

Interactive effects of elevated CO₂ concentration, nitrogen nutrition and UV-exclusion on yield, aboveground biomass and root development in winter wheat and spring barley

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Abstract: Within the manipulation experiment in open top chambers (Domanínek near Bystřice nad Pernštejnem) that allow simulation of elevated concentration of CO₂ ([CO₂]) (expected by the end of this century – 700 μmol mol⁻¹) and exclusion of solar UV radiation, the interactive effects of these environmental drivers together with nitrogen application were studied. Aboveground biomass at the time of harvest, grain yield and root area were studied in winter wheat (variety Bohemia) and spring barley (variety Bojos). From three replications of each treatment were the roots of four plants dug out from the soil and gently washed. Photos of roots taken using the digital camera were analysed by image processing software Image J (plugin SmartRoot). The result showed that the elevation of [CO₂] increased the above ground biomass and grain yield. High level of nitrogen increased the stimulatory effect of [CO₂] on the above-ground biomass and grain yield. Also UV exclusion stimulated the effect of the evaluated [CO₂] on above-ground biomass and grain yield. UV exclusion resulted in higher root area than the treatment of non-exclusion of UV.

Key-Words: winter wheat, spring barley, elevated CO₂ concentration, nitrogen nutrition, UV exclusion, root area

Introduction

Atmospheric carbon dioxide concentration ([CO₂]) has increased about 30% since pre-industrial times and during this century [CO₂] levels could double or triple compared to pre-industrial levels [1]. Numerous experiments have demonstrated that in many plant high atmospheric [CO₂] leads to increases in photosynthetic rate, whole-plant growth and water use efficiency (WUE) and decreases in transpiration. It is known that CO₂ enrichment causes partial stomatal closure and reduces stomatal conductance [2]. With elevation of [CO₂], stomata do not appear to limit photosynthesis any more than they do at normal ambient [CO₂], even though stomatal conductance usually decreases under these conditions [3]. But reduced stomatal conductance potentially leads to a decreased transpiration rate on a leaf area basis [3][4][5]. Elevated [CO₂] directly affects photosynthesis and has been shown to enhance growth and yield of crop plants under suitable conditions [6][7]. Moreover the effect of

elevated CO₂ on growth is strongly modulated by N supply. When nitrogen is high, elevated [CO₂] leads to a sustained stimulation of photosynthesis and growth [8]. Plants grown at elevation of [CO₂] also increase in root:shoot ratios that are symptomatic of N limited plants [9]. Elevation of [CO₂] cause reduction of nitrogen in plant [10]. Reductions of nitrogen concentrations in tissue under high [CO₂] grown plants might indicate physiological changes in the efficiency with which plants use nitrogen to gain biomass (i.e. increased nitrogen use efficiency).

The effects of [CO₂] on R/S ratio are contradictory due to complexity in accurate underground biomass estimation under diverse crops and conditions. Roots become more numerous, longer, thicker, and faster growing in crops exposed to high [CO₂] with increased root length in many plant species. Branching and extension of roots under elevated [CO₂] may lead to altered root architecture and ability of roots to acquire water and nutrients from the soil profile

with exploration of the soil volume. Root growth under elevated atmospheric $[\text{CO}_2]$ results in proportionally higher C allocation belowground, and increasing R/S ratios [11]. Many studies showed increased R/S [12, 13, 14, 15], while other studies showed decreased R/S [16, 17]. The direct effect of elevation $[\text{CO}_2]$ on R/S has no clearly defined conclusion [18, 19]. The underlying assumption that a larger proportion of dry matter produced under $[\text{CO}_2]$ enrichment is preferentially allocated to roots and increased under limiting water and nutrient supply needs to be evaluated [12].

Effects of ultraviolet (UV) irradiation on biological matter became an important issue over the past few decades since the man-made changes in atmosphere affected ozone layer which covers and protects the earth's surface from harmful ultraviolet radiation. Exposure to high UV-B radiation alters photosynthetic enzyme activities and disrupts PSII reaction centres [20]. Many researchers have reported reduction in biomass accumulation due to UV-B exposure in several trees [21, 22] and crop species [23]. Exclusion of UV significantly increased the total chlorophyll while Chl a/b ratio decreased. The efficiency of PS II (Fv/Fm), rate of photosynthesis and stomatal conductance significantly enhanced along with a remarkable increase in Carbonic anhydrase, PEP carboxylase and total soluble proteins. Thus UV excluded plants have higher reducing power and increased CO_2 fixation [24]. Moreover, exclusion of solar UV also enhanced root growth [25].

