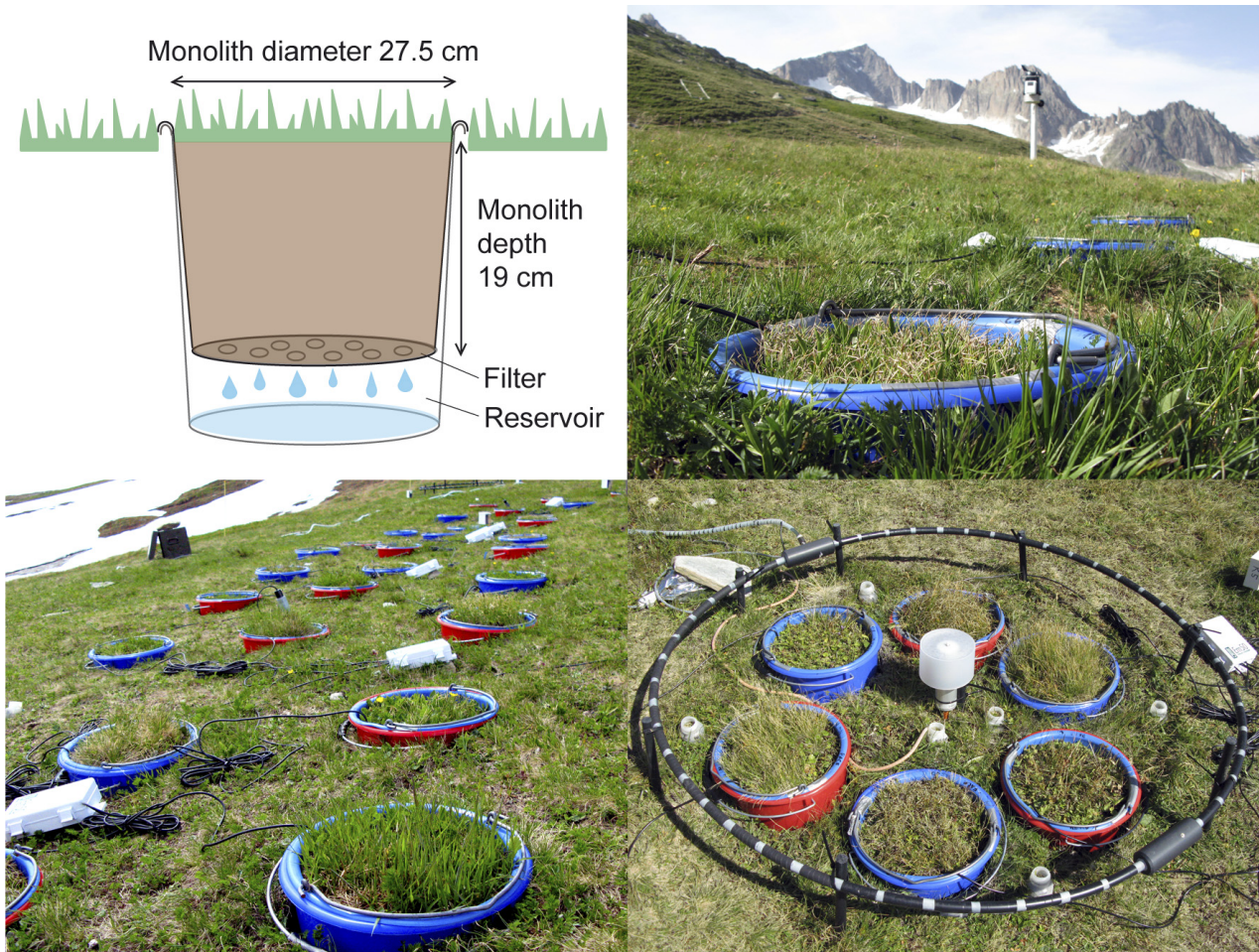
The lysimeters consisted of two plastic buckets of the same diameter (27.5 cm, surface area: 0.059 m<sup>2</sup>): a shallower (21 cm) inside a deeper one (30 cm; Fig. 1). The inner bucket was filled with a largely undisturbed alpine grassland monolith and had a perforated bottom, which permitted to collect seepage water in the outer bucket. The soil monoliths were all taken from the surrounding area (<1 km) of the study site and similar elevation (2440–2480 m a.s.l.). They were shaped with a knife to precisely fit into the slightly conical buckets avoiding any air gaps between the monolith and the bucket wall. Skeleton-free soil volume averaged at 10 L with soil

depths of c. 19 cm. Although rooting in alpine grassland is often deeper, c. 75% of all roots are confined to the top 10 cm, and c. 65% to the uppermost 2.5 cm (Körner 2003). Given the rainfall regime in this area, the water stored in that profile depth prevented any moisture limitation, except for longer rainless periods, when monoliths received known amounts of water (as was considered necessary once during an exceptionally dry period in Aug 2009). Soil moisture sensors (EC-5, EC-10, EM50, Decagon Devices Inc., Pullman, WA, USA) were inserted into the root zone of each lysimeter at 5–10 cm below soil surface and SWC was recorded hourly (plus seven probes outside the lysimeters served as controls). The lysimeters were sunk into the ground with the upper rim of the buckets slightly (1–2 cm) above the surrounding soil surface.

Daily ET was directly measured by weighing the grassland monoliths in the morning and evening hours of days without rain using a balance placed in an on-site shelter (BBK422-35LA, Mettler-Toledo Inc., Greifensee, Switzerland; precision  $\pm 0.1$  g). Monoliths were weighed at least once after sunrise at 6 a.m. and once after the sun disappeared from the study site between 6 and 7 p.m. depending on day length. On a few days, monolith weight was assessed every two hours for diurnal courses of ET. Bright day ET was recorded for 19, 31 and 15 days in 2008, 2009 and 2010, between end of June and mid September (except for 2008, when the experiment started in mid July). Seepage volumes were determined after major rain events: around 10 times per growing season (mid June–mid Sept) and summed up to seasonal sums. Two heavy rain events caused overnight flooding of the lysimeters in 2010. In these two cases, the amount of seepage of each monolith was thus estimated using SWC (see below) before and after the rain events and the amount of precipitation. However, this calculation likely underestimated the effect of clipping on the 2010 seepage sum.

### *Land use experiment*

For the land use experiment we chose four sward types representing the most abundant vegetation in the study area. The different types of swards were either dominated by the grasses *Agrostis schraderiana*



**Fig. 1.** A diagram and photographs of weighing lysimeters at Furka Pass, Switzerland (2440 m a.s.l.). The bottom of the inner bucket of the weighing lysimeters is perforated to allow collecting percolation water in the reservoir of the outer bucket. The filter pad at the bottom of the inner bucket prevents soil particles from being washed out with seepage water. Monolith volume averages at 10 L. The top right picture shows a clipped grassland monolith with the weather station in the background, and the bottom right picture shows six unclipped grassland monoliths in a FACE ring (Free-Air CO<sub>2</sub> Enrichment).

(sub-dominant species: *Ligusticum mutellina*; nomenclature following Lauber & Wagner 2007) or *Nardus stricta* (*Ligusticum mutellina*, *Leontodon helveticus*), by the sedge *Carex curvula* (*Ligusticum mutellina*, *Leontodon helveticus*) or by different forb species (*Geum montanum*, *Trifolium alpinum*, *Ranunculus montanus*, *Potentilla aurea*) to cover a wide range of vegetation structures (regarding vegetation height, density or the proportion of horizontal vs. vertical structures) and of plant biomass or leaf area index (LAI; Table 1). *Agrostis schraderiana*-dominated swards, although not as abundant as the other grassland types, were chosen to mirror the structure of grassland of the lower alpine belt under decreasing land use intensity, leading to tall grass canopies. In the following, we

address the grassland types by the abbreviations As for *A. schraderiana*, Ns for *N. stricta*, Cc for *C. curvula* and forb. We set up six monoliths of each of the four grassland types (24 in total), of which half ( $n = 3$ ) were clipped (to a vegetation height of 3–4 cm) at the peak of standing biomass (24 July 2008, 25 July 2009, 2 Aug 2010) as is the typical grazing by sheep (the common land use practice in the studied alpine grassland). Vegetation was kept short during the remaining weeks of the season. Our treatment is an approximation of sheep grazing, which is, by our experience in this area, more uniform than cattle grazing at lower elevation. Although the species *N. stricta* is avoided by most grazers as long as other fodder is abundant, we included vegetation with *N. stricta*, which is more

**Table 1.** Characterisation of the grassland types used in the land use experiment: plant dry matter partitioning, cryptogam mass, leaf area index (LAI; both assessed in autumn 2010, including phytomass removed by clipping) and maximum gross canopy height prior to clipping (means  $\pm$  SD;  $n = 6$  lysimeters)

	<i>Agrostis schraderiana</i>	<i>Nardus stricta</i>	<i>Carex curvula</i>	Forb	<i>P</i> -values
Plant mass partitioning (g m <sup>-2</sup> )					
Biomass	229 $\pm$ 44 <sup>a</sup>	208 $\pm$ 91 <sup>a</sup>	102 $\pm$ 33 <sup>b</sup>	246 $\pm$ 47 <sup>a</sup>	<b>&lt; 0.001</b>
Necromass	8 $\pm$ 3 <sup>a</sup>	40 $\pm$ 16 <sup>b</sup>	24 $\pm$ 9 <sup>c</sup>	14 $\pm$ 9 <sup>d</sup>	<b>&lt; 0.001</b>
Litter	136 $\pm$ 41	237 $\pm$ 120	138 $\pm$ 49	137 $\pm$ 50	<i>n.s.</i>
Cryptogams	19 $\pm$ 18 <sup>a</sup>	86 $\pm$ 89 <sup>ab</sup>	87 $\pm$ 34 <sup>b</sup>	90 $\pm$ 94 <sup>ab</sup>	<b>0.040</b>
LAI (m <sup>2</sup> m <sup>-2</sup> )	2.6 $\pm$ 0.5 <sup>a</sup>	1.2 $\pm$ 0.5 <sup>b</sup>	0.8 $\pm$ 0.3 <sup>b</sup>	2.5 $\pm$ 0.5 <sup>a</sup>	<b>&lt; 0.001</b>
Max. canopy height (cm)	11.8 $\pm$ 3.5 <sup>a</sup>	8.0 $\pm$ 1.8 <sup>b</sup>	5.8 $\pm$ 1.7 <sup>b</sup>	7.0 $\pm$ 2.6 <sup>b</sup>	<b>0.003</b>

*P*-values show results of ANOVA, bold values are statistically significant ( $P > 0.05$ ), means with different letters differ significantly based on Tukey's test ( $P < 0.05$ ).

abundant in the lower alpine zone, for the sake of completeness. The LAI of such swards is also reduced by grazing through the selective removal of all other species than *N. stricta*, thus, not what we could simulate. Clipped phytomass was separated into necromass (attached, dead material) and biomass of different plant functional types (grasses, sedges, forbs, dwarf shrubs), dried at 80°C and weighed. Leaf area was measured using the LI-3000C Portable Area Meter in combination with the LI-3050C Transparent Belt Conveyor Accessory (LI-COR Biosciences Inc., Lincoln, NE, USA). Maximum gross canopy height (excluding inflorescences) was recorded at peak season in each experimental year (before clipping).

#### *CO<sub>2</sub> enrichment experiment*

To explore ET responses to elevated CO<sub>2</sub>, we set up a second series of identically constructed weighing lysimeters in summer 2008 (12 *N. stricta*, 12 *C. curvula* and 12 forb-dominated) and exposed half of them to Free-Air CO<sub>2</sub> Enrichment (MiniFACE). Elevated CO<sub>2</sub> was provided by three 1.3 m diameter FACE rings, delivering pure CO<sub>2</sub> (Inauen, Körner & Hiltbrunner 2012), from 1 July to 9 September 2009. The target CO<sub>2</sub> concentration was 580 ppm, and CO<sub>2</sub> concentrations were recorded and controlled every second, for each FACE ring separately. FACE was switched off during 10 single days to obtain daily ET signals without CO<sub>2</sub> enrichment for standardisation, since the CO<sub>2</sub> effect on ET was expected to be small with

considerable variance among monoliths. The monoliths were randomly assigned to CO<sub>2</sub> treatments and two lysimeters of each grassland type were placed in each of the three FACE rings, adding up to a total of six lysimeters per ring (Fig. 1). For all monoliths in the CO<sub>2</sub> experiment, we randomised positions within treatment groups (ambient or elevated CO<sub>2</sub>) every two weeks, which allowed us testing each lysimeter as a spatially uncorrelated replicate ( $n = 6$ ). All monoliths in the CO<sub>2</sub> experiment remained unclipped. The unclipped control monoliths of the land use experiment (three of each grassland type *Ns*, *Cc* and forb) also served as controls for the CO<sub>2</sub> experiment, hence, the total number of monoliths across both experiments was: 6 *As*, 15 *Ns*, 15 *Cc* and 15 forb (51 lysimeters in total).

For the analysis of the ratio of heavy (<sup>18</sup>O) to light (<sup>16</sup>O) stable oxygen isotope concentration in leaf tissue under ambient and elevated CO<sub>2</sub> (expressed as  $\delta^{18}\text{O}$ , relative to the international standard VSMOW), we collected leaf samples of the most common species in all grassland types used in monoliths on 20 August 2009 (six graminoids and six forbs; for species names see Table 4). In order not to disturb lysimeters and given the same soil moisture readings inside and outside monoliths, leaf samples were taken immediately next to the monoliths from the native grassland growing inside and outside FACE rings. For each species, leaf samples (current season green foliage only) of several individuals were pooled (one pooled

sample per FACE ring and three pooled samples taken from control plots outside FACE rings,  $n=3$ ). The samples were dried for 48 h at 80 °C, ground to fine powder, put in tin capsules (0.5–0.7 mg) and after pyrolysis analysed on a continuous flow isotope ratio mass spectrometer (EA-1108, Carlo Erba Thermoquest, Milan, Italy; CONFLO II and DELTA PLUS XP, Thermo Finnigan, Bremen, Germany; precision of  $\delta^{18}\text{O}$  analyses  $\pm 0.3$  ‰).

After three seasons of operation (Sept 2010), aboveground plant material of all monoliths was harvested down to ground level in steps of 4 cm and treated as described above (categories: phytomass, cryptogams and litter, *i.e.* detached, dead material). The soil monoliths were subsequently oven-dried at 100 °C. Actual volumetric soil water content (SWC, mm) of each monolith was then calculated from dry weight for any weighing date during the experimental period. The highest soil water content after one day of drainage of saturated soils was assumed to represent the water content at ‘field capacity’ ( $\theta_{fc}$ ). For monoliths this  $\theta_{fc}$  is an approximation and does not necessarily reflect  $\theta_{fc}$  of soil outside lysimeters due to the interruption of the capillary continuum by the lysimeters, but served to standardize soil moisture data measured by soil moisture sensors. To explore relative differences in SWC among grassland types and treatments, all SWC data was set to the same initial soil water content (across all sensors) on the first weighing occasion of each growing season.

