



Full Length Article

Response of Semi-natural Wet Meadow to Natural Geogenic CO₂ Enrichment

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Abstract

A semi-natural wet meadow, dominated by *Carex rostrata*, experienced a natural geogenic CO₂ enrichment of soil [CO₂] and a small layer of air above the surface throughout the year, and occasional flooding in autumn. We expected such conditions to be tolerated by flood tolerant plant species, which might even benefit from CO₂ enrichment. We examined the influence of elevated [CO₂] and/or flooding on primary production of meadow vegetation and *C. rostrata*, measured through above- and below-ground biomass, LAI and photochemical efficiency (chlorophyll fluorescence Fv/Fm). Results showed that *C. rostrata* biomass increased under conditions of enhanced [CO₂] and elevated soil water content (SWC). The positive effect of increased [CO₂] was hampered by simultaneous increase in SWC. Additionally, patterns of *C. rostrata* senescence are changed, since dead biomass significantly decreased with the increase in [CO₂] levels and the contrary was shown for higher SWC. These results explain why *C. rostrata* was the dominant species. In summer, *Carex* responded with enhanced growth on plots with higher SWC, which represented its optimal habitat, while on drier sites it benefited from elevated [CO₂] because of improved water use efficiency. During autumn flooding, the positive effect of increased [CO₂] on biomass production was hampered by and corresponded with increased root biomass. *Carex rostrata*, well adapted to waterlogged soil and hypoxia, was able to tolerate O₂ deficiency irrespective of its origin: waterlogging or geogenic CO₂. Higher levels of [CO₂] in the soil increased the photochemical use of *Carex* in the afternoon with low-light conditions, even though the changes in photochemical efficiency were best explained by the erectophile canopy. In crux, *C. rostrata* is the dominant species in this particular semi-natural meadow, can tolerate high levels of geogenic [CO₂] in the soil and benefit from elevated [CO₂] in the air during drought and low-light. © 2013 Friends Science Publishers

Keywords: CO₂ enrichment; Waterlogging; *Carex*; Chlorophyll fluorescence; Biomass

Introduction

Steadily rising atmospheric CO₂ concentrations ([CO₂]), together with current projections predicting a continuous rise in the next decades, have attracted broad public interest. Attention has mainly been shifted to the consequences of elevated [CO₂] on global climate; however the rising [CO₂] is also likely to have a profound direct impact on ecosystems (Körner, 2003; Montoya and Raffaelli, 2010). Among ecosystems, grasslands often show detectable responses to increased [CO₂] (Tuba and Kaligarič, 2008). A majority of experiments involving CO₂ enrichment and grassland species have taken place in the controlled conditions, over a shorter period of time, lasting from days to a few months (Lee *et al.*, 2010). Accordingly, responses to CO₂ enrichment under controlled conditions may be significantly different to those observed in natural and semi-natural ecosystems (Wang, 2007). This is why every study in a natural environment, with a natural plant community adds an additional aspect to plant responses to elevated [CO₂]. This is especially so when plants are exposed to free-

air CO₂ enrichment (FACE), where the opportunity for long-term acclimation responses over multiple seasons (Leakey *et al.*, 2009) and for some generations exist (Paoletti *et al.*, 2005). On rare occasions in nature, emissions of geogenic CO₂ from mofettes generate plant communities in sites with locally increased [CO₂] in the soil and air. The best studied examples involve the Bossoleto spring in Italy and Strmec in Slovenia (e.g.; Vodnik *et al.*, 2002a, b; Marchi *et al.*, 2004; Pfanz *et al.*, 2007). Of course, results obtained in this way are not straightforward for describing the response of these plants to future atmospheric CO₂ increase.

Plants respond to increased [CO₂] with rapid physiological and biochemical responses, as well as with responses that become visible only after a longer period of time. A non-invasive method for defining fast response, before it becomes visible to the naked eye, is the monitoring of photosynthetic assimilation *in vivo* by measuring chlorophyll fluorescence, which is used for the calculation of maximum photochemical efficiency (F_v/F_m) of photosystem II (PSII). On a broader timescale, exposure to

elevated CO₂ can stimulate photosynthetic assimilation (Marek *et al.*, 2001) with increased leaf longevity in C₃ species (Craine and Reich, 2001). Because of its fertilization effect, the increase in [CO₂] can compensate for various environmental limitations influencing photosynthetic efficiency (Cherry *et al.*, 2009).

