



Effect of elevated CO₂ concentration, nitrogen nutrition and reduced water availability on malting quality of spring barley

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Abstract

A multifactorial field experiment in an open-top chamber facility at the experimental station Domaninek was conducted in 2014 to understand the potential effects of climate change factors on the malting quality of spring barley and to evaluate the interactions of these factors with nitrogen nutrition. The results showed a major effect of nitrogen nutrition on malting quality. Nitrogen increased the protein content and impaired the key malting quality parameters such as a relative extract or Kolbach index. Elevated CO₂ concentration had a generally positive effect on malting quality and alleviated the negative impact of nitrogen nutrition particularly. Reduced water availability had only a minor impact on malting quality; however, these effects were predominantly positive.

Keywords: CO₂ concentration, nitrogen, water availability, malting quality, barley

1 Introduction

Carbon emissions related to human activity contribute significantly to the elevation of atmospheric CO₂ concentration ([CO₂]), which consequently leads to the increase of temperature, a change in precipitation pattern and increased frequency of extreme weather events (O'Neill et al., 2017). [CO₂] steadily increased from pre-industrial levels of 280 ppm to the current 400 ppm with the actual increasing rate of 1.9 ppm per year (Tarasova et al., 2016). The [CO₂] levels are projected to reach between 445 and 640 ppm by 2050 and 720 and 1,020 ppm by 2100 (IPCC, 2007). There is a relatively broad consensus that many crops may respond positively to elevated [CO₂] (EC) levels in the absence of other stress conditions. However, the positive effect of EC can be offset by temperature rise, altered precipitation pattern and particularly by increased extreme weather conditions (Lobell and Gourdji, 2012).

For this reason, understanding the interactions between the effect of [CO₂] and other factors associated with climate change is crucial for predicting future food security. In addition to crop yield, climate change is also expected to alter crop quality. However, a relatively small amount of work has been performed on the combined effect of climate change variables on crop quality. Crop quality is thought to be a complex subject involving crop growth, CO₂ assimilation, biosynthesis and partitioning of carbohydrates, proteins and secondary metabolites (Da Matta et al., 2010). It has been shown that CO₂ enrichment increases starch significantly and reduces protein content in cereals (Erbs et al., 2010; Chaturvedi et al., 2017; Walker et al., 2017) which both determine the basic qualitative parameters of malting barley. The opposite effect is often reported for nitrogen (N) nutrition (e.g. Qi et al., 2006). The understanding of interactions

between $[\text{CO}_2]$ and N nutrition can be therefore crucial for mitigating the impact of climate change on the malting quality. However, sporadic results show that $[\text{CO}_2]$ induced reduction in protein content can be only partly counterbalanced by N fertilisation. Until now, the mechanisms by which $[\text{CO}_2]$ decreases grain protein content are not well understood (Taub and Wang, 2008). Although the reduction in grain protein content is generally beneficial for malting quality, it may also have a negative impact below a certain level resulting in reduced enzymatic activity and thus the impaired breakdown of starch (Qi et al., 2006; Erbs et al., 2010). Temperature rise, reduced water availability and particularly increasing variability and extremity of these two factors bring even more complexity to the impact of climate change on malting quality.

Besides long term changes in temperature, short periods of high temperatures above 30 °C during sensitive stages such as flowering or grain filling can also result in negative impacts on the grain quality of cereals (Passarella et al., 2008). Reduced water availability can have a negative impact on malt extract, β -glucan content (Coles et al., 1991), the proportion of grains retained on 2.5 mm sieve or protein content (Morgan and Riggs, 1981). Nevertheless, the response of qualitative parameters largely depends on the timing of water stress (Morgan and Riggs, 1981). Understanding the interactions between the effects of EC, water and nutrient availability are therefore essential for the reliable prediction of climate change impact on malting quality of barley and development of effective adaptation measures including the breeding of new varieties.

Therefore, the main objective of this study was to analyse the interactive effects of EC, N nutrition and reduced water availability on the main malting quality parameters of spring barley and thus to provide data for better prediction of climate change impact on malting quality.

2 Materials and Methods

The experiment was conducted in experimental station Domanínek, near Bystřice nad Pernštejnem in the Bohemian-Moravian Highlands (Czech Republic, 49°521'N, 16°235'E, altitude 575 m above sea level). Low fertile mineral soils characterize this area. The soil type is cambisol with a geological bedrock of weathered gneiss at a depth of 60–90 cm. The soil texture is sandy loam (45–60% sand and up to 16% clay) with a pH (KCl) between 4–5. This region is typical of a rain-fed area with a mean annual precipitation of 610 mm and a mean annual temperature of 7.2 °C within the period 1981–2010.