#### Statistical analyses

The statistical analyses of the results of the land use experiment and the  $\text{CO}_2$  experiment were performed separately using the open-source software R, version 2.13.2 (R Development Core Team 2011) with the package ‘nlme’ (Pinheiro *et al.* 2011). In the land use experiment, mean daily ET rates (averaged across all three seasons), sums of seasonal runoff and mean soil water contents (across the period after clipping) were analysed using analysis of variance (ANOVA) with the factors ‘grassland type’ and ‘clipping’. In all models, residuals were tested visually for normality and homogeneity of variances. In case of non-normality and inhomogeneity, we applied power or log trans-

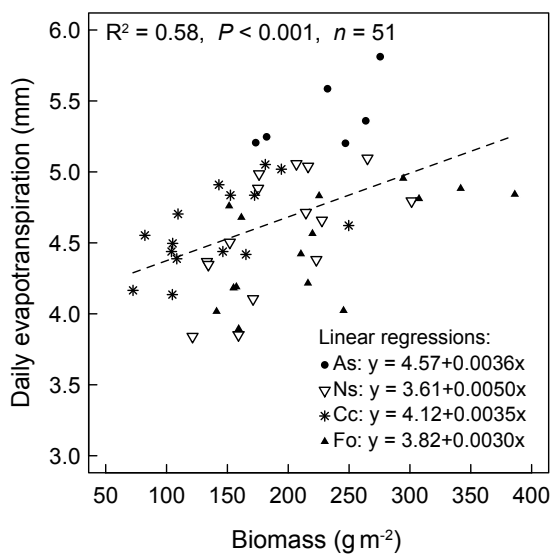
formations to the response variables or we fit a constant variance function using ‘gls’ (generalized least squares fit by ‘REML’). All models were subsequently reduced to the minimum adequate model by removing non-significant factors or interactions (not shown in tables of results of statistical tests) and merging grassland types that did not differ significantly in their response. Model selection was performed by comparing nested models, using log-likelihood ratio tests. For a *posteriori* comparison, we applied Tukey’s HSD to test multiple group means.  $\text{CO}_2$  effects on mean daily ET rates, mean SWC (both across the 2009 season) and the seasonal sums of deep seepage were analysed, employing models with the factors ‘grassland type’, ‘ $\text{CO}_2$  treatment’ and, for ET only, the covariate  $\text{ET}_{\text{CO}_2\text{off}}$  *i.e.* the mean ET rates of each monolith across all days when FACE was switched off. In all analyses,  $P$ -values  $< 0.05$  were considered statistically significant and  $P$ -values  $< 0.1$  marginally significant.

## Results

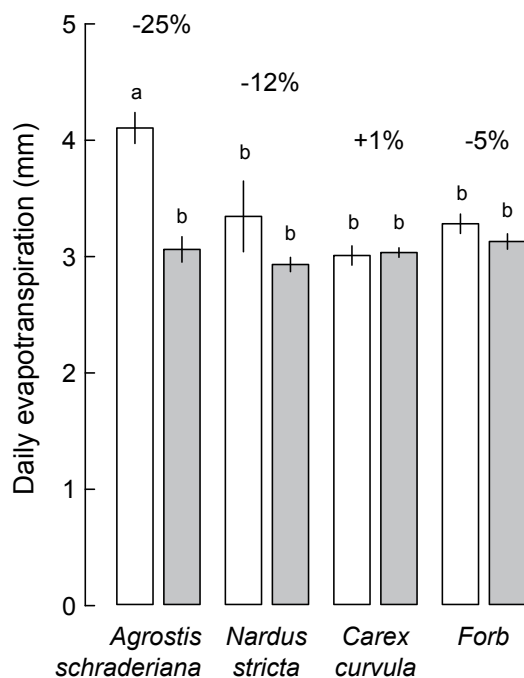
### *Effects of land use change on the water balance*

Mean total biomass and LAI in monoliths employed in the land use experiment were highest in *A. schraderiana* (As) and the forb-dominated communities (forb), slightly lower in *N. stricta* (Ns) and lowest in *C. curvula* swards (Cc) by the end of the experiment (autumn 2010; Table 1). In the graminoid-dominated swards, graminoid cover averaged at  $62 \pm 11\%$  (largely vertical structures), and in the forb-dominated swards, forb cover was  $69 \pm 7\%$  (largely horizontal structures; means  $\pm$  SD). The fraction of necromass in monoliths ranged from 4% (As) to 24% (Cc) of phytomass. The amount of plant litter plus cryptogams (lichens and mosses) surpassed the amount of phytomass in grassland types dominated by *N. stricta* and *C. curvula*. We found a statistically significant, positive linear correlation between the amount of biomass and mid-season ET (July) of these monoliths prior to the clipping treatment (Fig. 2). However, the grassland types behaved clearly differently ( $F_{3, 46} = 15.5$ ,  $P < 0.001$ ) with *A. schraderiana* showing higher ET at similar amounts of biomass than the other, lower-stature grassland types. Better atmospheric coupling of the taller stands is a





**Fig. 2.** Bright day rates of evapotranspiration (ET) of four alpine grassland types before clipping (July 2010) in relation to aboveground biomass (harvest in Sept 2010; dashed line: regression across all grassland types). Regressions by grassland type: As *Agrostis schraderiana*  $R^2 = 0.24$ ,  $n.s.$ ; Ns *Nardus stricta*  $R^2 = 0.32$ ,  $P = 0.016$ ; Cc *Carex curvula*  $R^2 = 0.28$ ,  $P = 0.024$ ; Fo forb-dominated  $R^2 = 0.33$ ,  $P = 0.014$ .



**Fig. 3.** Mean daily ET rates (means  $\pm$  SE,  $n = 3$  weighing lysimeters) of unclipped (white bars) and clipped (grey bars) alpine grassland swards across the years 2008–2010 (period after first clipping). Bars with different letters are significantly different.

likely explanation. Bright day courses of ET closely followed the course of vapour pressure deficit (VPD), with a peak between 11 a.m. and 1 p.m. (data not shown). ET was not reduced under dry weather conditions, even when monoliths reached the lowest soil water contents that were measured during the three test seasons, namely  $62 \pm 8\%$  of the water content at 'field capacity' ( $\theta_{ic}$ :  $103 \pm 9$  mm or  $54 \pm 5\%$  vol across all monoliths; means  $\pm$  SD). Hence, soil moisture never constrained ET at this elevation.

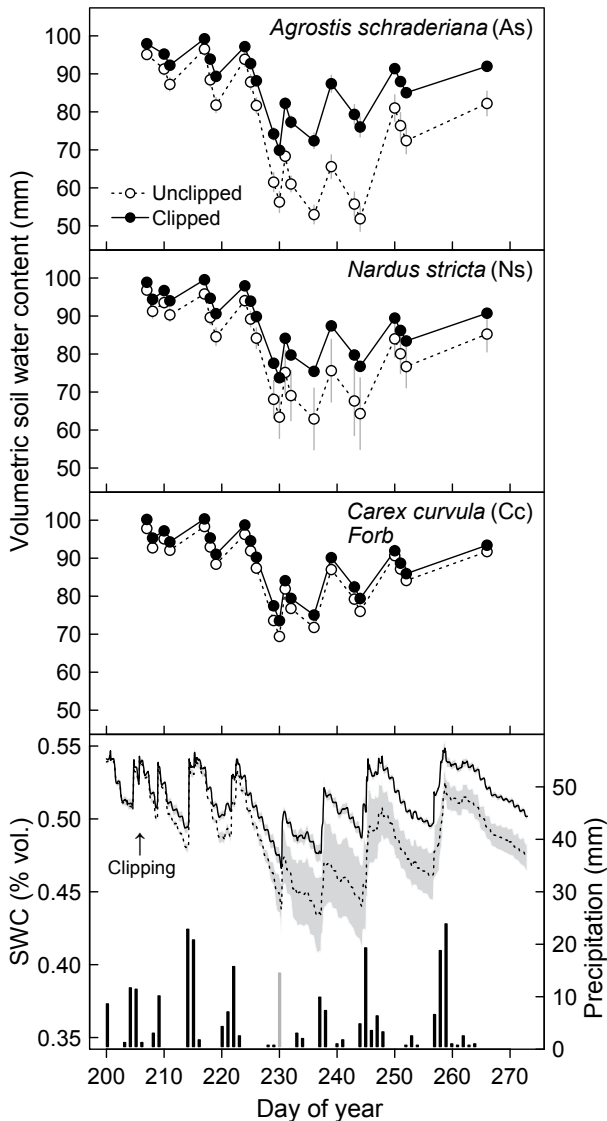
Depending on year, the clipping treatment reduced mean bright day ET by  $-21\%$  to  $-32\%$  in the high-stature *A. schraderiana*, by  $-8\%$  to  $-14\%$  in *N. stricta*, and least ( $+2\%$  to  $-8\%$ ) in the lowest-stature swards (sedge- or forb-dominated; Fig. 3, Table 2a). On average, this reduction by clipping amounted for  $-12\%$  of daily ET across all years and all grassland types. The difference in the clipping effect between grassland types corresponded to the amount

**Table 2.** The effect of grassland type (GT), simulated grazing (clipping) and their interaction on (a) mean daily evapotranspiration (ET; across all years, 2008–2010), (b) volumetric soil water content SWC (averaged across all dates in 2009) and (c) seasonal sums of deep seepage

	df	F	P-values
<i>a) Mean daily ET</i>			
GT	1, 20	4.4	<b>0.049</b>
Clipping	1, 20	16.9	<b>&lt;0.001</b>
GT x Clipping	1, 20	15.0	<b>&lt;0.001</b>
<i>b) Mean SWC</i>			
GT	2, 18	3.6	<b>0.047</b>
Clipping	1, 18	16.7	<b>&lt;0.001</b>
GT x Clipping	2, 18	3.1	0.070
<i>c) Deep seepage sum</i>			
<i>2008</i>			
GT	2, 18	5.3	<b>0.015</b>
Clipping	1, 18	39.2	<b>&lt;0.001</b>
GT x Clipping	2, 18	17.0	<b>&lt;0.001</b>
<i>2009</i>			
GT	2, 18	3.7	<b>0.046</b>
Clipping	1, 18	27.1	<b>&lt;0.001</b>
GT x Clipping	2, 18	5.9	<b>0.011</b>
<i>2010</i>			
GT	1, 20	8.7	<b>0.008</b>
Clipping	1, 20	14.7	<b>0.001</b>
GT x Clipping	1, 20	3.5	0.077

Bold  $P$ -values are statistically significant ( $P > 0.05$ ).

of biomass removed by clipping (As: 47%, Ns: 21%, Cc: 16%, forb: 18%). In general, clipping effects were highest during the first few weeks after clipping and diminished towards the end of the growing season, although the vegetation was kept short during the whole growing season after first clipping. Similarly, daily ET rates decreased by late season due to plant senescence and lower temperatures and lower daily irradiance sums (Table S1).

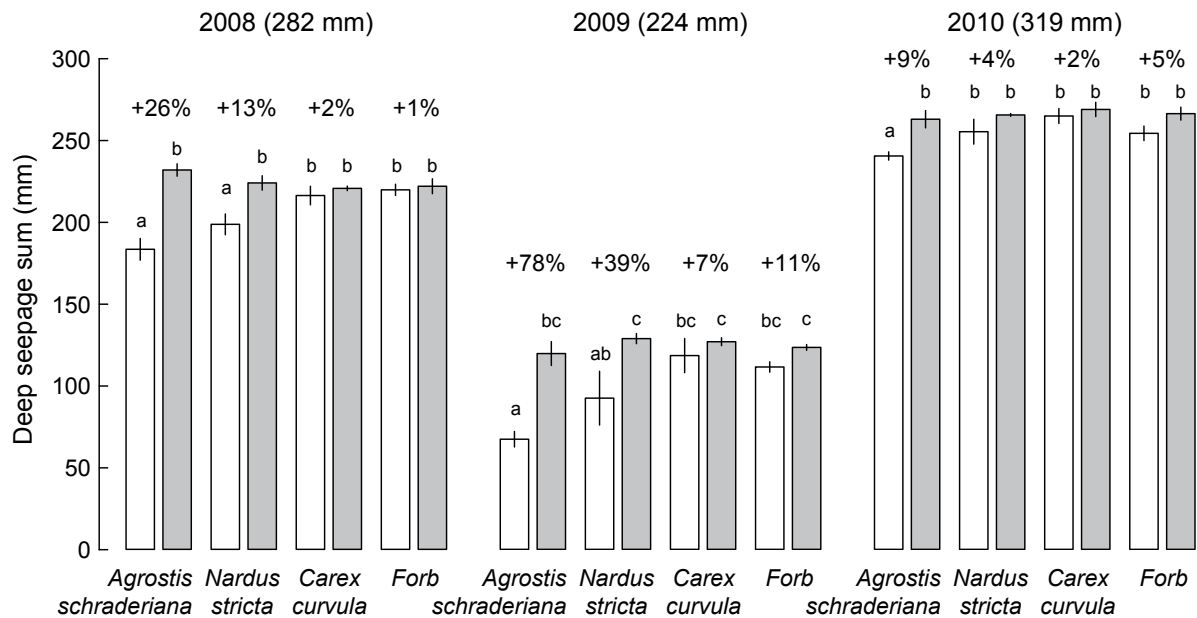


**Fig. 4.** Top panels: Volumetric soil water content (SWC) of unclipped and clipped lysimeters (means  $\pm$  SE,  $n = 3$ ; *Carex curvula* and forb pooled) on 22 weighing days after clipping on day 206 in 2009. Bottom panel: SWC measured by soil moisture sensors in unclipped (dashed line) and clipped (solid line) monoliths across all grassland types (means  $\pm$  SE,  $n = 4$ , SE: grey shaded area, standard error across grassland types) and precipitation (black bars; 14 mm of water added on day 230, grey bar).  $\theta_{ic}$ :  $103 \pm 9$  mm or  $54 \pm 5\%$  vol across all monoliths (means  $\pm$  SD).

The reduced water vapour loss due to clipping increased SWC in monoliths already a few days after the clipping treatment was applied (shown for 2009, the season when the monoliths were weighed most frequently; Fig. 4, top panels, Table 2b). These water savings were pronounced in *A. schraderiana* and *N. stricta*, but rather small in the small-stature *C. curvula* and forb-dominated lysimeters. The maximum differences in SWC due to clipping were reached during the warm and dry period in autumn 2009 and averaged at +24 mm (As), +13 mm (Ns), +4 mm (Cc and forb). During drying cycles, soil moisture of clipped monoliths decreased more slowly and remained at higher levels than unclipped ones (Fig. 4, bottom panel). Heavy rain events equalised SWC between clipped and unclipped lysimeters.