On the other hand, geogenic CO₂ enrichment causes hypoxic to anoxic conditions in the soil. It is well known that anoxia tolerance is positively correlated with flood tolerance (Fagerstedt and Crawford, 1987). We were interested in how plants respond to simultaneous stress by both flooding and carbon enrichment through a geogenic source. Can flood-tolerant plant species benefit from geogenic CO₂ enrichment, or does occasional flooding disturbance with hypoxia produce an even more stressful environment? The aim of the study was to examine the influence of elevated levels of [CO₂] combined with flooding as a limiting factor on primary production of flood-tolerant *Carex rostrata* (Cyperaceae), measured through both photochemical efficiency and above- and below-ground biomass. Because of the complexity of study site conditions, several other abiotic factors were also monitored in order to observe their potential influence on biomass. Additionally, some chlorophyll fluorescence measurements were made on the dominant *C. rostrata* along the transect to indirectly study different functional levels of photosynthesis under such specific environmental conditions.

Materials and Methods

Study Site and Target Species

The selected semi-natural wet meadow was located in the village of Rihtarovci (46°37'59"N, 16°3'19"E, 207 m.a.s.l.) in NE Slovenia (Central Europe), within the area of Radenci Spa, which is famous for its springs of mineral water containing dissolved CO₂ and for the occurrence of CO₂ mofettes. The CO₂, compressed in Miocene and Pliocene porous sediments under a pressure of 100 to 110 bars, results from post-volcanic activity (Žižek, 1999). Many studies involving elevated levels of [CO₂], have been performed in this area (Kaligarič, 2001; Pfnan *et al.*, 2004; Aschan *et al.*, 2005).

In 2001 and 2002 we selected a small area (200 m²) of a wet meadow with a natural CO₂ spring, one that had never been studied before. The gas vent was a mofette, which presented as a small hole flooded by rain water, giving it a puddled appearance. The leaking of geogenic CO₂ could be observed as small bubbles coming to the surface, as well as by the presence of many dead animals, which were attracted to the water and died because of high [CO₂]. The near proximity of the CO₂ spring in a radius of 2 m was without vegetation. The vegetation assemblage further away was a semi-natural wet meadow. The climate in this area is typically temperate, with a mean annual highest daytime temperature of 14.8°C and a mean annual minimum temperature of about 4.9°C. The total annual rainfall is

around 960 mm. During precipitation peaks in early spring and late autumn, this wet meadow was occasionally flooded. Because the flooding is a consequence of high precipitation and the clayey soil, preventing periodic flushing of nutrients, the productivity of this particular wet meadow type is low. This is why the meadow has been used as a litter meadow for several decades and has been regularly mown twice a year. Most of the standing biomass was composed of *Carex rostrata* Stokes (Cyperaceae), which in some sections even formed monodominant stands. According to the water content in the soil, *C. rostrata* prefers moist, partially submerged conditions (Visser *et al.*, 2000). Additionally, we have chosen to study *C. rostrata* as a representative of the entire C₃ genus (Hesla *et al.*, 1982), since this is the type of photosynthesis that shows the strongest response to enhanced [CO₂] (Poorter and Navas, 2003; Ainsworth and Long, 2005).

Establishing the Transect and [CO₂] Measurements

For the analysis of the response of wet meadow plants grown in elevated CO₂ conditions, we defined a transect with changing [CO₂], using a portable infrared gas analyser –IRGA (EGM-1, part of the porometer PMR-1, PP-Systems, Stotfold, UK) in the spring of 2001 and 2002. The distance between transect points was fixed so that the 10 m long transect consisted of 13 points. At points with greater changes in [CO₂] levels, six harvesting plots (0.25 m²) were marked on the left and right side of the transect line for summer and fall harvest.

Additional measurements of [CO₂] were performed at the time of harvest and during chlorophyll fluorescence measurements with two types of carbon dioxide diffusion tubes (Dräger, Vienna, Austria). For the measurements in the air (on the earth surface and between the canopy at 10 cm), the “carbon dioxide 500/a-D” (500-20000 ppm·h) was used. Measurements in the soil were performed with “carbon dioxide 1%/a-D” (1-30 Vol%·h) diffusion tubes, which we inserted in a 10 mm wide hole drilled into the soil. On the blind end of the tube, a stopper was placed, which entirely blocked the hole and prevented any CO₂ leakage. Readings were taken at different time intervals depending on the current [CO₂] levels. We made corrective calculations according to the diffusion tube instructions, when necessary.

Other Abiotic and Biotic Factors

The environmental variables, which we measured in one corner of each plot and along the [CO₂] gradient were as follows: water content in the soil (using TRIME-FM, Imko GmbH, Ettlingen, Germany); soil temperature 5 and 15 cm deep; and air temperature, and RH of the air (Humicap HM, Vaisala, Oy) above and between the canopy. For determining the photosynthetic photon flux density of photosynthetically active radiation (PAR) and global radiation, we took the readings with a quantum sensor (400-700 nm) and a pyranometer (350-900 nm) every hour.

