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Elevated CO_2 concentration affects vertical distribution of photosynthetic activity in *Calamagrostis arundinacea* (L.) Roth

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An understanding of photosynthetic responses to elevated CO_2 concentration (EC) across the entire vertical canopy profile will help to achieve more proper simulation of ecosystem responses to expected climate change. Accordingly, we studied changes in vertical gradients of photosynthetic parameters in the grass species *Calamagrostis arundinacea* under EC at the time of anthesis. Plants were transplanted and grown for 3 years under ambient (385 ppm; AC) and elevated (700 ppm; EC) atmospheric CO_2 concentrations in experimental glass domes. In general, EC reduced the variability of photosynthetic activity within the vertical profile of plants. Such changes also altered the differences between AC and EC in the vertical profile. While EC led to greater accumulation of chlorophylls, CO_2 assimilation rate, and efficiency of photochemical processes in lower leaves, EC primarily reduced stomatal conductance and transpiration rate in upper leaves. These changes resulted in higher water-use efficiency in lower leaves. On the other hand, we found slightly greater down-regulation of photosynthesis under EC in lower leaves indicated as the A_{700}/A_{385} ratio. Such knowledge may be important in upscaling EC effects from leaves to the canopy level and modelling impacts of expected climate change on grassland ecosystems.

Keywords: chlorophyll, CO_2 assimilation, elevated CO_2 concentration, transpiration, vertical gradient, water-use efficiency

Introduction

Atmospheric CO_2 is not only an important resource for photosynthesis but also the most important greenhouse gas, rapidly increasing due to anthropogenic activities. The current rate of CO_2 increase is ca 1.9 ppm year^{-1}. According to the Intergovernmental Panel on Climate Change (IPCC), the CO_2 concentration is projected to reach between 405 ppm and 460 ppm by 2025, between 445 ppm and 640 ppm by 2050, and between 720 ppm and 1,020 ppm by 2100 (IPCC 2007). To realistically estimate the future impacts of climate change, and in particular the impacts of elevated CO_2 concentrations on ecosystems, models must accurately represent the responses of whole plants or ecosystems in carbon and water exchange with the atmosphere (Smith and Dukes 2013). However, leaf-level measurements usually taken on the top (non-shaded) leaves scarcely can provide a true picture of gas exchange within the canopy because the light, microclimatic, and ontogenetic gradients occurring within the vertical canopy profiles (Urban et al. 2012) can greatly modify the response to changing conditions. This results in frequent disproportions between such leaf-level measurements and the true ecosystem response measured as total production or net ecosystem carbon exchange. One of the main reasons for this may relate to a lack of knowledge as to how lower parts of canopies contribute to total carbon or water exchange and respond to changing growth conditions. Greatly improved understanding as to the vertical distribution of photosynthetic activity and variability of responses to changing environmental conditions within the whole canopy is therefore needed.

To date, studies of the vertical gradient of photosynthetic parameters have been done particularly on forest tree species in relation to species-specific differences or seasonal courses (Koike et al. 2001). In annual crops, attention has been given to the relationship between vertical gradient of nitrogen content and photosynthetic parameters (Dreccer et al. 2000). According to available information, the impact of elevated CO_2 concentrations on the vertical gradient of photosynthetic parameters has not yet been studied either for grasses or for most plant species generally. In order to provide data for refining models focusing on the prediction of future climate impacts, we studied changes in vertical gradients as affected by elevated CO_2 concentration in the grass species *Calamagrostis arundinacea* (L.) Roth. This species was selected because grasses exhibit the most pronounced gradient of photosynthetic parameters given by the vertical differences in leaf ontogeny and translocation of nitrogen (Lötcher et al. 2003).

Materials and Methods

At the beginning of the 2007 growing season, *C. arundinacea* plants were collected from an open area near the Bílý Kříž experimental research site in the Beskydy Mts. (Czech Republic 49°33′N 18°32′E, 908 m a.s.l.) and then transplanted to two glass domes enabling cultivation under ambient (385 ppm; AC) and elevated (700 ppm; EC) CO_2 concentration for three growing seasons. The glass dome facility is described in detail by Urban et al. (2001). Plants were grown in the native soil. The geological bedrock is formed by Mesozoic Godula sandstone (flysch type) and is overlain by ferric podzols. Individual plants were cultivated as solitary with distance to other plants at least 0.5 m.