The amount of deep seepage water collected in lysimeters (equals total ecosystem water loss by runoff) provides a temporally integrated signal of the effects of vegetation cover or clipping on the seasonal water balance. Accordingly, clipping reduced ET and as a result led to higher seepage sums during the time after clipping, especially in the higher-stature As and Ns (Fig. 5, Table 2c). We found significantly reduced total seepage, but increased absolute and relative clipping effects in the warm and dry 2009 season compared to the relatively wet seasons 2008 and 2010 (meteorological conditions see Table S1). However, the effect of clipping on the 2010 seepage sum is likely to be underestimated (due to overnight flooding of the lysimeters). Across the full growing season (including the time before clipping; not available for 2008 due to installation), the clipping treatment significantly increased deep seepage by +5% to +38% in 2009 ( $F_{1,18} = 8.3$ ,  $P = 0.010$ ), depending on grassland type, and by only +4% to +8% in the rather wet 2010 season ( $F_{1,18} = 9.0$ ,  $P = 0.008$ ). In other words, declining land use or complete land abandonment (tall grass) reduced seepage by -13% (-35 mm) over the whole comparatively dry growing season in 2009 and by -5% (-22 mm) in 2010 (a relatively wet season).

In line with the higher plant water use in unclipped compared to clipped swards, mean bright day ET increased significantly with increasing canopy height (in unclipped monoliths), which is expected to



**Fig. 5.** The effect of clipping on the seasonal sum of deep seepage (means  $\pm$  SE,  $n = 3$ ; white bars: unclipped, grey bars: clipped) of four grassland types, measured after clipping (42 days in 2008; 55 days in 2009; 43 days in 2010, precipitation sums are given in brackets). Bars with different letters are significantly different within the same season. Note the relatively bigger signals in the warmer and drier 2009 season.

occur with extensification or after abandonment. Daily ET was +31% higher in the highest-stature *A. schraderiana* than in the lowest-stature *C. curvula* swards across all three growing seasons (2008: after mid July; 2009/2010: full growing seasons). These increased ET rates in *A. schraderiana* depleted soil water stores faster and caused the seasonal seepage sums to be significantly smaller than in *C. curvula*-dominated grassland: by  $-33$  mm ( $-15\%$ ) in 2008 (after mid July),  $-82$  mm ( $-30\%$ ) in 2009, and  $-42$  mm ( $-10\%$ ) in 2010 (full growing seasons). Hence, seasonal water yield was substantially reduced with increasing vegetation height.

From the seepage sums, precipitation and changes in SWC, we calculated evaporative water losses (ET) for unclipped monoliths during the full growing seasons from snowmelt to the last seepage measurement at the end of the 2009 and 2010 growing seasons: ET sums averaged at 288 mm (60% of total ecosystem water losses) in *A. schraderiana* (the highest-stature swards) and 196 mm (42%) in *C. curvula* (the lowest-stature swards) in the warm and dry 2009 season, and at 220 mm (37%) in *A. schraderiana* and 173 mm (29%) in *C. curvula* in the cool and wet

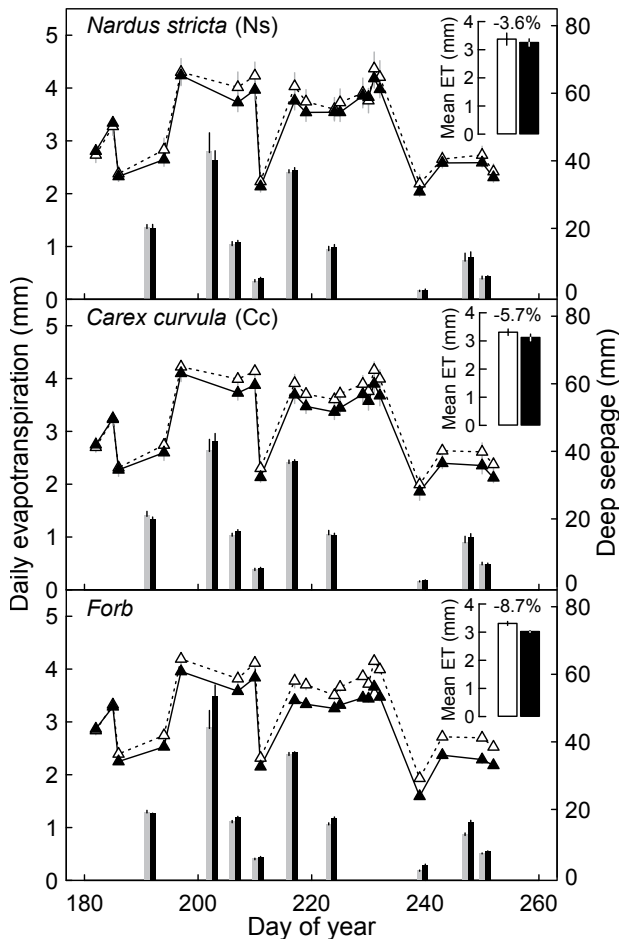
2010 season. Mean daily ET rates across all grassland types (unclipped monoliths) and the full growing season were thus 2.4 mm in 2009 (100 days) and 2.0 mm in 2010 (96 days).

#### Effects of elevated $CO_2$ on the water balance

FACE was operating on 59 out of 71 days between 1 July and 9 September 2009. On ten days in total, FACE was turned off to obtain ET signals without  $CO_2$  enrichment, and on another two days, FACE was interrupted because of snow cover. Across all enrichment days,  $CO_2$  concentration was very close to the target level in two rings ( $584 \pm 10$  ppm,  $592 \pm 14$  ppm), and slightly higher in one ring  $634 \pm 20$  ppm. Of all 1-s values 88%, 75% and 52% were within  $\pm 20\%$  of the target  $CO_2$  concentration of 580 ppm in the three rings. Potential influences of the differences between FACE ring  $CO_2$  concentration have been avoided by regular randomisation of the lysimeters between FACE rings.

$CO_2$  enrichment reduced daily ET rates consistently, in all three grassland types studied in the  $CO_2$  experiment and during the whole growing sea-





**Fig. 6.** The effect of elevated  $\text{CO}_2$  on daily ET rates (white triangles: ambient  $\text{CO}_2$ , black triangles: elevated  $\text{CO}_2$ ) and deep seepage (grey bars: ambient  $\text{CO}_2$ , black bars: elevated  $\text{CO}_2$ , right y-axes) of three grassland types in 2009 (means  $\pm$  SE,  $n=6$ ). The inset bar charts show mean daily ET rates (averaged across the growing season) under ambient (white) and elevated  $\text{CO}_2$  (black bars).

son by on average  $-3.6\%$  in *N. stricta*,  $-5.7\%$  in *C. curvula* and by  $-8.7\%$  in forb-dominated monoliths ( $F_{1,34} = 4.2$ ,  $P = 0.048$ ; Fig. 6). However, there was considerable residual variance, which was ascribed to single monolith properties that affect ET (e.g. biomass, vegetation structure). When the mean daily ET rates (across growing season) were standardized using the covariate  $\text{ET}_{\text{CO}_2\text{off}}$ , the ET reduction by elevated  $\text{CO}_2$  was  $-6.6\%$  in *N. stricta*,  $-3.2\%$  in *C. curvula* and  $-6.0\%$  in forbs and significantly different between grassland types (Table 3a). In forb-dominated monoliths subjected to elevated  $\text{CO}_2$ , the ET rates on days when FACE was switched off became steadily reduced with the progression of the growing season relative

**Table 3.** The effect of elevated  $\text{CO}_2$  on (a) mean daily evapotranspiration (ET, across all dates in 2009), (b)  $\delta^{18}\text{O}$  in foliage, (c) volumetric soil water content (SWC, averaged across all dates in 2009) and (d) seasonal sums of deep seepage in the three different grassland types (GT)

	df	F	P-values
<b>a) Mean daily ET</b>			
$\text{ET}_{\text{CO}_2\text{off}}$ (covariate)	1, 29	9758.2	<b>&lt;0.001</b>
GT	2, 29	64.9	<b>&lt;0.001</b>
$\text{CO}_2$	1, 29	150.4	<b>&lt;0.001</b>
$\text{GT} \times \text{CO}_2$	2, 29	11.1	<b>&lt;0.001</b>
<b>b) Leaf material <math>\delta^{18}\text{O}</math></b>			
Species	11, 49	14.3	<b>&lt;0.001</b>
$\text{CO}_2$	1, 4	11.2	<b>0.029</b>
Species $\times$ $\text{CO}_2$	11, 49	2.7	<b>0.009</b>
<b>c) Mean SWC</b>			
GT	1, 33	16.8	<b>&lt;0.001</b>
$\text{CO}_2$	1, 33	23.4	<b>&lt;0.001</b>
$\text{GT} \times \text{CO}_2$			<i>n.s.</i>
<b>d) Deep seepage sum</b>			
GT			<i>n.s.</i>
$\text{CO}_2$			<i>n.s.</i>
$\text{GT} \times \text{CO}_2$			<i>n.s.</i>

Bold *P*-values are statistically significant ( $P > 0.05$ ).

to the controls grown at ambient conditions (not in other grassland types). This could have been caused by slight seasonal reductions in LAI under elevated  $\text{CO}_2$  (in forb only; *n.s.*; data not shown). However, the  $\text{CO}_2$  effect on ET standardized by  $\text{ET}_{\text{CO}_2\text{off}}$  was significant irrespective of any possible biomass changes. Hence, across all grassland types, elevated  $\text{CO}_2$  clearly reduced ET by on average  $-5.3\%$ . This reduction in ET was also supported by a significantly higher stable oxygen isotope ratio ( $\delta^{18}\text{O}$ ) in organic leaf material in seven out of 12 tested species grown under elevated (580 ppm) compared to plants grown at ambient  $\text{CO}_2$  (Tables 3b and 4).  $\delta^{18}\text{O}$  in leaves as well as the  $\text{CO}_2$  effect on  $\delta^{18}\text{O}$  were highly species-specific.

Already a few days after the start of  $\text{CO}_2$  enrichment, the reduced ET rates under elevated  $\text{CO}_2$  resulted in higher SWC than in control monoliths in all grassland types, a difference that was perpetuated across the remaining part of the 2009 growing season (Table 3c, Fig. S1). The maximum increase in SWC under  $\text{CO}_2$  enrichment was 5.6 mm ( $+7.0\%$ ) in *N. stricta*, 4.7 mm ( $+6.5\%$ ) in *C. curvula* and 8.0 mm

**Table 4.**  $\delta^{18}\text{O}$  in leaf tissue under ambient and elevated  $\text{CO}_2$  (nine grassland species grown inside and outside FACE rings; means  $\pm$  SE,  $n = 3$  rings)

Species	$\delta^{18}\text{O}_{\text{ambient}}$ (‰)	$\delta^{18}\text{O}_{\text{elevated}}$ (‰)	<i>P</i> -values
Graminoid species			
<i>Agrostis schraderiana</i>	23.70 $\pm$ 0.70	24.17 $\pm$ 0.68	0.662
<i>Anthoxanthum odoratum</i>	24.16 $\pm$ 0.22	25.29 $\pm$ 0.38	0.061
<i>Carex curvula</i>	22.42 $\pm$ 0.59	23.51 $\pm$ 0.31	0.137
<i>Helictotrichon versicolor</i>	24.14 $\pm$ 0.30	25.30 $\pm$ 0.06	<b>0.019</b>
<i>Nardus stricta</i>	22.77 $\pm$ 0.13	23.99 $\pm$ 0.40	0.053
<i>Poa alpina</i>	25.44 $\pm$ 0.29	25.39 $\pm$ 0.54	0.931
Forb species			
<i>Geum montanum</i>	23.29 $\pm$ 0.28	23.32 $\pm$ 0.46	0.946
<i>Homogyne alpina</i>	22.01 $\pm$ 0.37	24.48 $\pm$ 0.50	<b>0.016</b>
<i>Leontodon helveticus</i>	22.73 $\pm$ 0.09	23.08 $\pm$ 0.03	<b>0.024</b>
<i>Ligusticum mutellina</i>	22.84 $\pm$ 0.08	22.48 $\pm$ 0.35	0.292
<i>Potentilla aurea</i>	23.49 $\pm$ 0.10	25.44 $\pm$ 0.44	<b>0.013</b>
<i>Trifolium alpinum</i>	22.08 $\pm$ 0.23	23.46 $\pm$ 0.37	<b>0.033</b>

Bold *P*-values are statistically significant ( $P > 0.05$ ).

(+11.0%) in the forb-dominated monoliths during the rather dry period in autumn 2009. The water savings due to higher atmospheric  $\text{CO}_2$  translated into slightly higher seasonal seepage sums in the forb-dominated grassland only (Fig. 6, Table 3d; forb-dominated: +18 mm;  $F_{1,10} = 3.6$ ,  $P = 0.086$ ).

## Discussion

This three-year field study provided clear evidence for reduced growing season water yield when grazing in alpine grassland is reduced or fully abandoned. Unexpectedly, simulated atmospheric  $\text{CO}_2$  enrichment did not affect the water yield significantly, even though we measured small, but significant reductions in ET of the different grassland canopies.

### *Water balance of alpine grassland*

The evapotranspiration rates presented here (2.0–2.4 mm per day and 173–288 mm per season) correspond to ET rates at similar elevation (2000–2550 m a.s.l.) collected in several lysimeter studies in the Austrian Alps, where seasonal mean ET of alpine grassland was 2.1 mm d<sup>-1</sup> and 165–255 mm per growing season (summarised by Wieser, Hammerle & Wohlfahrt 2008). In line with our observations, there were little, if any, indications of water stress on plant

transpiration in the Austrian Alps, despite reductions in soil water availability in combination with high evaporative demand, especially during the summer drought of 2003 (Brilli *et al.* 2011).

### *Effects of land use change on ET and the water balance*

Bright day ET rates were reduced by –12% across all years and grasslands types when grassland was kept short during the second half of the growing season (by simulated grazing), matching signals obtained in a small pilot study with *Carex curvula* in the Austrian Alps (–12% ET; Körner, Wieser & Cernusca 1989). The lower ET led to increased seasonal deep seepage in short grassland. The additional water yield, resulting from naturally small stature and/or clipping, was higher in the rather warm and dry 2009 season compared to the cooler and wetter growing seasons in 2008 and 2010. In other words, sustainable land use (*i.e.* non-destructive land use that maintains livestock carrying capacity) in this area produces higher water savings during dry summers, because soil moisture differences accumulate over longer periods.