Leaf area index (LAI) was obtained with an AccuPAR LP-80 Ceptometer (Decagon Devices, Pullman, WA, USA), which measures canopy interception of PAR and sun flecks, as well as calculating LAI. Light measurements were made directly above the canopy, and these values were compared to an average of 8 measurements below the canopy – at the soil surface between 11:00 and 14:00 h on cloudless days in July and September.

Chlorophyll Fluorescence

Chlorophyll a fluorescence was measured using a portable chlorophyll meter (PEA, Hansatech Ltd., King's Lynn, UK). The measurements were performed *in vivo* on the upper and lower sides of a selected, marked leaf. We choose to measure only the 2nd fully expanded leaves since chlorophyll fluorescence is usually leaf age-dependent (Lin *et al.*, 2011). Each measurement – the estimation of potential photochemical efficiency of PSII (Fv/Fm) – consisted of measuring the actual fluorescence and fluorescence after 15 min of dark adaptation. Measurements on the same leaf were done before dawn, in the morning, at noon, and in the afternoon. The leaf-clips of the measuring device were removed each time after the measurement of dark-adapted leaves to prevent leaf damage and were arranged on the leaves again immediately before the next measurements. Three leaves were measured on each plant in each plot along the transect representing the changing [CO₂]. Parameters of the fluorometer were identical for all measurements.

Pre-dawn Fv/Fm fluorescence ratios (Fv/Fm_{PD}) are expected to be the highest measured. Generally, the high pre-dawn values cannot be attained in later measurements, not even in the case of dark adapted leaves for 15 min, because of photochemical quenching, which increases along with rising levels of irradiance during the course of the day (Bolh ar-Nordenkampf and  quist, 1993). To present the fluorescence data, we used stacked bars, where the Fv/Fm_{PD} represents 100%. Therefore, we expressed actual (act. Fv/Fm) and dark adapted (d.a. Fv/Fm) morning, noon and afternoon fluorescence data as a ratio of pre-dawn value (e.g.

$$\frac{\text{act. Fv/Fm}_{\text{morning}}}{\text{act. Fv/Fm}_{\text{PD}}} \cdot 100\% \quad \text{and} \quad \frac{\text{d.a. Fv/Fm}_{\text{morning}}}{\text{d.a. Fv/Fm}_{\text{PD}}} \cdot 100\%$$

respectively). The black portion of the stacked bar is the difference between Fv/Fm_{PD} – d.a.Fv/Fm at a given time interval in % of Fv/Fm_{PD}

$$\left(100\% - \frac{\text{d.a. Fv/Fm}_{\text{morning}}}{\text{d.a. Fv/Fm}_{\text{PD}}} \cdot 100\% \right)$$

and represents the non-functional reaction centers of PSII. The grey part of the bar represents the difference between dark adapted and actual fluorescence in % of Fv/Fm_{PD} at a given time

$$\frac{\text{d.a. Fv/Fm}_{\text{morning}}}{\text{d.a. Fv/Fm}_{\text{PD}}} \cdot 100\% - \frac{\text{act. Fv/Fm}_{\text{morning}}}{\text{act. Fv/Fm}_{\text{PD}}} \cdot 100\%$$

In this way we can visualize photochemical use and the corresponding energy quenching, yielding an estimate of current photochemical capacity. The white portion of the stacked bar represents the remaining photochemical capacity, and usually at least 60% of PS II reaction centers are not in use.

Harvest

Beginning in 2001 and continuing to 2002, we harvested the aboveground biomass in the plots twice a year during the vegetation season (in July and September). Aboveground biomass was cut along transect from plots of 0.5 × 0.5 m. One side of the transect was used in the first harvest and the other side in the second harvest. We sorted the harvested plant material into three major categories: *C. rostrata*, grass species and dicots. Material from each category was further separated into live and dead material. We also collected the litter layer covering the ground in the plots. First, we weighed the fresh biomass (live and dead), and after the fresh material had been killed in an ordinary microwave oven, and oven-dried at 70°C for 48 h, we obtained the dried biomass in gm⁻². Additionally, we collected above-ground plant litter at each harvest. The litter was also oven dried and weighed.

We drilled soil cores (8 cm wide, 15 cm deep) from the centre of each plot to determine the belowground biomass. In the laboratory we separated roots from the soil by washing the soil sample through a fine sieve (Φ 0.1 mm) and collecting the bigger roots with tweezers. Belowground material was oven-dried at 70°C for 72 h.