The experiment was conducted in 24 open-top chambers (Figure 1). Each chamber is an equilateral hexagonal construction with the length of 2 m on each side, diameter of 4 m and height without roof of 2 m. Above these chambers a roof with rotating lamellas, that allow controlling chamber ventilation and precipitation manipulation, is placed. The facility is equipped with four infrared CO_2 analyzers Li 840 (Licor, USA) for feedback regulation of $[\text{CO}_2]$. Moreover, there is a set of sensors for measurements of microclimatic conditions such as air temperature and humidity, soil temperature and moisture or photosynthetically active radiation (PAR). Gaseous CO_2 is injected into a fan, mixed with air and then blown into the chamber by a bottom ventilation channel running along the whole perimeter of the chamber. The system allows the recirculation of CO_2 enriched air to reduce CO_2 consumption or the direct ventilation when the air leaves the chamber immediately through the opened roof lamellas. Switching between the recirculation or the direct ventilation is automatically regulated to avoid differences between outside and inside temperature larger than 1 °C. For technical details see Rajsner and Klem (2013).

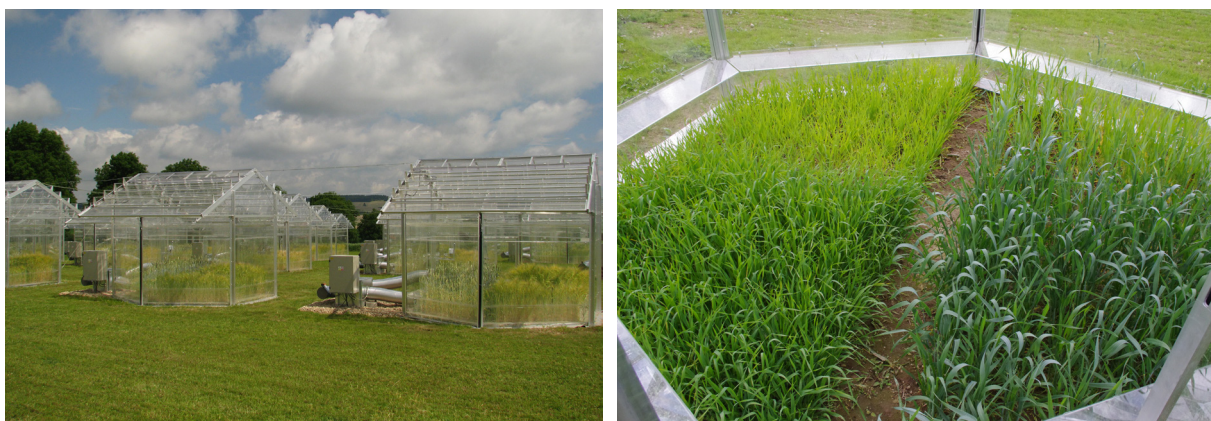


Figure 1 Open top chambers for the manipulation of CO_2 concentration and water availability (left) and a view of a chamber with plots unfertilised and fertilised with N.

Spring barley variety Bojos was sown on 19th March 2014 in the chambers with a density of 4 MGS (millions of germinating seeds). Fumigation with CO₂ started at the beginning of stem elongation (middle of May) with the concentration of 700 μmol CO₂ mol⁻¹ (EC). The control was represented by ambient CO₂ concentration (AC, about 390 μmol CO₂ mol⁻¹). The plots inside the chamber were divided into two subplots and one of them was fertilised by N (calcium nitrate, N+) at a dose 100 kg N ha⁻¹ at the growth stage of 2 leaves (DC 12). The second subplot remained unfertilised with N (N-). Reduced water availability was simulated by automatic closing the roof lamellas during rainfall for one month from the middle of stem elongation (end of May) to the middle of the grain ripening period. Ambient precipitation conditions were ensured by opening the roof lamellas during rainfall. Each combination of factors was replicated three times.

Harvesting was done manually at full ripening. The manual harvest was followed by the threshing of grain using a small plot harvester and consequently by grain yield assessment. The grain was sorted on a sieve and the fraction above 2.2 mm was used for subsequent malting while grain from three replicates was mixed thoroughly to one sample.

2.1 Malting quality

The samples of barley grain were malted in a micro malting plant of the company KVM (Uničov, CR). Laboratory malting was conducted by employing a procedure traditionally used in the RIBM which is based on the method published by [MEBAK \(2011\)](#).