The average, clipping-induced reduction in ET or the corresponding increase in water yield across all 24 monoliths used in the land use experiment are

attributing equal weight to the tall (*A. schraderiana*) and the three short (*N. stricta*, *C. curvula* and forb-dominated) grassland types. In reality, the abundance of tall grass is somewhat lower in the immediate surroundings of the study site, but much higher at only 100–200 m lower elevation in the lower alpine belt. Furthermore, the sedge-dominated monoliths employed were less lush (40% lower biomass) than average *Carex curvula* dominated swards examined elsewhere (Klug-Pümpel 1982; Körner *et al.* 1997), likely to cause an underestimation of the clipping effect in this highly abundant alpine grassland type. Since the clipping treatment was only applied by mid-season, in line with the recommended timing of sustainable sheep grazing in the region, it influenced the second half of the growing season only, as is apparent from the reduction of the treatment effect across the whole snow-free season compared to the period after clipping only. Overall, the –5% (–22 mm) to –13% (–35 mm) reduction in the seasonal sum of deep seepage by increased biomass, presented in this study, reflects a reasonable estimate of the potential, landscape-wide effects of land use reduction in alpine grassland. At lower elevation, in the lower alpine or upper montane belts, where grassland canopies are generally much higher than at the study site, effects of grazing are expected to be in the range observed here for *A. schraderiana*, *i.e.* arrive at reductions in ET of –25% and seasonal seepage gains of +8% (+30 mm) to +38% (+74 mm) for the two complete seasons (wet versus dry). Should future rates of atmospheric nitrogen deposition (currently 3.3 to 5 kg N ha<sup>-1</sup> a<sup>-1</sup> wet deposition in the studied region; Hiltbrunner, Schwikowski & Körner, 2005) exceed the ‘critical load’ for such alpine systems (5 to 10 kg N ha<sup>-1</sup> a<sup>-1</sup>; Bobbink *et al.* 2010), this may change vegetation in favour of more vigorous species, likely to enhance the land abandonment effect on ET, as shown here.

#### *Effects of elevated CO<sub>2</sub> on ET and the water balance*

At ecosystem scale, the widely reported reduction in stomatal conductance and thus, transpiration in grassland exposed to elevated CO<sub>2</sub> is diminished by soil evaporation (which is not directly affected), interception losses and a strong aerodynamic component of canopy resistance to transpiration (Field, Jackson &

Mooney 1995; Leuzinger & Körner 2010). For example, in Swiss calcareous grassland, CO<sub>2</sub> enrichment halved stomatal conductance in the dominant species *Bromus erectus* (Lauber & Körner 1997), which, due to other, less responsive species and the above processes, translated into a marginal reduction in ecosystem ET of c. 6% only (Stocker, Leadley & Körner 1997). In the present study, we found very similar ET savings under elevated CO<sub>2</sub>, which were supported by the increased δ<sup>18</sup>O in leaf tissue of plants exposed to 580 ppm CO<sub>2</sub>, providing an integrated, qualitative signal across the growing season. The increased δ<sup>18</sup>O most likely resulted from reduced stomatal conductance and thus, reduced leaf-level transpiration under CO<sub>2</sub> enrichment (Barbour 2007; Farquhar, Cernusak & Barnes 2007), as the isotope composition of soil water and the relative air humidity was assumed to be equal between treatments (same location and same meteorological conditions and isotopic signal in precipitation). In an isotope study in the Eastern Alps, an increase in leaf δ<sup>18</sup>O by 1.3‰ to 1.8‰ in the alpine species *Festuca rubra* and *Potentilla aurea* was linked to a significant reduction in stomatal conductance by around 30% (Scheidegger *et al.* 2000).

Similar to the data presented here, the small reduction in ET also resulted in higher soil water content under elevated CO<sub>2</sub> throughout the growing season in the calcareous grassland (Niklaus, Spinnler & Körner 1998). In another CO<sub>2</sub> experiment in alpine *Carex curvula* dominated grassland in the same area in the Swiss Alps, the CO<sub>2</sub> effect on stand ET was below detection limit of gas flux measurements (Diemer 1994; no weighing lysimeters). Soil water savings by CO<sub>2</sub> enrichment were commonly more pronounced in drier and more productive lowland grassland (Niklaus, Spinnler & Körner 1998; Nelson *et al.* 2004). We thus assume that, at ecosystem-level, soil moisture savings by elevated CO<sub>2</sub> are too weak to translate into significantly higher sums of deep seepage or runoff in non-water-limited, temperate systems, in which CO<sub>2</sub> effects on runoff mainly depend on day-to-day rainfall patterns (Leuzinger & Körner 2010). In addition, initial water savings by elevated CO<sub>2</sub> (after saturating rainfall) are consumed by plants during prolonged rainless periods, if water availability limits ET, and thus, do not result in increased runoff

(Morgan *et al.* 2004). Such effects of scaling (to higher levels of complexity, larger spatial scales or longer time spans) as well as possible atmospheric feedback should be considered in more detail in hydrological modelling of CO<sub>2</sub> effects (Leuzinger *et al.* 2011).

There are well-known drainage implications with lysimeters (Flury, Yates & Jury 1999). The interruption of the capillary continuum at the bottom of any lysimeter may cause the rate of deep seepage to slow, and thus, cause a periodic increase in soil moisture compared to control soils *in-situ*. Our fine resolution series of soil moisture readings inside versus outside lysimeters did not show such a slowed drainage effect. Hence, it seems, the generally high soil moisture and regular rainfall did not permit such signals to materialize, and the same relative depletion of soil moisture inside versus outside lysimeters during rainless periods indicated very similar water consumption by vegetation inside and outside lysimeters at this high alpine location.

#### *Ecological implications*

This paper quantifies the way vegetation interacts with the ecosystem water balance and it offers a basis for estimating the hydrological consequences of land transformation and atmospheric change at catchment scale. Our data evidence that the higher biomass and leaf area associated with extensification or abandonment of alpine pastures and meadows, which increase daily ecosystem vapour loss (ET), exhibit much stronger effects on the water balance than the slightly counteracting future (double pre-industrial) CO<sub>2</sub> concentrations. The higher evaporative water consumption reduces growing season deep seepage by –220 to –350 m<sup>3</sup> per hectare of unmanaged alpine grassland (wet versus dry summer). A decrease in catchment water yield following reduced land use implies more intense soil moisture depletion during dry periods and a reduction in fresh water provision of alpine catchments to rivers and lowland ecosystems. These changes also have repercussions for the potential of hydroelectric power production, all important ecosystem services provided by alpine regions (Viviroli *et al.* 2011; Grêt-Regamey, Brunner & Kienast 2012). While the effect of increased evaporative water con-

sumption on soil water availability is unlikely to cause plant water shortage in such humid alpine grasslands, our data illustrate the magnitude of such effects that would become much more important in drier regions or under future climate scenarios, which predict more frequent and prolonged dry spells in the Alps in combination with elevated summer temperatures and increased ET at high elevation (Calanca *et al.* 2006; Horton *et al.* 2006).

Assuming an average 1500 m vertical potential energy of water originating from the alpine belt of a mountain catchment (between 2400 m and sea level, of which potentially more than half is usable within the region, down to c. 800–1000 m a.s.l.), an overall efficiency of the hydroelectric installation of 8.5 kN m<sup>-3</sup> and a current average price of hydroelectric power yield (0.09 CHF per kWh; ElCom 2012; 1 CHF currently equals c. 0.83 Euros or 1.10 USD), the seasonal reduction in water yield of 220 to 350 m<sup>3</sup> (wet versus dry summers) per ha of abandoned high alpine grassland would correspond to a loss of revenues from power production of 70 to 112 CHF per ha and year. For the alpine zone of the currently studied test region in the headwaters of the river Reuss (9000 ha of vegetated area between 2100–2700 m a.s.l., mostly alpine grassland), these ‘costs’ for the loss of water for potential hydroelectric power production by tall versus regularly grazed alpine grassland would amount to 630 000 to 1 000 000 CHF per year (assuming that 100% of the ‘additional’ water by grazed terrain is used for power production). These estimated ‘costs’ of declining land use are probably an upper limit for low-stature alpine vegetation, since it is unlikely that all grassland of an alpine catchment is grazed uniformly. Furthermore, effects may get diminished with the accumulation of dead plant material in grassland that is dominated by certain species (e.g. *Festuca rubra*) and has been abandoned for several years (Tappeiner & Cernusca 1989; Rosset *et al.* 2001; Merz *et al.* 2009). On the other hand, the reduction in water yield by extensification or abandonment of grassland is likely to increase as vegetation gets taller at lower elevation and as shrubs encroach. Alterations in soil properties due to changes in land use, e.g. reduced or increased soil compaction or erosion induced by trampling of grazers (strongly depending on shep-

herding), will exert effects on the water balance that are not explored here. Our data show that the magnitude of land cover or land use effects on ET and runoff depend on climatic conditions (e.g. dry versus wet summers) as was suggested by hydrological (modelling) studies of alpine catchments (de Jong, Mundelius & Migala 2005; Verbunt, Groot Zwaartink & Gurtz 2005). Climatic warming is likely to reduce discharge of alpine catchments during prolonged summer seasons, due to earlier snowmelt, reduced summer precipitation, increased summer temperatures and declining contributions of glacier melt water to total discharge (Beniston, Stoffel & Hill 2011; Huss 2011; Viviroli *et al.* 2011). Under such conditions, the hydrological consequences of declining land use are likely to become significantly more important as is reported here for the rather dry 2009 season.

The data presented here substantiate the need to account for hydrological consequences in any judgement of land use change in alpine regions. Furthermore, our results permit to attribute economic value to pastoralism that goes beyond agronomic and ecological aspects (such as the loss of usable agricultural land, decrease in landscape diversity and species diversity). This broader view at the benefits of sustainable pastoralism in alpine catchments has obvious political implications including those for the energy sector and freshwater production, and thus, offers novel assets for the promotion of sustainable land use in remote areas (MacDonald *et al.* 2000; Flury, Gotsch & Rieder 2005; Marini, Klimek & Battisti 2011). We advocate that land use and change in vegetation cover should receive more attention in hydrological models of alpine catchments.

### Acknowledgments

This study contributes to the informal tri-national project Bio-CATCH on sustainable land use and catchment water yield. We are especially grateful to our project partners U. Tappeiner, and N. Obojes (EURAC, Bolzano and University of Innsbruck), S. Lavorel, J.C. Clément and J. Lochet (UJF Grenoble) for fruitful cooperation. This project received funding by BLW (Swiss Federal Office for Agriculture,

project Bio-CATCH, U. Gantner), the University of Basel, the SNF (Swiss National Science Foundation, project VALURsem, CR3013-124809), the FAG (Freiwillige Akademische Gesellschaft Basel) and profited from collaboration with the BAFU project N-alpin (Swiss Federal Office for the Environment, 09.0084. PJ/1225-1307). We thank T. van den Bergh, T. Bühlmann, G. Gisler, L. Reißig, D. Scherrer, M. Studer and T. Zehnder for their help with fieldwork, M. Saurer and R. Siegwolf for advice on and analyses of <sup>18</sup>O. We acknowledge the Korporation Ursern for allowing us to use their land for years and the Alpine Research and Education Station Furka (ALPFOR) for providing facilities and accommodation.

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## Supporting Information

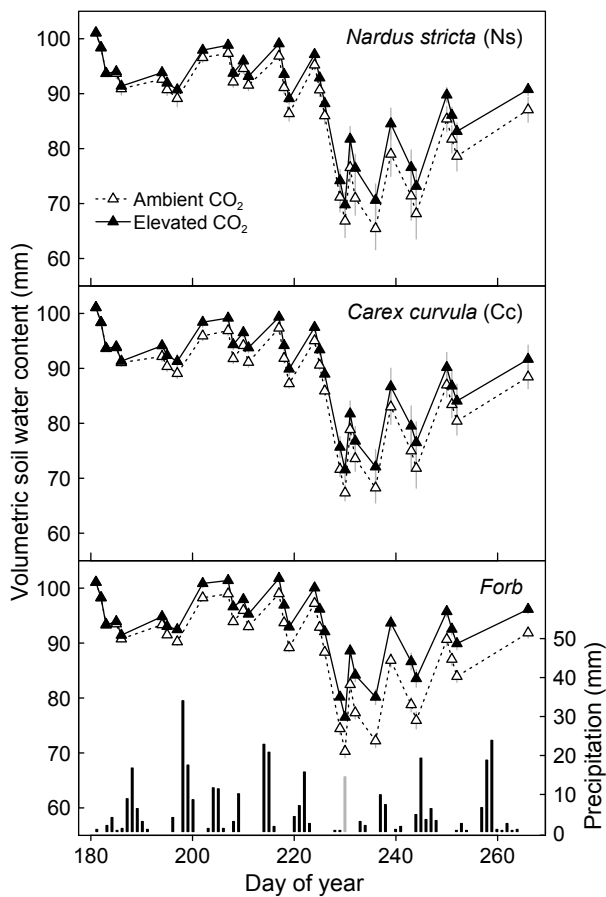
**Table S1.** Meteorological conditions during the experimental years, measured by the weather station set up at the study site near Furka Pass. Year-round data of a nearby alpine meteorological station (Gütsch) is provided by MeteoSwiss

		Furka Pass 2440 m a.s.l.			Gütsch 2287 m a.s.l.			
		2008	2009	2010	2008	2009	2010	norm*
Air temperature (°C)	annual				0.7	0.6	-0.6	-0.5
	June	†6.8	†5.2	†5.1	7.0	5.7	6.1	4.1
	July	7.2	7.5	9.1	8.1	8.5	10.1	7.3
	Aug.	7.8	9.5	6.3	8.6	10.5	7.5	7.1
	Sept.	3.0	5.7	3.6	3.8	6.9	4.4	5.1
Mean irradiance (Wm <sup>-2</sup> )	annual				167	174	167	162
	June	†265	†251	†203	240	247	217	242
	July	228	241	234	258	249	261	244
	Aug.	202	223	181	227	249	209	209
	Sept.	153	171	180	166	190	183	161
Precipitation (mm)	annual				1714	1296	1351	1479
	June	†56	†87	†102	117	94	108	116
	July	171	138	150	185	142	139	105
	Aug.	185	94	258	169	112	206	125
	Sept.	216	89	106	234	43	88	94
	total‡	628	408	616	649	355	512	

\* Long-term averages (1961-1990) by MeteoSwiss

† Data series starting after snowmelt (11 June 2008, 10 June 2009 and 8 June 2010)

‡ Growing season total (snowmelt in June to end of September)



**Fig. S1.** The effect of elevated CO<sub>2</sub> on volumetric soil water content (SWC) in monoliths of three grassland types (means  $\pm$  SE,  $n = 6$ ) on 31 weighing days in 2009 ( $\theta_{ic}$ :  $103 \pm 9$  mm across all monoliths; means  $\pm$  SD). Black bars represent daily precipitation (14 mm of water added on day 230).



# *The effect of land use and its decline on the water balance of montane and alpine grassland across the Alps*

Nicole Inauen and the Bio-CATCH consortium

This is a multi-site, multi-author synthesis of the Bio-CATCH network (Switzerland, Austria, France), a work, which is still in progress. I was appointed to lead author this synthesis. I offer it here as a part of my thesis in a not yet consolidated form (further data will be integrated).