Results

CO₂ Concentrations

Extremely high levels of [CO₂] in the air, about 2000 μmol·mol⁻¹ and sometimes even reaching the maximum range of IRGA at 2500 μmol·mol⁻¹, were measured only directly at the soil surface (Fig. 1). The values measured at one point were not constant and sometimes varied greatly during measurement; however, the measurements at each point were performed long enough to adequately capture the range of [CO₂] levels. At elevations higher above the soil surface, concentrations began to fall, and at 40 cm, no difference in [CO₂] levels was present, among neither heights nor transect points compared to control measurements further away. The [CO₂] in the air (measured with IRGA) did not change substantially during the vegetation season (Fig. 2a, b); however, [CO₂] in the soil (Fig. 2c, d), measured with diffusion tubes, increased more than fourfold, with occasional water logging in plot P4, and even more than eightfold in plots P5 and P6 in September.

Biomass Response to Increased [CO₂]

Correlations between dried biomass and [CO₂] differed according to the method used to obtain [CO₂] – IRGA or diffusion tubes. Measurements with IRGA showed

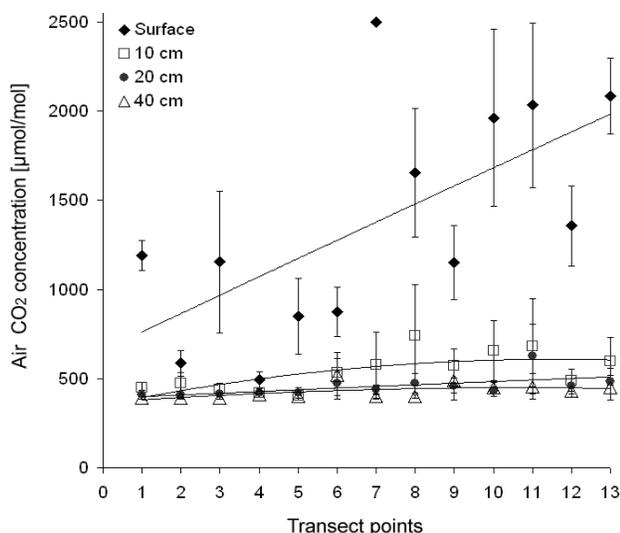


Fig. 1: Concentrations of CO₂ on soil surface and at different heights in spring, 2001

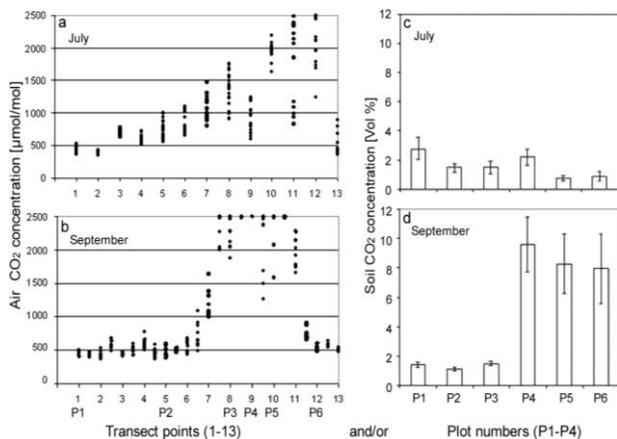


Fig. 2: Atmospheric [CO₂] (a, July; b, September) and soil [CO₂] (c, July; d, September) at the time of harvest and fluorescence measurements

significant correlation only for the dominant biomass formed by *C. rostrata*, with the [CO₂] at 10 cm above the soil surface within the canopy. The live above-ground biomass of *C. rostrata* increased significantly with increasing [CO₂] (linear regression; $y = 75.35 + 0.29x$, $r^2 = 0.53$, $r = 0.73$, $P = 0.01$; Fig. 3). On the other hand, the same increase in [CO₂] resulted in significantly less dead biomass (linear regression; $y = 225.00 - 0.15x$, $r^2 = 0.60$, $r = -0.78$, $P = 0.005$; Fig. 3). A contrary effect was observed for soil humidity (Fig. 4). With increasing soil humidity, the live above-ground biomass of *C. rostrata* declined significantly (linear regression; $y = 225.00 - 0.15x$, $r^2 = 0.44$, $r = -0.66$, $P = 0.019$), and more dead biomass accumulated (linear regression; $y = -66.25 + 7.54x$, $r^2 = 0.72$, $r = 0.85$, $P < 0.001$).