Light-saturated rates of CO_2 assimilation (A_{max}) and transpiration (Tr_{max}) as well as stomatal conductance (G_{Smax}) were measured at anthesis (12–14 July 2010) using a Li-6400 open infrared gas exchange system (Li-Cor, Lincoln, NE, USA). All measurements were made at a photosynthetic active radiation intensity of 1200 µmol $m^{-2} s^{-1}$, CO_2 concentrations of 385 and 700 ppm, leaf temperature of 25 °C, and ambient relative air humidity. Chlorophyll fluorescence parameters $1\text{-}V_j$ (complement to relative variable fluorescence at step J of the fast chlorophyll fluorescence kinetics) and F_V/F_M (maximum quantum yield of photosystem II) were determined after 25 min of dark adaptation (using leaf clips) by an FP-100 fluorometer (PSI, Brno, CZ). Chlorophyll content was determined *in vivo* using a SPAD502 chlorophyll meter (Konica Minolta Sensing, Osaka, JP).

All measurements were made on four leaves within the vertical profile of stems of five plants for each CO_2 treatment. The leaves were designated in the vertical profile of the plant as follows: flag (top) leaf (F), first leaf under flag leaf (F-1), second leaf under flag leaf (F-2), and third leaf under flag leaf (F-3).

The data were evaluated by analysis of variance, using the statistical package STATISTICA 12 (StatSoft, Tulsa, OK, USA). Two-way ANOVA analysis was used to test the effect of elevated CO_2 concentration and vertical leaf position on photosynthetic parameters. Tukey's post-hoc test was used to test differences between means.

Results and Discussion

Both chlorophyll fluorescence parameters, maximum quantum yield of PS II (F_V/F_M) and the parameter of fast fluorescence kinetics (1-*V*j), as well as chlorophyll content and CO_2 assimilation rate (A_{max}) show a typical decline in lower leaves of plants cultivated under AC (Fig. 1). Such decline is more evident in the two lowest leaves (F-2 and F-3). On the other hand, the changes in fluorescence parameters and A_{max} in the uppermost two leaves are very small and statistically insignificant. The decline under AC is particularly evident and also statistically significant in the cases of chlorophyll content, A_{max}, and F_V/F_M. On the other hand, EC led to less pronounced vertical distribution of both fluorescence parameters, chlorophyll content, and A_{max}. Such differences led to an increased response to EC in a downward direction within the vertical profile. This means that the resulting photosynthetic response of the whole plant to EC is different and significantly stronger as compared to measurements done on upper leaves, which are often the subject of leaf-level measurements. Based on the results of chlorophyll content and partly also chlorophyll fluorescence measurement, it can be assumed that these differences are to a large extent caused by the lesser decline in chlorophyll content in the lower leaves under EC conditions. This can be caused by the delayed senescence under EC which was reported by Curtis et al. (1988) in C3 plants. They had observed slower senescence and continued production in new shoots. Delayed senescence resulting in increased leaf longevity was also found by Tricker et al. (2004) in *Populus*. Taylor et al. (2008) documented a significant delay in the decline of autumnal canopy leaf area index and a significantly smaller decline in leaf chlorophyll content in elevated CO_2 concentrations at the end of season.

Nevertheless, the results describing effects of EC on plant senescence are not consistent. As reviewed by Wingler et al. (2006), senescence can be both delayed as well as accelerated by EC treatment. Leaf senescence under EC can be triggered by accumulation of sugars and reduced concentration of nitrogen. Accelerated senescence in older leaves provides release of nitrogen for young leaves and reproduction (Wingler et al. 2006). Environmental regulation of senescence is therefore a vital factor in the carbon and nitrogen economy of plants, and