Institute of Botany, University of Basel, Schönbeinstrasse 6, 4056 Basel, Switzerland; Institute for Alpine Environment, European Academy Bolzano/Bozen, Italy; Institute of Ecology, University of Innsbruck, Austria; Laboratoire d'Ecologie Alpine, Université Joseph Fourier, Grenoble, France; Station Alpine Joseph Fourier, Université Joseph Fourier, Grenoble, France



## Abstract

Land use has shaped alpine landscapes and habitats for centuries and has produced specialised and highly diverse alpine grassland communities. The current decline in agricultural use of alpine pastures and meadows across the Alps causes grassland canopies to grow taller and dead plant material to accumulate, due to cessation of regular biomass removal, and finally, leads to a shift in species composition. Since tall stands intercept and transpire more water, abandonment of alpine grassland most likely reduces runoff across the growing season due to increased evapotranspiration (ET). The Bio-CATCH consortium aimed at quantifying these hydrological consequences of land use changes in different montane and alpine grasslands along a W to E transect in the Alps (France, Switzerland and Austria). The water balance of grassland was assessed employing a total of 146 intact grassland monoliths in stainless steel deep seepage collectors, soil moisture sensors and weather stations at each site. The selected grassland types represented various land use types (recent and past) and differed in canopy height and biomass, reflected in the seasonal water balance. Three seasons of experimental land use simulation by clipping vegetation to a common height of 3 to 4 cm at and after the peak of the growing season yielded significantly increased sums of deep seepage (DS) due to reduced ET. The highest increase of DS was observed in tall grassland of the lower alpine belt in Austria, whereas DS gain by clipping was much smaller in naturally short vegetation of the upper alpine belt in Switzerland, all compared to unclipped swards. While runoff effects of land use in the alpine belt (Swiss site) were largest in dry summers (2009), they disappeared under summer drought in the montane and subalpine belt at the dry, continental French site. Overall, the additional water yield 'produced' by land use, calculated for the second half of the season after first clipping only, amounted to 13 mm ( $130\text{ m}^3\text{ ha}^{-1}$ ), 8 mm ( $80\text{ m}^3\text{ ha}^{-1}$ ) and 32 mm ( $320\text{ m}^3\text{ ha}^{-1}$ ) per season at the French, Swiss and Austrian site, respectively. Our results underline the significance of continued land care for catchment value in terms of fresh water supply and hydroelectric power production in different alpine regions across the Alps.

## Introduction

Abandonment of originally used agricultural land is an increasing concern across the European Alps, resulting in a loss of highly adapted mountain grassland, traditional alpine landscapes and their biodiversity. Economic and social changes have led to the abandonment of 33% of all farms across the entire arc of the Alps between 1980 and 2000 only (Streifeneder et al. 2007). However, major national and regional differences exist within the Alpine region: farm abandonment has been more severe in the southern (France -47%, Italy -44%, Slovenia -56%) than in the northern part of the Alps (Austria -9%, Germany -24%, Switzerland -34%; Streifeneder et al. 2007). Thus, large areas of poorly accessible agricultural land in the Alps have been abandoned, on average about 20%, in some areas as much as 70% (Tappeiner et al. 2003), especially, pastures and meadows at higher elevation (e.g. for Northern Italy, Tasser et al. 2009). Concurrently, land use has been intensified on more easily accessible and more productive parcels (Tasser and Tappeiner 2002). With globalisation and the mechanisation of alpine agriculture, traditional, labour intensive practices of land management are given up (e.g. substitution of hay meadows by permanent pastures, abandonment of shepherding or irrigation; MacDonald et al. 2000; Zimmermann et al. 2010).

Different management types and land use intensities have led to specialised, characteristic grassland communities: manuring in combination with frequent mowing favours fast-maturing, N-demanding plant species (forb-rich communities), while the cessation of these practices promotes dominance by graminoids with more conservative nutrient strategies (e.g. *Carex sempervirens*, *Festuca paniculata*; Tasser and Tappeiner 2002; Quétier et al. 2007; Lavorel and Grigulis 2012). More or less unattended grazing induces a small-scale mosaic of relatively species-poor grass-dominated communities (due to selective grazing) with invading dwarf shrubs (Tasser and Tappeiner 2002; Quétier et al. 2007). Further extensification or total abandonment of land use in mountain grassland results in fast encroachment by dwarf shrubs in former pastures and meadows up to a few hundred meters above treeline (Tasser and Tappeiner 2002) or by

trees and tall shrubs below the climatic treeline (Gellrich et al. 2007). Above the dwarf shrub belt, alpine swards dominated by sedges and grasses (depending on soil and bedrock type, e.g. *Carex spec.*, *Nardus stricta*, *Sesleria spec.*) form the natural climax vegetation in the Alps (Mertz 2008).

With decreasing land use intensity, plant height, litter accumulation and often the maximum standing biomass increase significantly (Tappeiner and Cernusca 1989; Wohlfahrt et al. 2003). Leaf traits associated with high photosynthetic assimilation and biomass production (SLA, leaf N) decrease, whereas leaf traits associated with slow decomposition and low palatability (high leaf dry matter) increase (Garnier et al. 2007; Quétier et al. 2007; Lavorel and Grigulis 2012). The abandonment furthermore induces a slowing of the biogeochemical cycles of carbon and nitrogen in the upper soil layers as well as other changes in soil characteristics with possible impacts on soil stability (Tasser et al. 2003, 2005).

The cessation of pasturing also influences hydrology. Soil compaction due to animal trampling increases surface runoff (Leitinger et al. 2010) and transpiration and interception are usually higher in tall vegetation. Therefore, these land cover changes affect evapotranspiration (ET) and the water balance (Tappeiner and Cernusca 1994; Asner et al. 2004; Garcia-Ruiz and Lana-Renault 2011). In a previous study, the simulation of sheep grazing in alpine swards dominated by *Carex curvula* in the Austrian Alps reduced bright day ET by –10% compared to ungrazed swards (Körner et al. 1989). Recent, more detailed data obtained in the Swiss Alps at 2440 m a.s.l. revealed similar grazing effects on daily ET in different grassland types in weighing lysimeters, with a significant increase in seasonal deep seepage in short grassland (Chapter 3). On the other hand, the accumulation of dead plant material within the canopy after land abandonment reduces evaporation from soils and thus reduce ET (Tappeiner and Cernusca 1998; Rosset et al. 2001). These side effects of land use decline on the hydrology in alpine grassland have not yet been accounted for in land use policy and deserve

quantification, given the importance of alpine hydrology for fresh water provision and hydroelectric power production.

Annual evapotranspiration (ET) in mountain grassland in the Alps generally decreases with elevation, whereas precipitation (P) increases (ET declines from ca. 70% of annual P at 500 m a.s.l. to 15% at 2500 m a.s.l.; Wieser et al. 2008). The remaining water runs off the surface or infiltrates the soils (deep seepage) and finally adds to river discharge, which therefore increases with elevation. This is the reason why the Alps are often designated the water towers of Europe and all major rivers of the world have their headwaters in highlands. In relation to their surface area, alpine catchments contribute over-proportionally to the total discharge of the river systems. Hence, more than half of humanity relies on the fresh water that accumulates in mountains (Messerli and Ives 1997, Viviroli et al. 2003; Weingartner et al. 2007). Hydrology in the Alps will most likely become more important in the future, as fresh water resources and hydropower production will be challenged by ongoing climatic change and rising renewable energy demands (Beniston et al. 2011; Viviroli et al. 2011).

This multi-site study aims at quantifying the influence of continued sustainable land use in alpine grassland *versus* abandonment on hydrological processes and catchment value across the Alps. For that purpose, the Bio-CATCH consortium selected field sites in the western, central and eastern part of the Alps, thus, differing in climate, past and present land use and consequently, in the species composition of grassland (near or above treeline). To resolve the water balance of different communities we adopted a standard protocol and employed a high number of stainless steel deep seepage collectors with intact grassland monoliths and applied a clipping treatment to half of the monoliths in order to simulate mowing or grazing.

## Materials and methods

### Study sites

The study sites are located along a West to East transect across the Alps: the westernmost study site is situated on S-facing slopes at Col du Lautaret in the French Western Alps, département Hautes-Alpes, with the montane sub-site Les Cours (6° 21' E 45° 03' N, 1810 m a.s.l.) and the subalpine sub-site Le Jardin (6° 24' E 45° 02' N, 2120 m a.s.l.). The Swiss site lies within the upper alpine zone at Furka Pass, canton Uri, in the Swiss central Alps (8° 25' E 46° 34' 2440 m a.s.l.), with a SE- and N-facing sub-site. The Austrian site in the central Eastern Alps is located near the climatic treeline at Kaserstattalm (11° 17' E 47° 07' N, 2000 m a.s.l.; SE aspect) in the Stubai Valley, North Tyrol. In the following, the study sites will be referred to as Stubai (A), Furka (CH) and Lautaret (F).

The sub-alpine climate at Lautaret is strongly continental, due to a rain shadow effect, with 956 mm of annual precipitation, of which ca. 60% occurs during autumn or winter, causing a pronounced summer drought. The growing season extends from mid April (lowest elevation), early May (highest elevation pastures) to late September or mid October. Mean monthly temperatures range between -2 to -4 °C in January, and 11 to 14 °C in July and August. The Furka Pass region is characterised by a humid temperate, alpine climate with annual sums of precipitation of about 1900 mm, 30 to 40% of which falling during a ca. 3 month growing season, which starts following snowmelt in June and ends in September. Mean summer air temperatures vary between 7 and 10 °C, however, frosts and snowfall may occur at any time during growing season. The Stubai site holds an intermediate position by exhibiting a mesic cool temperate, inner-alpine climate with 1100 mm of annual precipitation, about 35% of which falling as snow during the winter, and average summer air temperature around 8 to 12 °C.

Soils at Lautaret developed on schist and are 30-40 cm deep sandy loams with moderate skeletal fractions in the lower terraced areas (Les Cours, formerly ploughed) or >100 cm deep, more humid, clay

loams in the higher pasture areas (Le Jardin). Soils at the Furka site are humic and acidic at different developmental stages (on gneiss and granite), from alpine brown earths to podzolic soils. Similarly, the soils at the Stubai site developed on mica-schist, phyllit and gneiss, but there are also Triassic dolomites in the area causing calcareous soils.

The landscape at Lautaret is dominated by grassland ecosystems that are used for sheep and cattle grazing. At lower altitudes (1650 to 2000 m) former arable fields have been abandoned and converted to terraced grasslands used for hay making or grazing. At mid-slope (1800 to 2200 m) ancient hay meadows are currently converted to pastures for summer grazing of sheep or cattle, while some are no longer cut or grazed at all and are often dominated by the tussock grass *Festuca paniculata*. The upper slopes (2200 to 2500 m) are grazed by transhumant sheep flocks throughout summer. At Furka, the alpine grassland dominated by *Carex curvula* and *Nardus stricta* is grazed at different intensities by small stationary herds of sheep and bigger migrating flocks during summer and autumn (on average every second year). In the Stubai Valley vegetation is composed of (1) intensively managed meadows, mostly *Trisetum flavescens* -dominated communities in easily accessible and more productive areas, which are manured, mown and grazed annually, (2) more extensively used meadows and pastures (*Sieversio-Nardetum strictae* and *Seslerio-Caricetum sempervirentis*), which receive only low levels of fertilization, and (3) abandoned grassland on steep slopes or in inaccessible areas, encroached and later dominated by dwarf shrubs (*Vaccinio-Callunetum*, *Erico-Rhododendretum hirsuti*) and tree / shrub seedlings and saplings (Tasser and Tappeiner 2002).

### Meteorological instruments

All sites were equipped with weather stations, including tipping gauges installed at 2 m above ground at each study site (F: rain gauge: HOBO RG3\_M, air temperature and humidity: S-THB-M002, data logger: HOBO H21-001, Onset, Bourne, MA, USA; CH: rain gauge, sensors and logger: Wireless Vantage Pro2 Plus™, Davis Instruments, Hayward, CA,

USA; A: rain: Model 52203, R. M. YOUNG, Traverse City, MI, USA; air temp. and hum.: GWU KPC 1/6 – ME, Galltec GmbH, Bondorf, Germany; data logger: CR10X, Campbell Scientific). At Furka and Stubai, precipitation was additionally measured at 5 cm above ground level by totalisators (21 to 25 cm diameter funnels on belowground bottles).

#### *Deep seepage collectors*

To determine the water balance of different alpine grassland types, cylindrical buckets of stainless steel (deep seepage collectors, DSCs, diameter: 25 cm, surface area: 0.050 m<sup>2</sup>, depth: 40 cm) were filled with intact monoliths of the same diameter and of 28 cm depth, which were taken from grassland of the surrounding area of the respective study sites. The bottom 10 cm of the steel bucket was separated from the soil monolith by a steel sieve and a filter mat preventing soil particles from entering the bottom reservoir (ca. 5 L), where all percolation water was collected. This water was regularly pumped out of the DSC's reservoirs using portable vacuum systems (VK-lite, UMS, Munich, Germany) in order to determine seepage volume. DSCs were emptied after major rain events or at least every two weeks during the snow-free period at each site. The DSCs were sunk into the ground in such a way that the ca. 2 cm collar at the upper edge of the steel cylinder stayed above the soil surface to prevent lateral in- and outflow of surface water, so that deep seepage in DSCs equalled total runoff (R, i.e. the sum of surface runoff and deep seepage) of the simplified ecosystem water balance equation:

$$P = ET + R + \Delta S$$

This system allows calculating seasonal evapotranspiration (ET, incl. interception) from the seasonal sums of precipitation (P), deep seepage (which equals R) and the seasonal change in soil water content ( $\Delta S$ ). Volumetric soil water content (SWC) was measured by soil moisture sensors (EC-10, Decagon, Pullman, WA, USA) inserted in the root zone of each DSC (–5 to –10 cm). SWC data was recorded as hourly means (F and A: HOBO H21-001, Onset; CH: EM50, Decagon). The highest SWC after drainage from saturated soils (after snowmelt  $\theta_s$ ) was assumed to represent the

water content at field capacity ( $\theta_{fc}$ ). SWC data were standardized in relation to  $\theta_{fc}$  of each sensor, which was set to 100% SWC. For the computation of seasonal ET,  $\Delta SWC$  was calculated as the difference of the actual standardized SWC at the end of season and after snowmelt at the beginning of the season. This difference was multiplied by average  $\theta_{fc}$  for the different soils at different sites (determined in vol%) and by soil depth in DSCs (28 mm).

#### *Grassland types and study design*

The selected monoliths taken from the surrounding area (< 1 km) of each study site represent a wide range of land uses and vegetation structures. Eight monoliths of abandoned and sixteen of mown terraced grassland were selected at the lower French sub-site Les Cours (abandoned or mown terrace), and eight monoliths of abandoned and eight of mown meadows at the higher Le Jardin. As grazing is the only land use type at Furka, we used alpine pastures of different structure for the DSCs. For the SE-exposed sub-site: 16 monoliths dominated by tall grasses (*Deschampsia cespitosa* (L.) P. BEAUV. or *Agrostis schraderiana* BECH., both preferentially occurring at humid locations), 16 dominated by short graminoids (*Carex curvula* ALL. and *Nardus stricta* L.) and 8 dominated by the forb species *Trifolium alpinum* L.. And for the N-exposed sub-site: 16 monoliths dominated by the same short graminoid species. At Stubai, we employed 16 abandoned monoliths without and 10 abandoned monoliths with significant dwarf shrub cover (mainly *Vaccinium gaultherioides* BIGELOW and *Vaccinium myrtillus* L.), as well as 20 monoliths originating from meadows (mown twice a year) and 20 from pastures (cattle grazing).