Steeping was conducted in a steeping box. The temperature of water and air during air rests was 14.0 °C. The duration of steps was 5 hours on the first day and 4 hours on the second day. On the third day the water content in the germinating grain was adjusted by steeping or spraying to the value of 45%.

The germination was conducted in a germination box. The temperature during the steeping was 14.0 °C. The total steeping time, including air rests and germination, was 144 hours.

Kilning was performed in a one-floor electrically heated kiln. The total kilning time was 22 hours, the pre-kilning temperature was 55 °C, kilning at the temperature of 80 °C for four hours followed.

The parameters studied in the non-malted grain, malt and wort were protein content of barley grain (%), starch content of barley grain (%), friability (%), partly unmodified grains (%), whole unmodified grains (%), homogeneity by friabilimeter (%), β-glucan content of wort (mg l⁻¹), viscosity of wort 8.6 % (mPa.s), Kolbach index (%), protein content of malt (%), soluble nitrogen substances (%), relative extract at 45 °C (%), extract of malt (%), dia-

static power (WK u.), α-amylase activity (D.U.), apparent final attenuation (%), malting quality index ([Psota and Kosař, 2002](#)), haze of wort 15° (EBC u.), haze of wort 90° (EBC u.), total polyphenols in wort (mg l⁻¹). The produced malt was analysed according to the methods given in publications of [EBC \(2010\)](#) and [MEBAK \(2011\)](#).

3 Results and Discussion

3.1 Effect on chemical grain composition

The protein content was generally reduced by EC and enhanced by the N nutrition ([Figure 2](#)). N nutrition had a dominant effect on protein content. N nutrition increased the grain protein content, particularly under AC. The effect of EC on the decrease of protein content was thus more pronounced in N+ variants. These results correspond to the results on the effect of EC on the reduction of protein content in wheat grain ([Wieser et al., 2008](#); [Fernando et al., 2012](#); [Walker et al., 2017](#)) and other crops ([Taub et al., 2008](#); [Chaturvedi et al., 2017](#)).

Conversely, the impact of reduced water availability on the grain protein content was relatively low, while reduced water availability slightly decreased the grain protein content with the simultaneous increase of starch content. Such effect is to some extent contradictory in comparison with the literature, which more often indicates the increase in the grain protein content due to terminal drought, i.e. drought in the second half of ripening ([Kimball et al., 2001](#)). Protein deposition to the grain is generally less susceptible to drought than starch deposition, which leads to an increasing ratio of protein to starch and thus also to the relative increase in grain protein content under reduced water availability ([Jenner et al., 1991](#)). The most available results are, however, based on late (terminal) drought, occurring during grain filling and ripening. In the case of our experiment, reduced water availability was induced earlier, taking part from the middle of the stem elongation to the middle of the grain filling. In this case, reduced water availability could lead to reduced N availability for plants and a decline in the N use efficiency due to lack of water ([Klem et al., 2018](#)). As reported by [Ercoli et al. \(2008\)](#), the effect of N nutrition on the accumulation of proteins in the grain is reduced by more than a half and, at the same time, the ability to remobilise N from leaves into grain is reduced when plants are exposed to conditions of limited water availability. In their experiment with winter wheat [Ozturk and Aydin \(2004\)](#) demonstrated that the impact of late drought on the grain protein content is significantly higher than the impact of early drought. Even slight decrease of protein content may occur under early drought. According to [Ro-](#)

bređo et al. (2011), drought also causes a decline in N metabolism of the plant (especially nitrate reductase activity), but EC alleviates such adverse effect. However, due to the relatively low impact of reduced water availability and the same way of the EC effect and reduced water availability on the grain protein content, this alleviating effect of EC was not demonstrated in our experiment.

Our experiment also showed a well-known inverse correlation between the protein and starch contents in the grain. The main effect on starch content was recorded for [CO₂] and N nutrition (Figure 2). EC caused a slight increase in starch content under conditions of ambient precipitation, but this effect was more pronounced under reduced water availability and also in N- treatment. Xie et al. (2003) found

that the relationship between starch and protein content in grain at reduced water availability is primarily linked to endogenous abscisic acid (ABA). The increase in ABA due to lack of water is negatively correlated with starch content and positively with protein content in the grain. The role of ABA, induced by drought stress on the ratio between starch and protein accumulation in grain, could be complex and could involve e.g. stomata closure and reduced photosynthetic production of assimilates or acceleration of senescence and the shortening of the grain filling period.