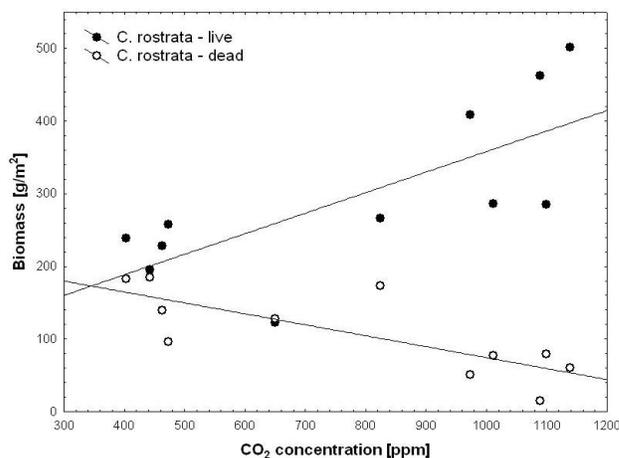


Fig. 3: Correlation of live and dead *C. rostrata* biomass, harvested in July, and atmospheric [CO₂]

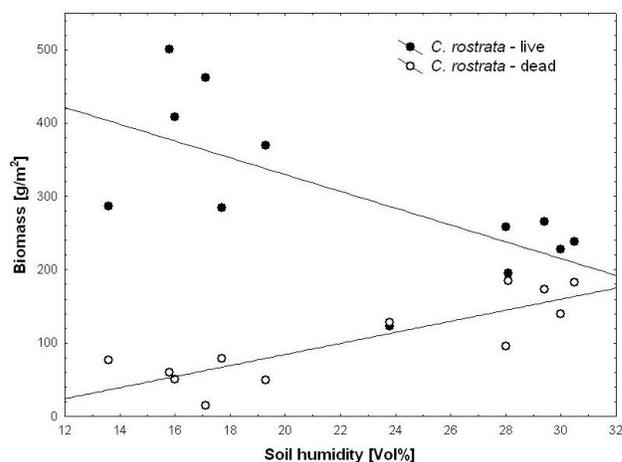


Fig. 4: Correlation of live and dead *C. rostrata* biomass, harvested in July, and soil water content

The dead biomass was formed by dead leaves still attached to live plants within the tussock, and it can, undoubtedly, be assigned to *C. rostrata*.

When [CO₂] were measured with diffusion tubes, the total annual yield (biomass of *C. rostrata*, grasses and other Dicots together) was smaller on plots with higher levels of [CO₂] measured at 30 cm ($r = -0.90$, $P = 0.15$). Live *C. rostrata* biomass in June was positively correlated to the [CO₂] at the soil surface ($r = 0.88$, $P = 0.019$), while in September it correlated positively with SWC ($r = 0.84$, $P = 0.038$). Grasses were also correlated positively with [CO₂] directly at the soil surface in July (live biomass: $r = 0.84$, $P = 0.037$; dead biomass: $r = 0.90$, $P = 0.016$); however, their biomass was negatively correlated with SWC in September ($r = -0.85$, $P = 0.031$), which was also true for the live biomass of other Dicots ($r = -0.08$, $P = 0.045$).

Our results showed that the effect of increased [CO₂] was hampered by increased SWC observed in the case of *C. rostrata* standing biomass (Fig. 5). For *C. rostrata*, biomass is increased by enhanced [CO₂] and by increased SWC.

However, on occasions when levels of [CO₂] and SWC are both higher, we observed a decrease in *C. rostrata* biomass (Fig. 5).

One indication of standing biomass and canopy density is the calculation of LAI, which in July corresponded positively to [CO₂] at the soil surface; however, the correlation was only close to significance ($r=0.77$, $P=0.074$). Again, in late season (September), LAI ($r=0.96$, $P=0.003$), sun flecks ($r=-0.93$, $P=0.007$), and canopy PAR interceptions ($r=-0.84$, $P=0.035$) were correlated with SWC.

Root biomass, collected in September and calculated for 0.15 m³, was twice to almost five times larger than total above-ground biomass (annual yield). This is most likely related to high levels of [CO₂] in the soil at the time of harvest, since the correlation was close to significance ($r=0.79$, $P=0.059$). The vast majority of the belowground biomass could be attributed to *C. rostrata* roots.

Chlorophyll Fluorescence

In the presentation of chlorophyll fluorescence data in Fig. 6 three types of reaction centers (PS II) are shown, and in this way light conversion to chemical energy, meaning assimilating power is described. We observed differences during the day as well as between the upper and lower sides of the leaf. The highest current photochemical use (CPU, shown in grey), about 30%, was observed in the morning for the upper leaf side; the percentage declined at noon and did not improve much in the afternoon. Throughout the day the upper sides of the leaf had higher CPU than the lower sides. Although the lower sides evidently decline in CPU at midday, their CPU in the afternoon was slightly better (between 7-11%) compared to morning (4-9%). The second type of PS II reaction centers involves downward regulated centers and centers triggered for repair, meaning reaction centers (PI, shown in black) that are non-functional, mostly because of photoinhibition. Daily course of PI centers showed low values in the morning and at the same time exhibiting the greatest differences between upper and lower leaf sides. During the day, PI rose and increased, especially for the lower leaf side compared to morning. In the afternoon, PI did not decline, representing lack of repair or replacement of non-functional PS II reaction centers. The ratio between CPU and PI was <1 in the morning, changing to >1 at noon and again falling close to 1.