Material and Methods

The experiment was conducted in experimental station Domaníněk, near Bystřice nad Pernštejnem in Bohemian-Moravian highlands (Czech Republic, 49°52'N, 16°23'E, altitude 575 m a. s. l.). The soil type is modal cambisol, with geological bedrock weathered gneiss in depth 60-90 cm. Soil texture is sandy loam (45-60% sand and up to 16% clay) and pH(KCl) is between 4-5. This region is characterized as rain-fed area with mean annual precipitation 610 mm and mean annual temperature 7.2°C. The experiment consists of 24 open-top chambers, which allows manipulation of $[\text{CO}_2]$ and precipitation (Fig. 1). UV radiation was excluded by using the non-transparent plastic roof. Spring Barley variety Bojos was sown on 19th March 2014 in the chambers with density 4 MGS (millions of germinating seeds). Winter wheat variety Bohemia with bread quality A was sown on 9th October 2013 in the chambers with density 4 MGS (millions of germinating seeds). Fumigation with elevated $[\text{CO}_2]$ (EC; 700 $\mu\text{mol mol}^{-1}$) started at the beginning of

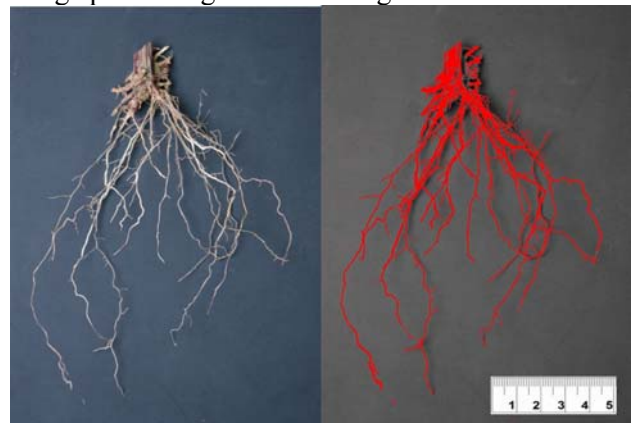
stem elongation (middle of May). The plots inside chamber were divided to two subplots and one of them fertilized with calcium nitrate in a dose 200 kgN ha^{-1} for winter wheat and dose 100 kgN ha^{-1} for spring barley at the growth stage end of tillering. The second subplot remained unfertilized with nitrogen. Each combination of factors was three times replicated.

The aboveground biomass was harvested manually at full ripening and weighted. This was followed by threshing of grain using a small plot harvester. The roots of four plants were dug out from the soil by excavation of soil around the root system in depth about 20 cm and gently washed them on 0.05 mm mesh sieve. Photos of roots taken using the digital camera were analysed by image processing software Image J (Fig. 2).

Fig. 1 Experimental site with 24 open-top chambers, which allows manipulation of $[\text{CO}_2]$ and precipitation



Fig. 2 Photos of roots of winter wheat were taken by digital camera and the root which were analysed by image processing software Image J.



Results and Discussion

The assessment of the effect of elevated $[\text{CO}_2]$ in the interaction with the effect of nitrogen and UV exclusion reveals similar results for above-ground

biomass and grain yield for both of spring barley and winter wheat (Fig. 3, 4, 5 and 6). The results indicated that elevated [CO₂] increased the above ground biomass and grain yield for both, with and without nitrogen fertilizer. In case of the UV exclusion (UV-), the elevated [CO₂] had higher effect to above-ground biomass and grain yield than non-exclusion of UV (UV+) probably because exclusion of UV increased rate of photosynthesis and CO₂ fixation [24]. However, the treatment without nitrogen fertilizer in conditions with present UV radiation (UV+) slightly decreased the above-ground biomass and grain yield of winter wheat. It was probably due to decrease of nitrogen uptake caused by UV radiation. Therefore UV exclusion combined with application of nitrogen fertilizer resulted in the highest above-ground biomass and grain yield when compared with the others treatment for both spring barley and winter wheat.