#### *Clipping treatment and vegetation assessment*

Vegetation of half of all monoliths was clipped to a common vegetation height of 3 to 4 cm at the respective time of the standing biomass peak at each site in order to simulate site-typical mowing or grazing. In the dwarf shrub rich, abandoned grassland monoliths at Stubai, all vegetation other than dwarf shrubs was clipped. Vegetation was kept short during the rest of the growing season by repeated clippings. Clipped

phytomass of the first clipping each year was separated into biomass and necromass (attached, dead material), oven-dried at 80°C and weighed. Maximum gross canopy height as well as plant species composition, total green plant cover and total litter cover were estimated at the peak of standing biomass at each site and in each experimental year (before clipping).

#### *Statistical analyses*

Differences in seasonal DS sums, clipped phytomass or biomass were tested by analyses of variance (ANOVA) with the nested factors 'site' or 'sub-site' and 'grassland/land use type'. For posteriori comparison, we applied Tukey's HSD to test multiple group means. The clipping effects on DS were analysed separately for different sites or sub-sites, years and grassland/land use types. To test differences in DS sums between the three study years, repeated measures ANOVA was employed. In all analyses, residuals were tested visually for normality and homogeneity of variances. In case of non-normality and inhomogeneity, we applied power or log transformations to the response variables. All statistical analyses were performed using the open-source software R, version 2.13.2 (R Development Core Team 2011). *P*-values <0.05 were considered statistically significant and *P*-values <0.1 marginally significant.

## Results

### *Seasonal water balance*

The sum of precipitation and deep seepage across the growing season 2008 was highest at the Austrian site (Stubai), intermediate at the Swiss (Furka) and lowest at the French (Lautaret) sites (Table 1). However, on a daily basis, thus, accounting for growing season length, precipitation and deep seepage were highest at the N-facing Swiss sub-site and slightly lower at the SE-facing Swiss and the Austrian sites. Grassland at Les Cours and Stubai showed the highest sums of evapotranspiration (ET) during the growing season 2008. On a daily basis, though, ET rates at the subalpine sub-site Le Jardin were similar to those at Stubai and Les Cours (2.6 mm d<sup>-1</sup> on average across land use types), while swards at the alpine site Furka lost sig-

nificantly lower amounts of water via ET, 2.3 and 1.7 mm d<sup>-1</sup> at SE- and the N-facing sub-site, respectively. The amount of ET relative to the sum of precipitation was 66 to 77% in rather dry, montane and subalpine grassland at Lautaret, 38 to 39% in alpine swards at Furka (though only 25% at the N-facing sub-site), and 40 to 47% in rather humid, subalpine grassland at Stubai.

At Lautaret, ET was slightly lower in abandoned grassland (abandoned terrace and meadow) than in mown grassland (mown terrace and meadow; Table 1). At the Furka site, the tall grassland types (dominated by *Deschampsia cespitosa* or *Agrostis schraderiana*) showed the lowest DS sums and thus, highest ET rates compared to the other grassland types, however, the lower-stature *Carex curvula* and *Nardus stricta* -dominated grassland types (short grass) and *Trifolium alpinum* -dominated ones (forb) showed similarly high ET rates. Evaporative water losses in the same short grassland types at the N-facing sub-site were significantly lower. At Stubai, meadows and pastures lost more water via ET than abandoned grassland, however, in abandoned grassland dominated by dwarf shrubs, ET was found to be highest of all vegetation types at the Stubai site. However, all these vegetation-related trends in DS and thus, ET in non-clipped DSCs were not statistically significantly different within the same site or sub-site when accounting for multiple comparisons (Tukey's test), but the differences in DS between sites or sub-sites were statistically significant ( $F_{4,63} = 391$ ,  $P < 0.001$ ), except for the SE- and N-facing Swiss sites.

### *Effects of clipping*

The peak-season clipping treatment in 2008 removed the highest amount of phytomass (biomass and necromass) at Stubai and the lowest at Furka ( $F_{2,69} = 25.0$ ,  $P < 0.001$ ), still, the phytomass in tall vegetation at Furka was similar to that of abandoned terraces or mown meadows at Lautaret (Fig. 1, top panels; for the French site, only the 2009 clipped phytomass data was available). The within-site differences in phytomass or biomass between sub-sites and grassland types were not statistically significant. The highest necromass to biomass ratio in clipped plant material was found in short vegetation at the SE-facing sub-



**Table 1** Seasonal water balance 2008: seasonal sums of precipitation (P), deep seepage (DS; mean  $\pm$  SE), seasonal changes in soil water content (SWC; snowmelt to end of season), seasonal evapotranspiration (ET), daily ET and seasonal ET relative to seasonal P in different grassland types along the W-E transect (sites in France, Switzerland and Austria; unclipped DSCs). The growing season was defined as the time from the first DS measurement after snowmelt to the last seepage event of the season at each site

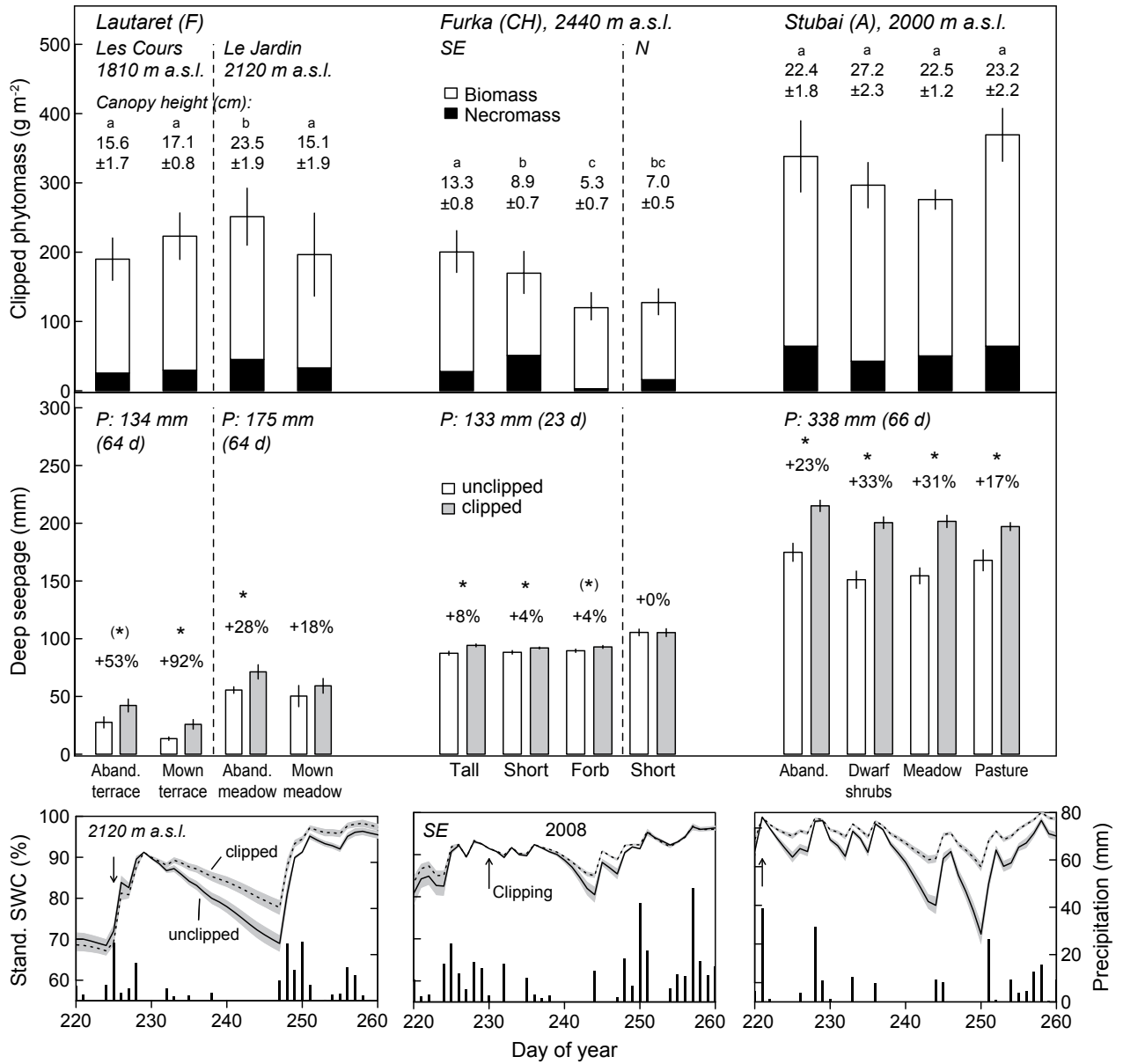
Grassland/land use type	<i>n</i>	P (mm)	DS <sub>unclipped</sub> (mm)	$\Delta$ SWC (mm)	ET (mm)	ET (mm d <sup>-1</sup> )	ET (% P)	Season length (d)	Growing season (doy)
<i>Lautaret (F)</i>									
Les Cours, 1810 m a.s.l.									
Abandoned terrace	4	501	175 $\pm$ 14	-6	332	2.6	66	128	136–263
Mown terrace	8	501	156 $\pm$ 8	-6	351	2.7	70	128	136–263
Le Jardin, 2120 m a.s.l.									
Abandoned meadow	4	337	83 $\pm$ 5	-4	257	2.6	76	100	164–263
Mown meadow	4	337	79 $\pm$ 14	-4	261	2.6	77	100	164–263
<i>Furka (CH), 2440 m a.s.l.</i>									
Tall grass <sup>a</sup>	8	577	358 $\pm$ 6	-8	226	2.3	39	99	162–260
Short grass <sup>a</sup>	8	577	361 $\pm$ 6	-9	224	2.3	39	99	162–260
Forbs <sup>a</sup>	4	577	363 $\pm$ 13	-4	218	2.2	38	99	162–260
Short grass <sup>b</sup>	8	468	349 $\pm$ 8	2	118	1.7	25	71	190–260
<i>Stubai (A), 2000 m a.s.l.</i>									
Abandoned	8	841	512 $\pm$ 15	-6	335	2.4	40	138	143–280
Abandoned (+dwarf shrubs)	5	841	458 $\pm$ 21	-9	392	2.8	47	138	143–280
Meadow	10	841	476 $\pm$ 12	-7	372	2.7	44	138	143–280
Pasture	10	841	491 $\pm$ 23	-5	355	2.6	42	138	143–280

<sup>a</sup>SE- and <sup>b</sup>N-facing sub-sites at Furka (CH).

site at Furka. Canopy height was tallest at Stubai and lowest at Furka ( $F_{2,150} = 159$ ,  $P < 0.001$ ), with significant within-site differences between grassland types at Lautaret and Furka.

Clipping significantly increased volumetric soil water content (SWC) during the time after clipping (Fig. 1, bottom panels), apparently due to reduced ET after biomass removal. At the Lautaret sites, clipping evoked a maximum increase in SWC (across all grassland types) of +6.3% (6.8 mm) at Les Cours and of +8.8% (10.0 mm) at Le Jardin. At the upper French sub-site Le Jardin, the effect persisted until the end of season, yet, it disappeared after a rather heavy rain event (DOY 250) at the lower sub-site Les Cours (not shown). At the Furka site (SWC data available for the SE-facing sub-site only), where clipping was applied latest of all sites, the soil water savings due to clipping were lowest (max. +4.5%, 6.6 mm) and were reset several times by rain events. The clipping effect was most pronounced at Stubai with a maximum increase in SWC of +17.7% (21.5 mm) in clipped compared to unclipped grassland, and it persisted until the end of the season. Deep seepage sums during the time after clipping to the end of the growing season were highest

at Stubai (highest P), intermediate at Furka and lowest at Lautaret, because precipitation (although equal or higher) was distributed across a much longer period at the French compared to the Swiss site (DS:  $F_{2,134} = 1459$ ,  $P < 0.001$ ; Fig. 1, mid panels). Biomass removal by clipping significantly increased deep seepage in almost all grassland types and sites. In absolute terms, the clipping effect was highest at Stubai (+29 to 49 mm), intermediate at Lautaret (+9 to 16 mm) and rather small at Furka (0 to +7 mm). In relative terms, though, the increase in DS by clipping was highest at Lautaret, as the DS sums were generally low in these mostly water-limited swards. Abandoned grassland (abandoned terraces and meadows) showed the highest absolute reductions in water consumption due to clipping at Lautaret, and the tall swards exhibited the most pronounced clipping effect at Furka. At Stubai, DS was increased most by clipping in dwarf shrub-dominated grassland and in meadows, although biomass removal in these two grassland types was slightly lower than in abandoned grassland without dwarf shrubs or in pastures. However, these interactions between clipping and grassland/land use type were not statistically significant at any site.



**Fig. 1** Top panels: phytomass (biomass plus necromass; mean ± SE) removed at peak season (clipping treatment) in different grassland types of the three study regions along the W-E transect in the Alps in 2008 (the dashed lines separate sub-sites). The numbers above the bars give maximum gross canopy height prior to clipping (means ± SE; 2008), means with different letters differ significantly (Tukey's test within site,  $P < 0.05$ ). Mid panels: deep seepage sums (mean ± SE) of unclipped and clipped grassland across the time after clipping in 2008 (clipping effect: \*  $P < 0.05$ ; (\*)  $P < 0.1$ ). P indicates the precipitation sum, and the number of days after the first clipping is given in brackets (vegetation was kept short). Bottom panels: standardized volumetric soil water content (across grassland types; mean ± SE) in unclipped and clipped grassland and precipitation (daily resolution; black bars, right y-axis) during the period after clipping (arrows) in 2008 (Lautaret: Le Jardin, Furka: SE-facing sub-site only). 100% SWC corresponds to water content at field capacity (vol%): Le Jardin (2120 m a.s.l.) 40.7, Furka 51.7, Stubai 43.5.