In relation to [CO₂], we found a correlation only with soil [CO₂]. Two plots with the highest upper leaf side CPU (26 and 31%) in the morning had [CO₂] levels of 1.48 Vol% and 1.74 Vol%, respectively (Fig. 6a). At midday photoinhibition of reaction centers in the upper leaf side decreased with increasing levels of [CO₂] in the soil (linear regression; $y = 18.1 - 4.2 \cdot x$). Similarly, the remaining current photochemical capacity (RCPC shown in white; Fig. 6) increased with [CO₂] for the upper leaf side, while a trend was not observed for the lower leaf side.

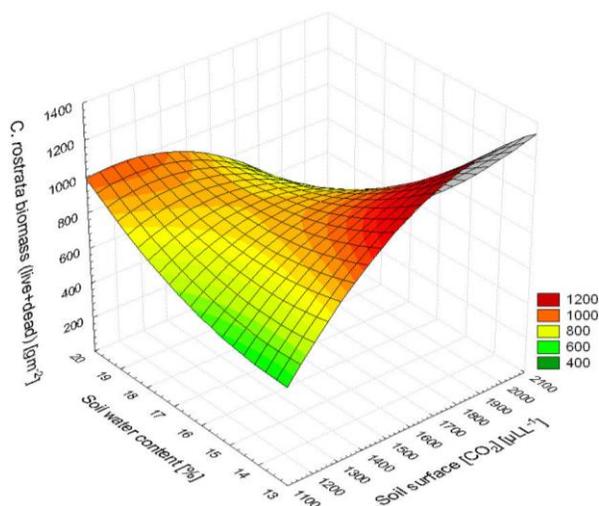


Fig. 5: Correlation of total *C. rostrata* to atmospheric [CO₂] on the soil surface and soil water content

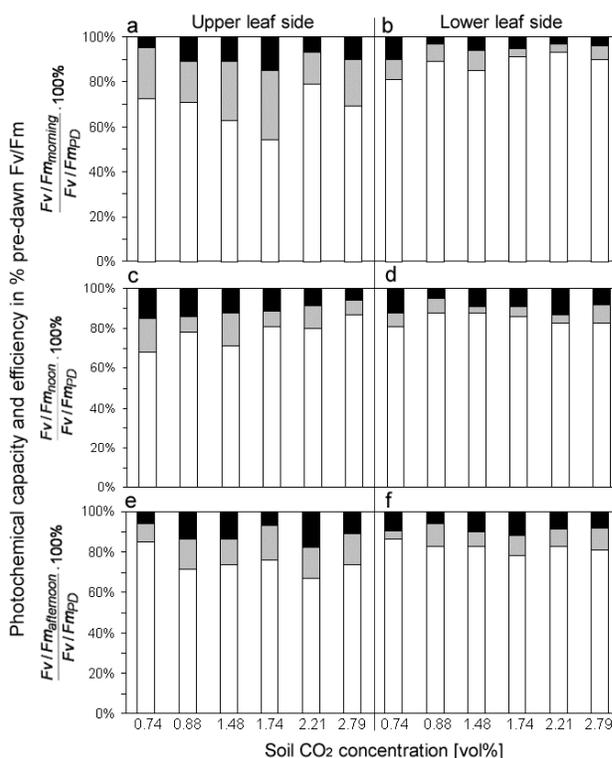


Fig. 6: Daily course of photochemical capacity and efficiency (Fv/Fm) in % predawn (Fv/Fm_{ppd}) for the upper and lower leaf sides in relation to soil [CO₂]. Black: photoinhibition (PI); grey: current photochemical use (CPU); white: remaining current photochemical capacity (RCPC)

In the afternoon CPU of the upper leaf side increased significantly (by 4 to 9%) only on plots with soil [CO₂] about 2 Vol% or higher, even though the lower leaf side showed an increase in CPU irrespective of [CO₂].

Discussion

Geogenic CO₂ at the study site was the source of carbon enrichment in the air, while it created hypoxic conditions in the soil. As measured with IRGA, CO₂ release from the soil did not occur constantly but in fluctuating bursts, resulting in high variation of measurements along the transect (Fig. 1). The mofettes often release CO₂ in fluctuating bursts and in similar locations in Slovenia, soil [CO₂] has varied by as much as almost 30% (Vodnik *et al.*, 2006; 2009). However, if the surface morphology allows transient accumulation of CO₂ at the surface, this can greatly influence the vegetation (Pfanzen and Sassmannshausen, 2008). Because of the more or less even surface of the study site, measurements of [CO₂] declined sharply with height, diminishing already at 20 cm above the soil surface. Unless we performed an excessive number of measurements with IRGA, values obtained from the diffusion tubes gave more reliable information about the [CO₂] and were used at the time of harvest instead. However, despite the variability in CO₂ fluctuations, for some plots higher levels of [CO₂] remained permanent.