Fig. 3 Effect of CO₂ concentration, nitrogen and UV exclusion on above-ground biomass of winter wheat. AC (ambient CO₂ concentration; 390 μmol mol⁻¹), EC (elevated CO₂ concentration; 700 μmol mol⁻¹), N- (unfertilized with nitrogen), N+ (fertilized with nitrogen 200 kg ha⁻¹), UV- (UV exclusion), UV+ (non UV exclusion). Means (columns) and 95% confidence intervals (error bars) are presented (n=3) ± SD. The F-value was shown in table 1.

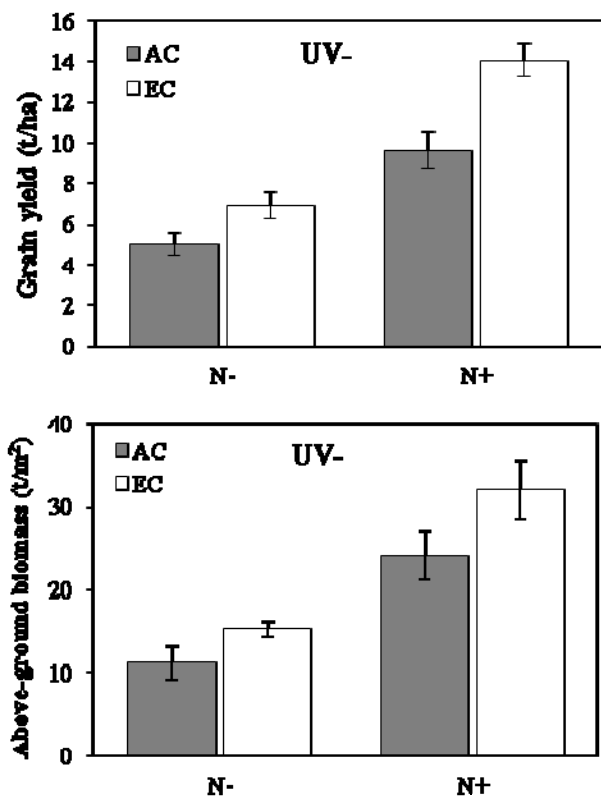


Fig. 4 Effect of CO₂ concentration, nitrogen and UV exclusion on grain yield of winter wheat. AC (ambient CO₂ concentration; 390 μmol mol⁻¹), EC (elevated CO₂ concentration; 700 μmol mol⁻¹), N- (unfertilized with nitrogen), N+ (fertilized with nitrogen 200 kg ha⁻¹), UV- (UV exclusion), UV+ (non UV exclusion). Means (columns) and 95% confidence intervals (error bars) are presented (n=3) ± SD. The F-value was shown in table 1.

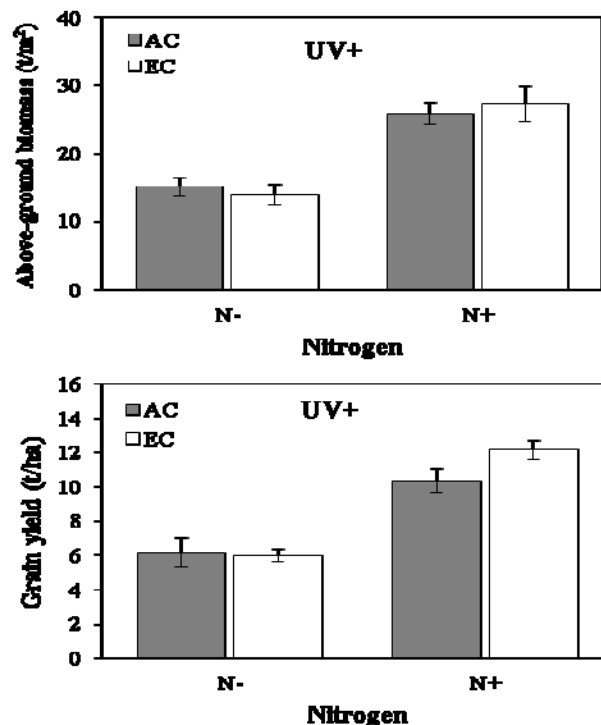
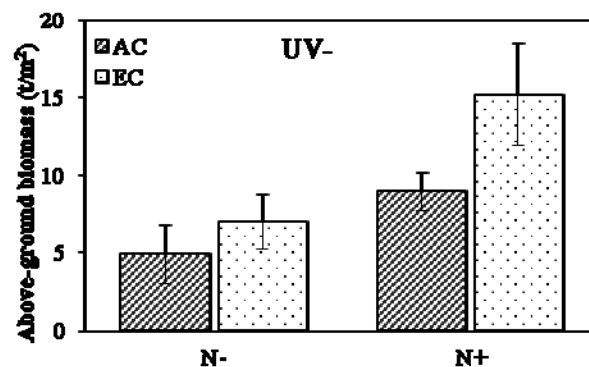