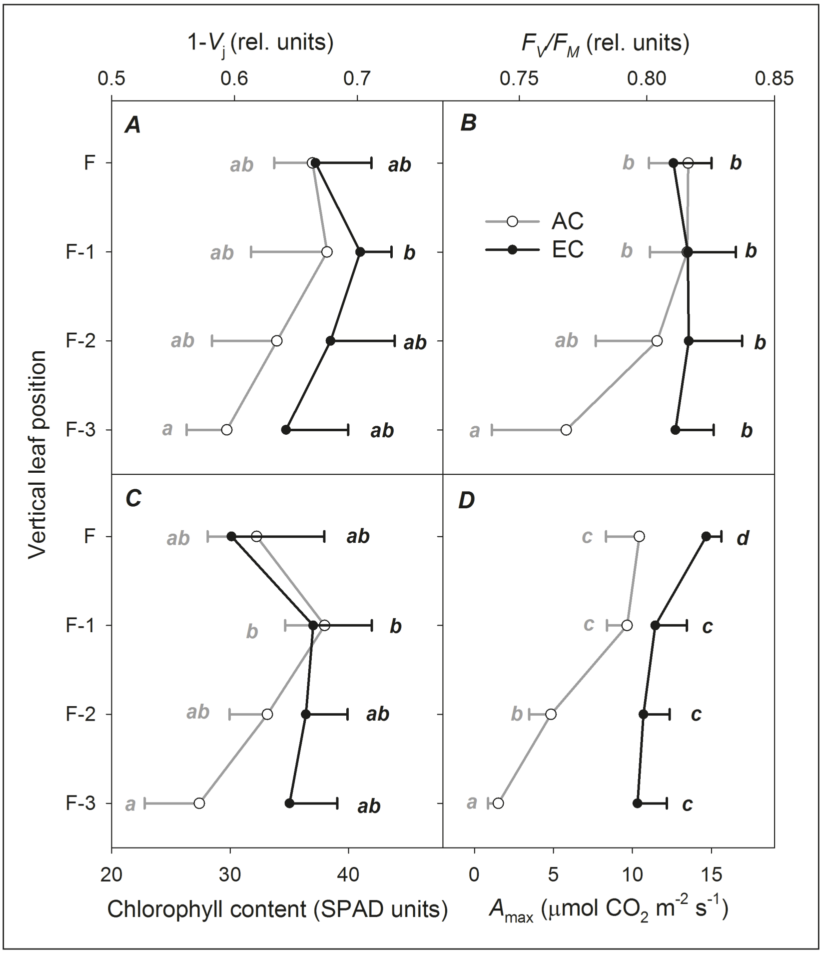

Fig. 1: Effect of elevated CO_2 concentration (EC) in comparison with ambient CO_2 concentration (AC) on vertical distribution of relative variable fluorescence at step J (1–V_j; A), maximum quantum yield of photosystem II (F_V/F_M; B), chlorophyll content (C), and light-saturated CO_2 assimilation rate (A_{max}; D) in vertical profile of Calamagrostis arundinacea plants (F – flag/top leaf, F-1 – first leaf under flag leaf, F-2 – second leaf under flag leaf, and F-3 – third leaf under flag leaf). Means (points) and SD (error bars) are presented (n = 5). Different letters denote statistically significant differences ($p \leq 0.05$) among leaves and CO_2 treatments using Tukey's post-hoc test.

sugar-induced senescence is particularly important under low nitrogen availability (Leakey et al. 2009). Accelerated hexose accumulation likely plays a major role in photosynthetic acclimation under elevated CO_2 (Stitt and Krapp 1999). The effect of elevated CO_2 on hexose accumulation and sugar-induced senescence is inconsistent, however (as reviewed by Wingler et al. 2006). Ludewig and Sonnewald (2000) concluded that early senescence is caused by accelerated leaf ontogeny, not by sugar-dependent changes in gene expression. Older leaves are more sensitive to sugar accumulation than are young leaves (Araya et al. 2008). As acclimation to EC depends on nitrogen availability, CO_2-induced senescence is observed to a greater extent under nitrogen-limiting conditions but not under high nitrogen availability (Sigurdsson 2001). Warren et al. (2011) also found accelerated senescence under EC during stress conditions, such as drought. On the other hand, an alleviating effect of EC under moderate drought stress has been demonstrated as a result of improved water-use efficiency (Allen et al. 2011, Keenan et al. 2013).