**Table 2** Deep seepage sums (DS; mean  $\pm$  SE) in different grassland types along the W-E transect during the period after clipping treatment in 2008–2010. The difference in seepage ( $\Delta$ DS) between clipped and unclipped vegetation is given in mm and% (bold lettering indicates significant differences,  $P < 0.1$ ). Sites differ in the amount of precipitation (P) and the number of days after the first clipping (to the end of the growing season)

Grassland/land use type	<i>n</i>	DS <sub>unclipped</sub> (mm)	DS <sub>clipped</sub> (mm)	$\Delta$ DS (mm)	$\Delta$ DS (%)	P (mm)	P (mm d <sup>-1</sup> )	Time (d)
<i>Lautaret (F), 1810 m<sup>a</sup> and 2120 m<sup>b</sup> a.s.l.</i>								
2008								
Abandoned terrace <sup>a</sup>	8	28 $\pm$ 5	42 $\pm$ 6	<b>15 <math>\pm</math> 5</b>	<b>53</b>	134	2.1	64
Mown terrace <sup>a</sup>	16	13 $\pm$ 1	26 $\pm$ 4	<b>12 <math>\pm</math> 3</b>	<b>92</b>			
Abandoned meadow <sup>b</sup>	8	56 $\pm$ 3	71 $\pm$ 6	<b>16 <math>\pm</math> 5</b>	<b>28</b>	175	2.7	64
Mown meadow <sup>b</sup>	8	50 $\pm$ 9	59 $\pm$ 6	9 $\pm$ 8	18			
2009								
Abandoned terrace <sup>a</sup>	8	0	0	n.a.	n.a.	108	1.1	100
Mown terrace <sup>a</sup>	16	0	0	n.a.	n.a.			
Abandoned meadow <sup>b</sup>	8	0	0	n.a.	n.a.	114	1.4	80
Mown meadow <sup>b</sup>	8	0	0	n.a.	n.a.			
2010								
Abandoned terrace <sup>a</sup>	8	0.0	0.0	n.a.	n.a.	147	1.7	85
Mown terrace <sup>a</sup>	16	0.0	0.0	n.a.	n.a.			
Abandoned meadow <sup>b</sup>	8	38 $\pm$ 11	47 $\pm$ 7	9 $\pm$ 9	23	142	2.6	55
Mown meadow <sup>b</sup>	8	19 $\pm$ 6	34 $\pm$ 3	<b>15 <math>\pm</math> 5</b>	<b>76</b>			
<i>Furka<sup>c</sup> (CH), 2440 m a.s.l.</i>								
2008								
Tall grass	16	87 $\pm$ 2	94 $\pm$ 1	<b>7 <math>\pm</math> 1</b>	<b>8</b>	133	5.8	23
Short grass	16	88 $\pm$ 1	92 $\pm$ 1	<b>4 <math>\pm</math> 1</b>	<b>4</b>			
Forbs	8	90 $\pm$ 1	93 $\pm$ 1	<b>3 <math>\pm</math> 1</b>	<b>4</b>			
2009								
Tall grass	16	49 $\pm$ 3	68 $\pm$ 5	<b>19 <math>\pm</math> 4</b>	<b>39</b>	184	3.7	50
Short grass	16	55 $\pm$ 4	65 $\pm$ 2	<b>10 <math>\pm</math> 3</b>	<b>18</b>			
Forbs	8	52 $\pm$ 3	56 $\pm$ 5	4 $\pm$ 4	8			
2010								
Tall grass	16	67 $\pm$ 3	72 $\pm$ 3	4 $\pm$ 3	7	115	5.0	23
Short grass	16	67 $\pm$ 1	66 $\pm$ 1	0 $\pm$ 1	-1			
Forbs	8	73 $\pm$ 3	64 $\pm$ 4	-9 $\pm$ 3	-13			
<i>Stubai (A), 2000 m a.s.l.</i>								
2008								
Abandoned	16	175 $\pm$ 8	215 $\pm$ 5	<b>40 <math>\pm</math> 6</b>	<b>23</b>	357	5.4	66
Abandoned (+dwarf shrubs)	10	151 $\pm$ 7	200 $\pm$ 5	<b>49 <math>\pm</math> 6</b>	<b>33</b>			
Meadow	20	154 $\pm$ 7	202 $\pm$ 5	<b>47 <math>\pm</math> 6</b>	<b>31</b>			
Pasture	20	168 $\pm$ 9	197 $\pm$ 3	<b>29 <math>\pm</math> 7</b>	<b>17</b>			
2009								
Abandoned	16	92 $\pm$ 3	111 $\pm$ 2	<b>19 <math>\pm</math> 3</b>	<b>20</b>	249	3.7	68
Abandoned (+dwarf shrubs)	10	83 $\pm$ 3	102 $\pm$ 3	<b>18 <math>\pm</math> 3</b>	<b>22</b>			
Meadow	20	92 $\pm$ 3	103 $\pm$ 2	<b>11 <math>\pm</math> 3</b>	<b>12</b>			
Pasture	20	95 $\pm$ 3	100 $\pm$ 2	5 $\pm$ 3	6			
2010								
Abandoned	16	107 $\pm$ 6	144 $\pm$ 9	<b>36 <math>\pm</math> 8</b>	<b>34</b>	236	4.4	54
Abandoned (+dwarf shrubs)	10	87 $\pm$ 6	145 $\pm$ 8	<b>58 <math>\pm</math> 7</b>	<b>66</b>			
Meadow	20	103 $\pm$ 8	144 $\pm$ 6	<b>41 <math>\pm</math> 7</b>	<b>40</b>			
Pasture	20	101 $\pm$ 4	128 $\pm$ 6	<b>27 <math>\pm</math> 5</b>	<b>27</b>			

<sup>a</sup>Les Cours and <sup>b</sup>Le Jardin, sub-sites at Lautaret (F).

<sup>c</sup>SE-facing sub-site at Furka.

A comparison of the three study years 2008 to 2010 and the three sites across the Alps illustrated that P and thus, DS during the time after clipping were highest in 2008 and lowest in 2009 at all sites (P is given per day to standardize for different lengths of measurement periods; DS:  $F_{10,319} = 363$ ,  $P < 0.001$ ; Table 2). Furthermore, P (per day) was highest at Furka and lowest at Lautaret during all seasons, except for 2009, when P at Furka and Stubai were equal. Within the Lautaret site, P was always higher at the subalpine sub-site Le Jardin than at the lower-elevation sub-site Les Cours. At Lautaret, late seasons in 2009 and 2010 (Les Cours) were too dry to permit any deep seepage even in clipped DSCs, which means that all water input by rain was lost through ET during these periods. At Le Jardin (2120 m a.s.l.), the 2010 clipping effects were roughly similar to those of 2008. At the more humid Swiss site, the most pronounced evaporative water savings by biomass removal were found during the long and exceptionally dry 2009 period, while clipping effects were smaller in the more rainy 2008 period and even lacking in 2010, when snowfall events were rather frequent. At Stubai, clipping led to a clear increase in DS in all years, with the weakest absolute and relative effects in the driest season 2009. More pronounced effects were found in the moister seasons 2008 and 2010, with the highest relative increase in DS by clipping in 2010. In the years 2009 and 2010, the trends in the magnitude of clipping effects between different grassland types at Furka and at Stubai remained roughly the same as in 2008.

## Discussion

The assessment of the water balance of alpine grassland using a large set of contained monoliths in three regions across the Alps revealed that deep seepage (DS) and evapotranspiration (ET) are both influenced by the land cover type. Furthermore, experimental biomass removal similar to mowing or grazing caused pronounced water savings (more runoff). These results underpin the significance of land care for catchment value in alpine terrain. The robustness of the findings across this wide geographical range offers broad ap-

plicability of the results. In the following, I will first discuss land cover effects and differences across sites and will then compare the effects of biomass removal by land use (clipping) in different grassland types.

### *Seasonal water balance*

The rates of grassland ET across the Alps presented in this study (full data sets for all regions for the 2008 season) are within the range or slightly higher than those found in earlier lysimeter studies in the Austrian Alps (including more eastern parts). Wieser et al. (2008) arrived at 165 to 360 mm per season and, if divided by the number of days per growing season, 1.9 to 2.5 mm per day in grassland at 1800 m to 2500 m a.s.l.. In the present study, the highest ET was measured at Stubai (2000 m a.s.l., Austrian Alps), with 335 to 392 mm per growing season (2.4 to 2.8 mm per day), depending on land use type. The Stubai site also showed the highest biomass as well as canopy height of all sites. In view of the drier climate in the studied region of the French Alps, we expected water shortage and reduced ET during the growing season, especially, at the lower sub-site. However, in 2008, ET at both Lautaret sub-sites was not lower than ET in the other regions. An equivalent study with weighing lysimeters in the Swiss Alps (2440 m a.s.l.) arrived at mean daily ET rates (calculated by the water balance across the growing season) of 2.0 mm in short to 2.9 mm in tall grassland for the growing season 2009, and of 1.8 to 2.3 mm for 2010 (Chapter 3). In the present study with deep seepage collectors, plants at the N-exposed sub-site at Furka showed much lower daily ET rates (1.7 mm) than very similar grassland communities situated at the SE-facing sub-site (2.3 mm). This difference was largely a result of late snowmelt, shorter season and thus, reduced biomass at the northern slope. Furthermore, the lower radiation sums result in reduced soil warming by insolation at the northern slopes. Soil temperature measured at -10 cm across the growing season 2008 averaged at  $8.2 \pm 3.9^\circ\text{C}$  at the N-facing site and at  $11.4 \pm 3.2^\circ\text{C}$  at the SE-facing sub-site (mean  $\pm$  SD).

While mean daily ET of grassland in the Austrian Alps did not follow any elevational trends, so that seasonal sums were in essence determined by the length of the growing season (Wieser et al. 2008), a more recent experiment using weighing lysimeters in the Swiss Alps along an elevation gradient of ca. 1000 m, evidenced clear elevational trends. Bright day ET rates decreased over-proportionally compared to the atmospheric evaporative demand between 1500 and 2440 m a.s.l. (van den Bergh et al., 2013). This discrepancy is likely due to the inclusion of periodically dry periods at lower elevations (<1000 m a.s.l.) in the Austrian study, whereas the Swiss transect was confined to continuously humid conditions. The decline in ET was explained by a combination of lower transpiring biomass and reduced evaporative forcing at higher elevation. The present study also suggests an elevational decline of daily ET given the higher rates at Stubai (2000 m a.s.l.) compared to those at Furka (2440 m a.s.l.), both receiving similar daily precipitation input across the growing season 2008, but substantially differing in plant biomass per unit area.

The calculation of seasonal ET rates based on the water balance equation requires correct measurements of precipitation. In this study, we found that P measured at 5 to 10 cm above ground level yields 15 to 24% higher seasonal sums than precipitation measured at 2 m above ground at Furka (2008 to 2010) and 14 to 19% at Stubai. For reasons related to the logarithmic vertical wind profile over grassland, ground-level precipitation, which is relevant for the water balance, is typically higher than P measured at 2 m above ground (+6% to +11%, Körner et al. 1978; Wieser et al. 2008). However, our P measurements at Furka and Stubai near ground level may also have been prone to errors by net gains in splash water. P measured at the French site (at 2 m, no near ground-level data available) is thus potentially underestimating the actual rain input at ground level, which would further increase calculated ET rates in both the montane and subalpine sites at Lautaret.

#### *Seasonal water balance of different land use / grassland types*

The various grassland types (unclipped monoliths) selected across the three mountain regions mirror the different past and present land management, its intensity as well as the vegetation responses to the recent land use decline (i.e. grassland at different stages of succession). In unclipped stage, ET at the lower and the higher French sites was slightly higher in grassland still regularly mown compared to the abandoned grassland types, although biomass and canopy height were higher in the abandoned compared to the mown meadow, most likely because of the accumulation of dead plant material in abandoned grassland. A shift in species composition after abandonment towards less vigorous and thus, less transpiring species could be another reason for the slightly lower ET in intact abandoned grassland (Gross et al. 2008). At the alpine Furka site, ET in tall, short and forb-dominated swards averaged at surprisingly similar values, although biomass and canopy height differed significantly among grassland types. At Stubai, the dwarf shrub dominated swards showed the highest ET, likely due to better aerodynamic coupling (highest and more open canopies). The accumulation of litter and standing dead dry matter are the likely causes why the abandoned grassland without dwarf shrubs showed lowest ET (Tappeiner and Cernusca 1998). However, when seasonal ET sums of abandoned grassland at Lautaret and Stubai were compared to ET of clipped variants of both, meadows and pastures (the analogue to the normal management), ET in abandoned grassland was always higher or equal than in grazed or cut grassland.

The experimentally removed biomass down to a standardised stubble height of 3 to 4 cm represents a much smaller fraction of biomass in low-stature grassland types, especially, at the alpine site Furka, than in tall swards. However, stubble of otherwise tall swards contains large amounts of dry matter that hardly contributes to transpiration. For the dwarf shrub-rich, abandoned grassland (Stubai), the predictive power of clipped biomass for total stand-

ing biomass is limited, since only the grass fraction was clipped but not the dwarf shrubs of the same monolith. Grasslands at Furka and Stubai are stocked by biomass well in the range published in literature (Körner 2003; Schmitt et al. 2010), while grassland at Lautaret (outside DSCs) commonly accumulates much more biomass and grows much taller (Quétier et al. 2007).

#### *The effect of biomass removal on the water balance*

Results of three years of land use simulation by applying a clipping treatment in different grassland types at different sites across the Alps obviously reflected the amount of biomass removed. Hence, increases in total runoff were highest in lush, subalpine grassland at Stubai. Lower biomass and plant stature, as at the alpine site Furka, yielded much less seasonal water savings by clipping. While runoff effects of land use in the alpine belt (Furka) were largest in dry summers (2009), they disappeared under summer drought in the dry and continental montane and subalpine site at Lautaret. At the Furka site, the water savings by land use were reduced during very moist periods, during which heavy rain events prevented the build-up of soil moisture differences between clipped and unclipped vegetation. Excluding drought at Lautaret (2009 and, for the montane sub-site, 2010) and frequent snowfall periods at Furka (2010), we arrive at additional water yield 'produced' by land use of 13 mm ( $130\text{ m}^3\text{ ha}^{-1}$ ), 8 mm ( $80\text{ m}^3\text{ ha}^{-1}$ ) and 32 mm ( $320\text{ m}^3\text{ ha}^{-1}$ ) per season at the French, Swiss and Austrian site, respectively. These water savings caused by clipping were calculated for the second part of the growing seasons only, i.e. for the period during which grazing or mowing effects would come into action (cutting at peak biomass at each site). So, when considering the whole season's water balance, the relative effects of clipping on DS, but not the absolute ones, will be reduced, since the first half of the growing season is not affected by the treatment. The effect of mowing on DS may even be enlarged in intensively used meadows, which are often mown earlier and cuts are repeated several times during the growing season.

We conclude that the higher biomass and taller plant canopies (increased leaf area) associated with extensification or abandonment of alpine pastures and meadows increase daily ecosystem vapour loss compared to regularly used land, mainly during non-water-limited periods and more so in the sub-alpine than in the alpine belt. Signals also diminish when standing dead material accumulates. As most of the high mountain terrain receives plentiful precipitation, effects of land use on water yield are significant. The impacts on catchment value can be assessed by an economic valuation considering the 'costs' of the additional water that is lost through ET, when land use is reduced or fully abandoned. Such an assessment would permit to attribute economic value to pastoralism that goes beyond the presently widely perceived agronomic and ecological aspects (see Chapter 3). Such economic implications are region-specific and depend on elevation (potential energy of water) and thus, await evaluation at larger scales in space and time (e.g. employing geographical information systems to assess total discharge of river systems for several years or decades).