Biomass sampling, which we applied, represents a measure for plant resource capture and allows the monitoring of plant growth and mortality. At the peak of the vegetation period in July, *C. rostrata* responded with enhanced growth in plots with higher SWC, which is its natural habitat, and in drier sites with high soil [CO₂]. Our findings are congruent with other studies showing that elevated [CO₂] can compensate for lower SWC (Lee *et al.*, 2010), foreseeing that the positive effect of higher [CO₂] would be more pronounced in dry environments because of plants' reduced water use. According to Volk *et al.* (2000), plant responses to higher levels of [CO₂] can be separated into direct responses to [CO₂] and responses that are driven by the CO₂-induced water saving effect, because of stomatal conductance reduction and consequently lower transpiration.

Even though the positive effect of CO₂ on biomass production is broadly accepted, it is hypothesized that this increase in biomass may be moderated by climate. This means that reduced water use will not typically benefit a plant's biomass production when sufficient soil moisture is available (Leakey *et al.*, 2006). Such conditions, with high SWC, occurred when our study site was occasionally flooded by rainfall in autumn, which caused O₂ depletion in the soil, as well. Our results showed that grasses, sedges and Dicots respond differently to such specific conditions. Volk *et al.* (2000) stressed that the interacting effect of soil moisture and CO₂ enrichment caused a species-specific response when they studied *Bromus erectus* and *C. flacca*, which showed a strong positive effect. It is not surprising that we found *C. rostrata* to be the dominant species in our study, since it is able to tolerate hypoxia in its natural habitat, often represented by waterlogged soils (Visser *et al.*, 2000). In the late vegetation period in September, when

plants simultaneously experienced elevated [CO₂] and high water content, even waterlogging, then the *C. rostrata* above-ground biomass decreased (Fig. 5) and corresponded with increased root biomass. Our results showed that the positive effect of increased [CO₂] on biomass production was hampered by increased SWC; this effect was especially marked in *C. rostrata* (Fig. 5), even though it is well adapted to waterlogged soil and hypoxic soil conditions.

An indication of the diminishing effect of increased [CO₂] under conditions of high SWC can be seen through changes in the correlation of overall biomass (dicots, grasses, *C. rostrata*). At the peak of the vegetation season (in July), when the biomass-related parameters responded better with the [CO₂] at the surface, we observed the fertilizing effect of CO₂. In the autumn (September), precipitation increased and the occasionally water-logged soil restrained biomass production. Because of the even surface at the study site, the effects of flooding influenced all plots in a similar manner, while [CO₂] levels in the soil and at the surface differed greatly.

Elevated [CO₂] in the soil with simultaneously waterlogged soil constrained *C. rostrata* to invest in aboveground biomass, but not however, the allocation to belowground biomass. Knowledge is scarce about the belowground traits of plants in relation to their response to increased [CO₂] (Cornelissen *et al.*, 2003). Our approach showed that it was much easier to define the effect of CO₂ enrichment aboveground than below. Even though the technique of soil cores underestimates the production in grasslands (Higgins *et al.*, 2002), our results showed enormous belowground biomass. It is broadly accepted that aboveground species composition is reflected below ground; thus, we can conclude that almost all root biomass at our study site belonged to the dominant *C. rostrata*. Previous investigations showed numerous occasions when *Carex* species invested in the biomass of their belowground organs: for instance, when a species living in an ecosystem with low availability of nutrients received additional nutrients (van Wijk *et al.*, 2003). Measurements made by these authors showed greater below- vs. above-ground biomass ratio on fertilized plots and the 76% relative amount of the biomass belonging to the below-ground organs. Enhanced air [CO₂] has a fertilizing effect, and if we compare van Wijk *et al.* (2003) data with our observations, we see that the ratio of below-ground vs. above-ground biomass of *C. rostrata* was about 5.3 at the end of the vegetation season in 2001, and 4.5 on average throughout 2002. *Carex rostrata* root matter contributed 78 to 85% to the total biomass in July and 72 to 95% in September. We observed the specific root architecture, where fine roots grew densely in the upper few centimetres, some even above the surface if a litter layer was present, and only a few larger roots led into deeper soil. In waterlogged soils, plants aerate their belowground tissue by oxygen diffusion

through intercellular spaces and by formation of specialized parenchyma (aerenchyma) in the root cortex (Visser *et al.*, 2000).