Fig. 5 Effect of CO₂ concentration, nitrogen and UV exclusion on above-ground biomass of spring barley. AC (ambient CO₂ concentration; 390 μmol mol⁻¹), EC (elevated CO₂ concentration; 700 μmol mol⁻¹), N- (unfertilized with nitrogen), N+ (fertilized with nitrogen 100 kg ha⁻¹), UV- (UV exclusion), UV+ (non UV exclusion). Means (columns) and 95% confidence intervals (error bars) are presented (n=3) ± SD. The F-value was shown in table 1.



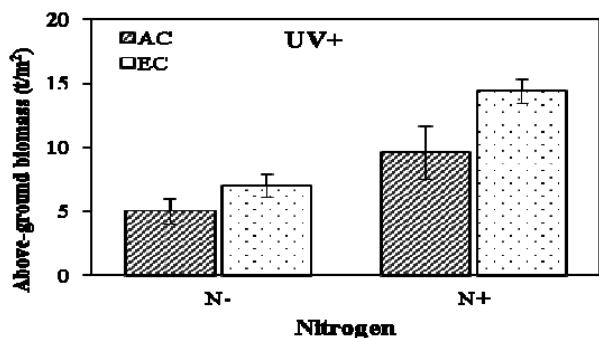
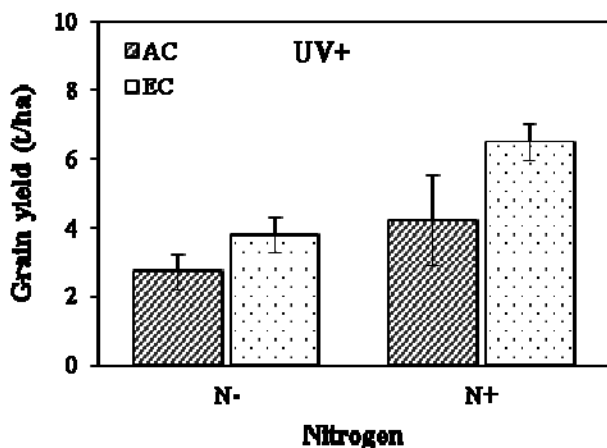
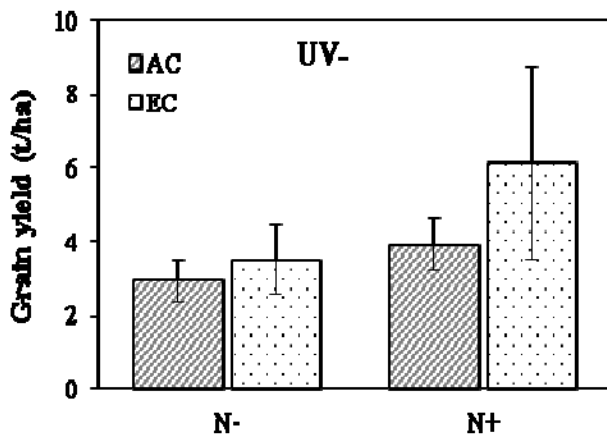


Fig. 6 Effect of CO₂ concentration, nitrogen and UV exclusion on grain yield of spring barley. AC (ambient CO₂ concentration; 390 μmol mol⁻¹), EC (elevated CO₂ concentration; 700 μmol mol⁻¹), N- (unfertilized with nitrogen), N+ (fertilized with nitrogen 100 kg ha⁻¹), UV- (UV exclusion), UV+ (non UV exclusion). Means (columns) and 95% confidence intervals (error bars) are presented (n=3) ± SD. The F-value was shown in table 1.