3.2 Effect on amyolytic modification

The content of extract in malt corresponds to the starch content in the non-malted grain (Figure 2). Variants

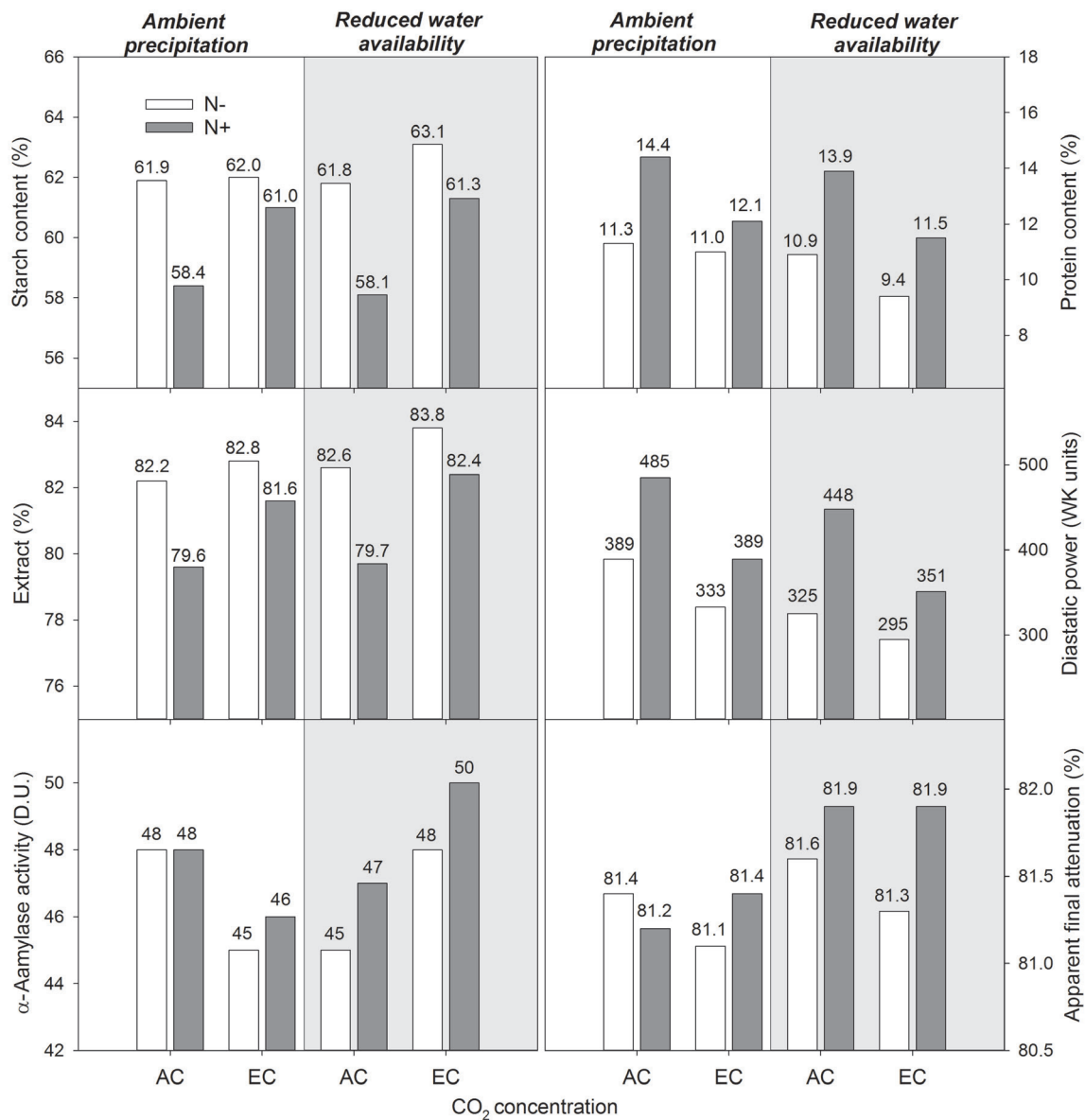


Figure 2 Effect of elevated CO₂ concentration (EC, 700 $\mu\text{mol CO}_2 \text{ mol}^{-1}$) compared to ambient CO₂ concentration (AC, 400 $\mu\text{mol CO}_2 \text{ mol}^{-1}$) and interactions with N nutrition (N+, 100 kg N ha⁻¹; N-, 0 kg N ha⁻¹) or water availability (ambient precipitation and reduced water availability) on the chemical composition of grain and parameters of amyolytic modification of starch.