Nitrogen and water availability as well as leaf age appear to be the main factors determining whether elevated CO_2 concentrations accelerate or delay leaf senescence. The response is not fixed, however, but changes with species, genotype, or other environmental factors. It is nevertheless evident from our results that the responses to elevated CO_2 concentration that are associated with senescence should be studied within the whole vertical profile of the plant and not just in the upper leaves, as has often been the case.

Similarly to chlorophyll fluorescence parameters, A_{max}, and chlorophyll content, we also found a greater gradient of stomatal conductance (G_S) and transpiration rate (Tr) within the vertical profile under AC than EC conditions (Fig. 2A,B). The values of G_{Smax} and Tr_{max} were lower under EC as compared to AC, however, as a result of stomata regulation by CO_2 concentration. This contradictory effect of EC on stomata closure and less pronounced vertical stratification of G_{Smax} resulted in large and statistically significant differences in G_{Smax} and Tr_{max} between AC and EC in the upper leaves and decreasing differences in a downward direction.

Such different responses of G_{Smax} or Tr_{max} and A_{max} to EC within the vertical profile substantially influenced the vertical distribution of water-use efficiency (WUE), defined as the ratio between CO_2 assimilation rate and transpiration (Fig. 2C). This results in a less pronounced and statistically insignificant gradient of WUE for both the AC and EC treatments. However, a decreasing WUE under AC and increasing WUE under EC in a downward direction were observed. In general, the WUE values were significantly higher (except flag leaf) within the whole vertical profile under EC and the differences increased in the lower leaves. Although the decrease in G_S under elevated CO_2 concentration is evident from numerous studies, they also document a wide range of responses (as reviewed by Leakey et al. 2009). Because G_S can be strongly affected by leaf ontogeny (Grassi and Magnani 2005), differences in the vertical profile of grasses, representing the ontological gradient, can be expected, including interactive effects with EC. Lewis et al. (2002) had suggested that the effect of leaf ontogeny on photosynthetic response to EC is mediated through effects on the balance between source activity and sink capacity. After the start of senescence, the environmental control of senescence becomes negligible because of ontogenetic decline in the range of stomatal conductance (Winkel et al. 2001). This is in agreement with our results, wherein older (lower) leaves exhibited small, statistically insignificant effects of EC on G_{Smax} and Tr_{max}. The more significant effect of EC on WUE in lower leaves is likely due to the combined effect of the onset of leaf senescence and EC on stomatal closure but still high stimulatory effect of EC on the CO_2 assimilation rate.

Finally, we evaluated the proxy of Rubisco carboxylation activity as a ratio of CO_2 assimilation rate measured at CO_2 concentration 700 and 385 ppm for each leaf (Fig 2D). Although this stimulation effect is weaker through the whole vertical profile under EC, the differences between EC and AC are significant only in the lowest leaf (F-3). It is also evident that stimulation by CO_2 concentration increases in a downward direction and particularly in plants grown under AC conditions. This means that downregulation of photosynthesis under EC is associated with advanced ontogeny and the onset of leaf senescence.

Lewis et al. (2002) had demonstrated that CO_2 assimilation rates were stimulated by EC less during anthesis, which represents minimum sink activity, but the stimulation rates increased during fruit production, which represents an active sink for carbohydrates. Down-regulation of photosynthesis is thus associated with the sink capacity not only of the whole plant but also of individual leaves. Older leaves, which change from being significant sinks to sources of carbohydrates, are thus subjected to a more pronounced down-regulation of photosynthesis under EC.

Changes in vertical gradients of photosynthetic processes reflect a number of processes and environmental factors. According to the theory of optimal allocation within plant canopies, the photosynthetic capacity scales linearly with gradients of light penetration (Kull and Tulva 2002). The vertical allocation of photosynthetic activity changes during plant development (Bertheloot et al. 2008), however, and it is strongly responsive to nitrogen status (Lötscher et al. 2003), density (Dreccer et al. 2000), and drought (Xu and Zhou 2005). Such changes in the vertical gradient of photosynthetic capacity have the aim of preventing upper, non-shaded leaves from stress impacts and maintaining reproduction processes. Changes in the vertical gradient due to environmental stresses appear earlier than in the upper leaves and can also serve as an early indicator of stress effects detected by, for example, spectral reflectance (Klem et al. 2014).