High level of nitrogen increased the stimulatory effect of CO₂ on the above-ground biomass and grain yield. Nitrogen supply strongly interact with elevated [CO₂] effect influencing plant metabolism and grain yield [10]. When nitrogen nutrition is high, elevated [CO₂] leads to a sustained stimulation of photosynthesis and growth [8].

The effects of [CO₂], nitrogen nutrition and UV-exclusion on root area was generally smaller compare to effects on aboveground biomass and grain yield. UV exclusion resulted in higher root area than the treatment of non-exclusion of UV (Fig. 7 and 8).

Fig. 7 Effect of CO₂ concentration, nitrogen and UV exclusion on root area of winter wheat. AC (ambient CO₂ concentration; 390 μmol mol⁻¹), EC (elevated CO₂ concentration; 700 μmol mol⁻¹), N- (unfertilized with nitrogen), N+ (fertilized with nitrogen 200 kg ha⁻¹), UV- (UV exclusion), UV+ (non UV exclusion). Means (columns) and 95% confidence intervals (error bars) are presented (n=3) ± SD. The F-value was shown in table 1.

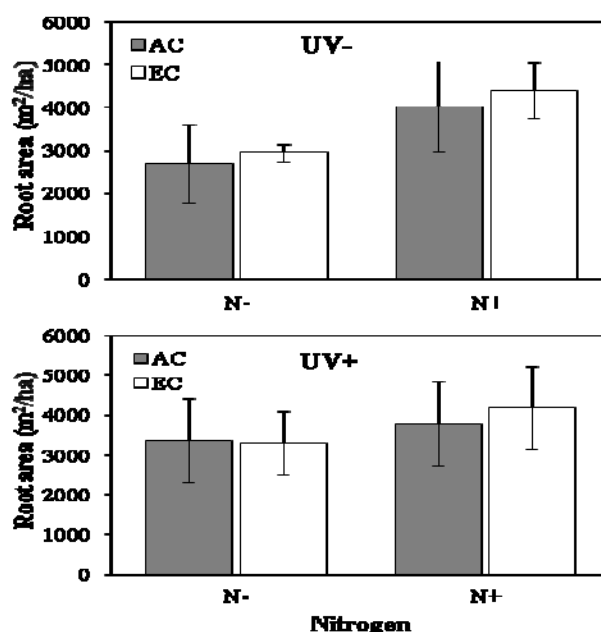
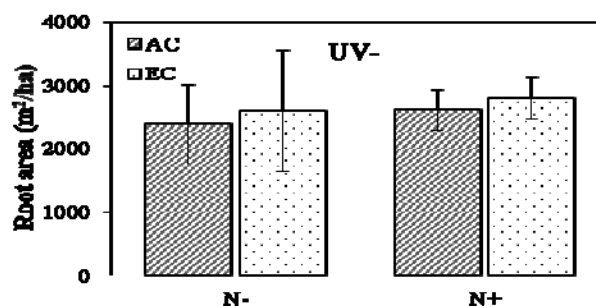


Fig. 8 Effect of CO₂ concentration, nitrogen and UV exclusion on root area of spring barley. AC (ambient CO₂ concentration; 390 μmol mol⁻¹), EC (elevated CO₂ concentration; 700 μmol mol⁻¹), N- (unfertilized with nitrogen), N+ (fertilized with nitrogen 100 kg ha⁻¹), UV- (UV exclusion), UV+ (non UV exclusion). Means (columns) and 95% confidence intervals (error bars) are presented (n=3) ± SD. The F-value was shown in table 1.



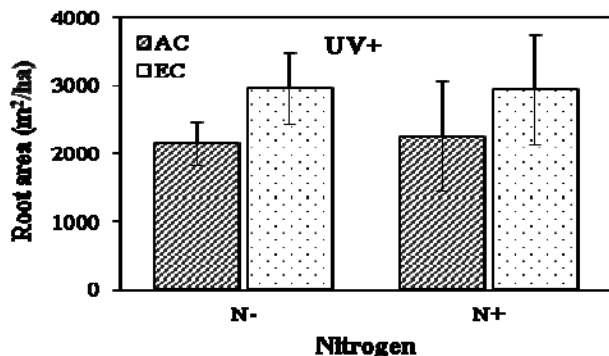


Fig. 9 The relationship between the above-ground biomass and root area of winter wheat from the effect of elevated [CO₂] in the interaction with the effect of nitrogen and UV exclusion.