However, extraordinary conditions arise when waterlogging occurs in soil, where hypoxic conditions already exist because of a geogenic CO₂ spring. In our study the [CO₂] in the soil increased up to 11 times to around 8 vol%, resulting in stressful conditions even for flood tolerant species (Fig. 2 d). Plants capable of tolerating anoxia, like *C. rostrata*, obviously respond similarly to oxygen depletion, irrespective of whether the anoxic conditions are caused by water logging or by enhanced [CO₂] levels in the soil. In a similar manner, we may expect the increased [CO₂] in the soil to influence the amount of litter as well. In an environment where oxygen is a limiting resource, its availability interferes with microbes and governs rates of decomposition, methane production and other processes (Verville *et al.*, 1998). Our results show a large mass of litter present, even though this meadow had been regularly mown in previous years. Hoorens *et al.* (2002) stressed that the litter quality of *C. rostrata* grown under elevated [CO₂] levels changed in the direction of significantly less nitrogen and more phenolics, which slow down decomposition. Their results were case sensitive, since this was true only for *Carex* and not for *Sphagnum* or *Vicia*. We believe the litter accumulation in our observation was higher because the high levels of [CO₂] on the soil surface interfered with the soil decomposition community. This should be especially true in cases when CO₂ origin is geogenic.

Chlorophyll fluorescence parameters can be easily measured and provide useful tools for probing photosynthetic performance *in vivo*, even though the most frequently used ratio – dark-adapted Fv/Fm – is a relative measure not an accurate quantitative value of the maximum quantum yield of PSII (Baker, 2008).

Our measurements of chlorophyll fluorescence (Fv/Fm) during the day (Fig. 6) corresponded only to [CO₂] in the soil, and not to any other [CO₂] or soil humidity. Measurements showed a general decline in current photochemical use (CPU) from morning until afternoon, with the lowest values measured at midday. We performed measurements on days with high irradiance, when *C. rostrata* showed a decline in photosynthetic rates around midday. Bolh ar-Nordenkamp and  quist (1993) suggest that this decline can be partly attributed to photoinhibition. Besides photoinhibition, the reduced photochemical conversion in *C. rostrata* can be explained by its vertical-leaved plant architecture, with erectophile leaves in clumps, since we measured a similar decline in photochemical use on the upper, as well as on the lower sides of the leaf. Additionally, at noon the photochemical conversion was reduced as the plants experienced a midday depression causing more non-functional PS II centers on the lower side of the leaf. No prominent repair or recovery of photoinhibited PS II centers occurred during low light

conditions in the afternoon, again because of the erectophile leaves, which get similar amount of PAR compared to midday on both upper and lower sides of the leaf.

In the morning, when optimal irradiance conditions existed, chlorophyll fluorescence (Fv/Fm) did not show a significant relation to [CO₂] in the soil. However, later at midday the upper leaf side showed fewer PI reaction centers, which needed to be repaired if plants grew in conditions of higher soil [CO₂]. In the afternoon plants growing on plots with higher levels of [CO₂] in the soil had a greater increase in photochemical use. Our results show that changes in fluorescence measurements throughout the day can best be described in terms of erectophile canopy structure, and the positive effect of [CO₂] can be observed only when conditions decline from optimal and become more stressful e.g., high irradiance at midday. In the afternoon erectophile leaves received more light on both the upper and lower sides of the leaf. Irradiance increased the ratio of PI reaction centers compared to midday, since photoinhibition repair takes several hours (Bolh ar-Nordenkamp and  quist 1993). However, even though the PI ratio increased irrespective of [CO₂], the CPU increased only on plots with high [CO₂] levels.

Our results suggest that elevated [CO₂] levels might increase CPU in low light and could therefore compensate for the increasing number of PI reaction centers during the day. Even though the changes in daily course of photochemical efficiency can best be explained with reference to the erectophile canopy, we found that plants experiencing higher [CO₂] had increased photochemical use in the afternoon, even though that the portion of photoinhibition, which accumulates during the day, is not repaired.

In conclusion, *Carex rostrata* biomass responds to increased levels of [CO₂] in a manner of FACE, even though the increasing [CO₂] was geogenic in origin. At vegetation period, *C. rostrata* responds with enhanced growth on plots with higher SWC, which represented its optimal habitat, while on drier sites it benefits from elevated [CO₂] levels because of improved *C. rostrata* water-use efficiency and outcompeted grasses. During autumn flooding the in *C. rostrata* positive effect of increased [CO₂] levels on biomass production was impeded; above-ground biomass decreased and corresponded with increased root biomass. *Carex rostrata*, which is well adapted to waterlogged soil and hypoxic soil conditions, was able to tolerate hypoxia, whether it arose from waterlogging or is a consequence of geogenic CO₂ enrichment.

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