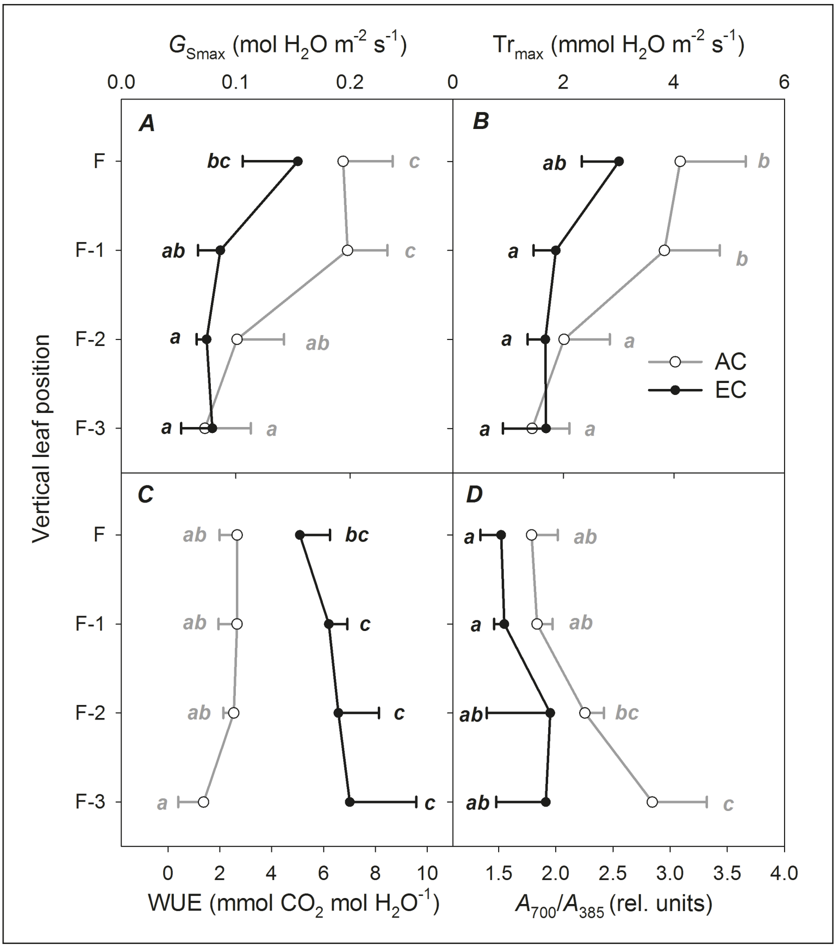

Fig. 2: Effect of elevated CO_2 concentration (EC) in comparison with ambient CO_2 concentration (AC) on distribution of light-saturated stomatal conductance (G_S; A), light-saturated transpiration rate (Tr; B), water-use efficiency (WUE; C), and the stimulation effect of CO_2 concentration calculated as A_{700}/A_{385} ratio (D) in vertical profile of Calamagrostis arundinacea plants (F – flag/top leaf, F-1 – first leaf under flag leaf, F-2 – second leaf under flag leaf, and F-3 – third leaf under flag leaf). Means (points) and SD (error bars) are presented (n = 5). Different letters denote statistically significant differences ($p \leq 0.05$) among leaves and CO_2 treatments using Tukey's post-hoc test.

Conclusions

In general, elevated CO_2 concentration (EC) reduces the differences in photosynthetic activity within a plant's vertical profile. While EC stimulates more chlorophyll content, CO_2 assimilation, and photochemical processes in lower leaves, a stronger effect on stomata closure and transpiration rate was found in upper leaves. These changes resulted in higher water-use efficiency in lower leaves. Such knowledge may be important in upscaling EC effects from leaf level to whole canopy level and in modelling the impacts of expected climate change on grassland ecosystems.

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