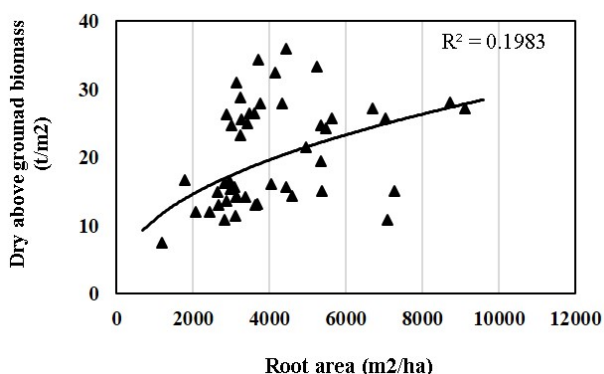
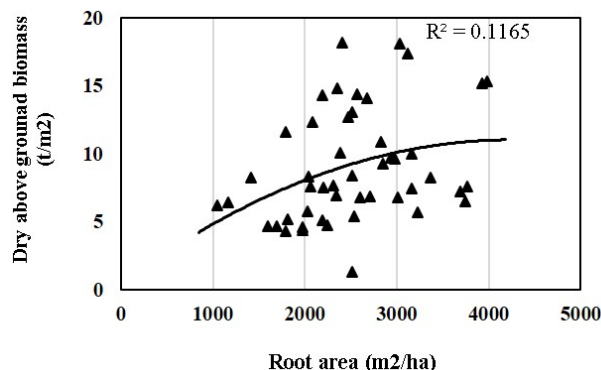


Fig. 10 The relationship between the above-ground biomass and root area of spring barley from the effect of elevated [CO₂] in the interaction with the effect of nitrogen and UV exclusion.



The figure 9 and 10 showed the relationship between the above-ground biomass and root area. The results show that increase of root area is related to increase of aboveground biomass.

Table 1 The F-value from ANOVA test. Significant values was indicated: **, P<0.01; *, P<0.05.

Source	Biomass		Grain yield		Root areas	
	Winter wheat	Spring barley	Winter wheat	Spring barley	Winter wheat	Spring barley
CO ₂ concentration	34.067**	61.467**	104.610**	21.238**	0.658 ^{ns}	7.203*
Nitrogen	651.625**	157.071**	803.937**	34.140**	12.231**	0.239 ^{ns}
UV exclusion	0.052 ^{ns}	0.000 ^{ns}	1.661 ^{ns}	0.225 ^{ns}	0.177 ^{ns}	2.156 ^{ns}
UV * N	7.132*	0.019 ^{ns}	3.340 ^{ns}	0.193 ^{ns}	1.537 ^{ns}	3.373 ^{ns}
UV * CO ₂	30.339**	0.610 ^{ns}	36.617**	0.209 ^{ns}	0.084 ^{ns}	1.923 ^{ns}
N * CO ₂	9.197**	13.117**	33.728**	4.642*	0.276 ^{ns}	0.317 ^{ns}
UV * N * CO ₂	0.273 ^{ns}	0.522 ^{ns}	0.366 ^{ns}	0.0956 ^{ns}	0.063 ^{ns}	0.249 ^{ns}
REP	11.505**	4.283*	2.283 ^{ns}	1.982 ^{ns}	0.548 ^{ns}	0.041 ^{ns}

Conclusion

The effect of elevated [CO₂] in the interaction with the effect of nitrogen and UV exclusion reveals similar results for above-ground biomass and grain yield for both spring barley and winter wheat. The elevation of [CO₂] increased the yield of above ground biomass and grain yield. High level of nitrogen increased the stimulatory effect of CO₂ on the above-ground biomass and grain yield. UV exclusion stimulated the effect of the elevated [CO₂] on above-ground biomass and grain yield. The

effect of elevated [CO₂] in the interaction with the nitrogen application and UV radiation on root area is smaller compare to aboveground biomass and grain yield.

Acknowledgement

This research was supported by projects NAZV QJ1310123 and NAZV QI111A133

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