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## General Summary and Conclusions

This PhD thesis addressed two main aspects of ‘Global Change’ and their effects on alpine vegetation, the rising CO<sub>2</sub> concentration in the atmosphere as well as land use and its current decline. Current and future rises of CO<sub>2</sub> concentrations are commonly expected to stimulate photosynthesis and to reduce carbon limitation of growth. Whether this supposition holds for alpine pioneer species in glacier forefields was explored in a project, the results of which are presented in **chapter 2**. The second part of this thesis tested the hypothesis that elevated CO<sub>2</sub> (through its influence on stomata) reduces evapotranspiration, which in turn should enhance runoff. This question was combined with a second driver of evapotranspiration, the density and height of vegetation. It was expected that land use (grazing) reduces biomass and thus, water vapour losses (evapotranspiration), which should lead to increased runoff as well (**chapter 3**). Since land use changes are a widespread across the Alps, this latter question was explored over an East to West gradient across the Alpine Arc together with partners in France and Austria. In **chapter 4**, I present a first synthesis of this consortial undertaking.

### *Chapter 2: No growth stimulation by CO<sub>2</sub> enrichment in alpine glacier forefield plants*

Three seasons of free-air CO<sub>2</sub> enrichment (580ppm) at 2440m a.s.l. in the Swiss Alps revealed that none of the nine glacier forefield species grown in assemblages were stimulated by elevated CO<sub>2</sub> throughout the course of the experiment. Which came as a surprise, as one would expect high-elevation plants, naturally growing under reduced partial pressure of CO<sub>2</sub>, and especially alpine pioneer vegetation, growing in open terrain and provided with ample space to expand, to be particularly responsive to atmospheric CO<sub>2</sub> enrichment. The four species that were additionally grown in isolation were significantly stimulated (34% higher biomass) by a low dose of full mineral fertilizer (equivalent to 25 kg N ha<sup>-1</sup> a<sup>-1</sup>) that was applied to half of the individuals in order to explore a potential nutrient limitation of the CO<sub>2</sub> response. However, the combination with elevated CO<sub>2</sub> did

not further enhance plant growth either. Total (above- plus belowground) biomass was not affected by increased CO<sub>2</sub> concentration, but unexpectedly, elevated CO<sub>2</sub> reduced aboveground biomass by –35% across all species grown together in microcosms or in isolation. Even in the fast growing, more competitive species showed negative growth responses, although they were hypothesised to show strongest positive CO<sub>2</sub> responses. The reduced aboveground biomass was associated with higher biomass partitioning to roots, which was often observed in plants exposed to elevated CO<sub>2</sub> growing under relatively low nutrient availability. We measured several leaf traits as explanatory variables for growth responses to the CO<sub>2</sub> and the fertilizer treatments. The foliar nonstructural carbohydrate concentration (NSC) increased under elevated CO<sub>2</sub>, reflecting an imbalance between carbon sources and sinks. The nitrogen concentration in leaves decreased significantly, even when correcting for the higher NSC concentration, suggesting down-regulation of Rubisco synthesis. Accordingly, we observed a significant downward adjustment of photosynthetic capacity by on average –26% under long-term exposure to 580ppm CO<sub>2</sub>, which was assessed in graminoids only, as leaves of the other species were too tiny. These leaf trait responses to experimentally enriched atmospheric CO<sub>2</sub> were in line with the earlier experiment on late-successional alpine grassland in the same area of the Swiss Alps (Körner *et al.* 1997). This suggests, together with the consistent reduction of leaf N in almost all species examined here, that the associated down-regulation of leaf-level photosynthesis is likely to be common to alpine plant species under long-term exposure to elevated CO<sub>2</sub>. The absence of any CO<sub>2</sub>-driven growth stimulation may be related to an enhanced microbial nutrient immobilization induced by increased plant C inputs (so called exudates) to the rhizosphere (Diaz *et al.* 1993), or to the fact that positive growth responses to CO<sub>2</sub> enrichment, when they were observed in lowland grassland, were caused by CO<sub>2</sub>-induced stomatal responses leading to soil moisture savings (Morgan *et al.* 2004), which can not materialize in these permanently moist alpine systems. In the alpine belt, low temperature, at least

during the night, determinate growth in a short growing season and the inherently slow growth of some species may further constrain positive CO<sub>2</sub> effects on plant growth. With a great robustness across species and growth conditions, our results thus suggest that glacier forefield pioneers, growing under harsh climatic conditions are not carbon limited at current atmospheric CO<sub>2</sub> concentration.

*Chapter 3: Hydrological consequences of declining land use and elevated CO<sub>2</sub> in alpine grassland*

To quantify the partly opposing effects of land use abandonment (less runoff) and elevated CO<sub>2</sub> (more runoff) on the water balance of alpine grassland we used undisturbed monoliths of four alpine grassland types, either dominated by the grasses *Agrostis schradariana* or *Nardus stricta*, by the sedge *Carex curvula* or by forbs, in 51 weighing lysimeters in a field experiment in the Swiss Alps (2440 m a.s.l.). The weighing lysimeters allowed measuring rates of daily evapotranspiration (ET) directly on bright days, determining soil water contents from actual water content minus monolith dry weights and calculating the seasonal ET sums from deep seepage collected under the lysimeters. Part of the monoliths was clipped to a common vegetation height of 3 to 4 cm in order to simulate sheep grazing during three seasons (2008 to 2010). Another set was exposed to elevated CO<sub>2</sub> (580 ppm) using free-air CO<sub>2</sub> enrichment (FACE) during the 2009 growing season. Simulated grazing reduced bright day ET by on average –12% across all years, ranging from +1% to –25%, depending on grassland type. The highest-stature *Agrostis* dominated swards showed the most pronounced water savings due to clipping. As a consequence, the higher biomass and LAI in unclipped grassland also lowered the seasonal sum of deep seepage (equivalent to total runoff) by on average –13% across all grassland types in a comparatively dry year (2009) and by –5% in a wet year (2010), compared to clipped swards. The experimental enrichment of the atmosphere with CO<sub>2</sub> reduced ET by –4% to –9% depending on grassland type. Furthermore, the isotopic ratio of <sup>18</sup>O in foliage was significantly increased under elevated CO<sub>2</sub>, providing an integrated signal of reduced stomatal conductance

and thus, diminished leaf-level transpiration across the whole growing season. Consequently, soil moisture was significantly higher in monoliths subjected to 580 ppm CO<sub>2</sub>, however, effects of elevated CO<sub>2</sub> on deep seepage were not statistically significant. Hence, future (double pre-industrial) CO<sub>2</sub> slightly counteracts the land use effects at canopy level, however, not in terms of water yield, so that the net effect of declining land use or abandonment and elevated CO<sub>2</sub> is still clearly negative for catchment water yield and thus, for potential hydroelectric power production. Although these economic ‘costs’ of water loss due to declining land use or abandonment are moderate per hectare of alpine grassland, the sums are substantial when scaled to the vast areas potentially affected in the Alps. For the Ursern valley (9000 ha of vegetated area), our test region in the headwaters of the river Reuss, the seasonal potential loss in yield amounted to –220 to –350 m<sup>3</sup> per hectare of alpine grassland, corresponding to CHF 70 to 112 per hectare and year and to a total of CHF 630 000 to 1 000 000 per year for the whole catchment, for an assumed 1.5 km vertical gradient of potential energy. Our results advocate the inclusion of water yield costs in assessments of the consequences of reduced pastoralism in alpine catchments. Furthermore, they highlight the importance of land use effects for alpine hydrology, especially under future climatic warming, which is likely to reduce discharge of alpine catchments during prolonged summer seasons (Beniston *et al.* 2011; Viviroli *et al.* 2011).

*Chapter 4: The effect of land use and its decline on the water balance of montane and alpine grassland across the Alps*

This synthesis of the Bio-CATCH network summarizes the effects of land use and its current decline on the vegetation cover and the water balance of former montane and alpine pastures and meadows along a W to E transect across the Alps (France, Switzerland and Austria). Deep seepage (DS) and evapotranspiration (ET) of grassland was assessed using a total of 146 intact grassland monoliths in stainless steel deep seepage collectors (DSC), soil moisture sensors and weather stations at each site. The seasonal water

balance reflected differences in canopy height and biomass among the different grassland or land use types employed in DSCs. The highest ET was measured in the Austrian Alps (2000 m.a.s.l.; 2.4 to 2.8 mm per day), where also grassland biomass and canopy height were highest. The lowest ET was found in the lowest-stature swards, at the true alpine site in the Swiss Alps (2440 m.a.s.l.; 1.7 to 2.3 mm per day). We expected water shortage and reduced ET at the drier French sites, however, ET at the montane and sub-alpine sub-sites was not lower than ET in the other regions (2.6 to 2.7 mm per day). In unclipped stage, ET at the lower and the higher French sub-sites was slightly higher in grassland still regularly mown compared to the abandoned grassland types, most likely because of the accumulation of dead plant material within the canopy of abandoned grassland. ET in tall, short and forb-dominated swards (unclipped monoliths) averaged at surprisingly similar values at the alpine Swiss site, although biomass and canopy height differed significantly among grassland types. At the Austrian site, the dwarf shrub dominated swards showed the highest ET, likely due to better aerodynamic coupling, whereas litter accumulation caused the lowest ET in abandoned grassland without dwarf shrubs. Three seasons of experimental simulation of land use by clipping vegetation to a common height of 3 to 4 cm at and after the peak of the growing season yielded significantly increased sums of deep seepage (DS) due to reduced ET. The size of the clipping effect on DS obviously reflected the amount of biomass removed. The highest increase of DS was observed in tall grassland of the lower alpine belt in Austria, while the increase of DS due to clipping was much smaller in naturally short vegetation of the upper alpine belt in Switzerland, all compared to unclipped swards. While runoff effects of land use in the alpine belt (Swiss site) were largest in dry summers (2009), they disappeared under summer drought in the montane and subalpine belt at the dry, continental French site. At the Swiss site, the water savings by land use were reduced during very moist periods, during which heavy rain events prevent the build-up of soil moisture differences between clipped and unclipped vegetation. Overall, excluding the drought season at the French site (without seepage, 2009) and a period

with frequent snowfall at the Swiss site (2010), the additional water yield 'produced' by land use, calculated for the second half of the season after first clipping only, amounted to 13 mm ( $130\text{ m}^3\text{ ha}^{-1}$ ), 8 mm ( $80\text{ m}^3\text{ ha}^{-1}$ ) and 32 mm ( $320\text{ m}^3\text{ ha}^{-1}$ ) per season at the French, Swiss and Austrian site, respectively. The impacts on catchment value across the Alps can be assessed by an economic valuation considering the 'costs' of the additional water lost through ET, when land use is reduced or fully abandoned (as has been done for the Ursern Valley in chapter 3). Our results underline the significance of continued land care for catchment value in terms of fresh water supply and hydroelectric power production in different alpine regions across the Alps.

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## Curriculum vitae of *Nicole Inauen*

### Address

Stückelbergstrasse 4  
CH-9000 St.Gallen  
Switzerland

**Phone** +41 (0)79 764 21 33  
**Email** nicole.inauen@bluewin.ch  
**Date of birth** 20 June 1983  
**Place of origin** Appenzell, AI  
**Nationality** Swiss

### Education

**2008 – 2012** PhD at the Institute of Botany, University of Basel, Thesis: 'The consequences of land use and elevated CO<sub>2</sub> in alpine ecosystems', supervised by Prof. Christian Körner

**2006 – 2008** MSc in Ecology at the Institute of Botany, University of Basel, Thesis: 'No growth stimulation in glacier forefield plants by elevated atmospheric CO<sub>2</sub> concentration', supervised by Prof. Christian Körner

**2006** Tropical Biology Association – Field course in savannah ecology and conservation in Kenya

**2003 – 2006** BSc in Biology with Major in Animal and Plant Sciences, University of Basel

### Professional Experience

**2013 – present** Project manager, Landscape Quality, Landwirtschaftliches Zentrum St.Gallen LZSG

**2012 – 2013** Scientific assistant, Ecology section, Swiss Federal Office for Agriculture FOAG

**2008 – 2011** Teaching assistant for the lab and field course 'Blockkurs Ökologie'

**2008** Editorial assistant, Oecologia Office, Institute of Botany, University of Basel

**2007** Field assistance for the 'Swiss canopy crane' project, Institute of Botany, University of Basel

## Publications

Inauen N, Körner C, Hiltbrunner E (2013) Hydrological consequences of declining land use and elevated CO<sub>2</sub> in alpine grassland. *Journal of Ecology*, **101**, 86–96.

Inauen N, Körner C, Hiltbrunner E (2012) No growth stimulation by CO<sub>2</sub> enrichment in alpine glacier forefield plants. *Global Change Biology*, **18**, 985–999.

## Awards

Prix de Quervain 2013, Young Scientist Award of the Swiss Committee on Polar and High Altitude Research, Swiss Academies of Arts and Sciences

## Conferences and Meetings

LATSIS Symposium 2010, Lausanne – Poster: ‘Land use change and elevated CO<sub>2</sub> affect the water balance of alpine grassland’, N. Inauen, E. Hiltbrunner, Ch. Körner

GMBA Conference, 2010, Chandolin VS – Talk: ‘Diversity responses to elevated CO<sub>2</sub> in alpine plant communities’, N. Inauen, E. Hiltbrunner, Ch. Körner

11th Swiss Global Change Day 2010, Bern – Poster: ‘Land use change and elevated CO<sub>2</sub> affect the water balance of alpine grassland’, N. Inauen, E. Hiltbrunner, Ch. Körner

Diversitas Open Science Conference 2, 2009, Cape Town, South Africa – Talk: ‘Biodiversity responses to elevated CO<sub>2</sub> in glacier forefield plant communities’, N. Inauen, E. Hiltbrunner, Ch. Körner

Phil.Alp 2008, Brig – Talk: ‘Wie reagieren Pflanzen im Gletschervorfeld auf die erhöhte CO<sub>2</sub>-Konzentration in der Atmosphäre’, N. Inauen, E. Hiltbrunner, Ch. Körner

## Coursework during PhD

Ecology of alpine plants, field course, July 2008, Furka, Ch. Körner and J. Stöcklin

Contemporary applied statistics for ecology, January 2009, Zurich, A. Hector

Scientific illustrations using R, January 2010, Zurich, A. Hector

Scientific writing practice 2: writing up research, Feb/Mar 2010, Zurich, P. Barnett

Project management, January 2011, Zurich, S. Eichenberger

Research seminar ‘plant ecology’, 2008–2011, Basel, Ch. Körner and J. Stöcklin

Russian: elementary language course, 2011, Basel, E. Weisskopf