fertilised by N during cultivation (N+) always showed a lower extract content in the malt. However, grain from variants grown under EC exhibited a higher extract compared to AC counterparts, particularly in N+ treatments. The effect of reduced water availability was manifested by a slight increase of the extract and the highest extract content in malt was found in the variant grown under reduced water availability and EC. Studies on the effect of EC on extract content in barley malt practically do not exist. Erbs et al. (2010) only documented the effect of EC on the reduction of viscosity of water extract. As the extract content in the malt is closely related to starch content in grain, the effect of EC on extract content in the malt can be derived from this parameter. However, the response of starch content to EC is rather variable with a high effect of genotype (reviewed by Beckles and Thitisaksakul, 2014), which allows us to assume that also the effect of EC on extract in the malt will be genotype-dependent. From our results, the interactive effect of N nutrition and [CO₂] on starch and extract content in the malt is also evident. If the conditions for starch synthesis and accumulation in grain are favourable (N-), the EC changes starch and also extract content only to a small extent, while at higher N doses (N+) the effect of EC is more pronounced. This may indicate the existence of an upper limit for extract content in malt given by genotype. The variety used in our experiment (Bojos) is a genotype characterized by a generally high extract content (Psota et al., 2005) resulting in a higher range of N nutrition and EC effects. Tester (1997) also demonstrated a higher genetic variability in starch (and particularly amylose) and less variation caused by environmental conditions in barley.

The N+ treatment exhibits a higher diastatic power, which is an indicator of the activity of amylolytic starch-hydrolysing enzymes, predominantly β -amylases and α -amylases that break down starch during mashing (Figure 2). Reduced water avail β ability decreased the value of diastatic power. Also, the EC treatment reduced the level of diastatic power. The lowest value of diastatic power was found under combined reduced water availability and EC. The activity of α -amylase was increased by N nutrition. However, such an effect was less evident for well-watered variants. Water availability also completely changed the effect of EC on α -amylase activity. While EC increased α -amylase activity under reduced water availability, the effect was opposite under ambient precipitation. Such interactions resulted in the highest α -amylase activity under a combination of EC, N+ and reduced water availability. However, the level of changes in α -amylase activity was relatively low, which corresponds to the results previously achieved for the effect of drought

(Morgan and Riggs, 1981), [CO₂] and N nutrition (Erbs et al., 2010). The effect of EC and N nutrition on α -amylase activity was also found to be strongly dependent on the year (Erbs et al., 2010).

Apparent final attenuation, which corresponds to the quality of the wort, was highest in samples from reduced water availability and N+ treatment (Figure 2). EC reduced the apparent final attenuation in N+ treatments, while without N fertilisation it remained stable.

3.3 Effect on proteolytic modification

Protein content in malt corresponds to the protein content in non-malted grain. N+ treatments exhibit a higher protein content in malt (Figure 3). EC generally reduced protein content in malt, and this effect was more pronounced in N+ treatments. The soluble N content also corresponds to the protein content in non-malted grain and malt.

The values of Kolbach index, which indirectly express the activity of proteolytic enzymes, were always lower in N+ treatments (Figure 3). EC, on the other hand, increases Kolbach index compared to AC. The increase of Kobach index under EC is higher in N+ treatments. A negative relationship between N nutrition and Kolbach index has already been described in the literature (e.g. Béndek and Kádár, 1988; Edney et al., 2012). However, less is known about the effect of water availability on proteolytic enzyme activity. Verma et al. (2003) showed that water availability effect on Kolbach index varies between genotypes, but in general lower water availability is associated with reduced Kolbach index. However, there is no literature discussing the effect of EC on proteolytic modification on malting barley. Our data indicate that EC has a consistently positive effect on proteolytic modification. As Kolbach index is negatively associated with protein content and the ratio of soluble N and total N in malt, EC leads to increase of Kolbach index.

In the case of a relative extract at 45 °C, which also indicates the level of proteolytic modification that occurs during the malting process, the N nutrition raised the levels compared to N- treatment. EC reduced the value of relative extract at 45 °C, and this effect was more pronounced in N+ treatment.

3.4 Effect on cytolytic modification

Increased protein content in non-malted grain due to N nutrition negatively affected degradation of cell walls characterised by friability, occurrence of partly unmodified and whole unmodified grains in malt, and homogeneity determined by a friabilimeter (Figure 4). The highest friability was recorded for variants grown under EC conditions and reduced water availability. The friability decreased primarily with N nutrition. EC eliminated this

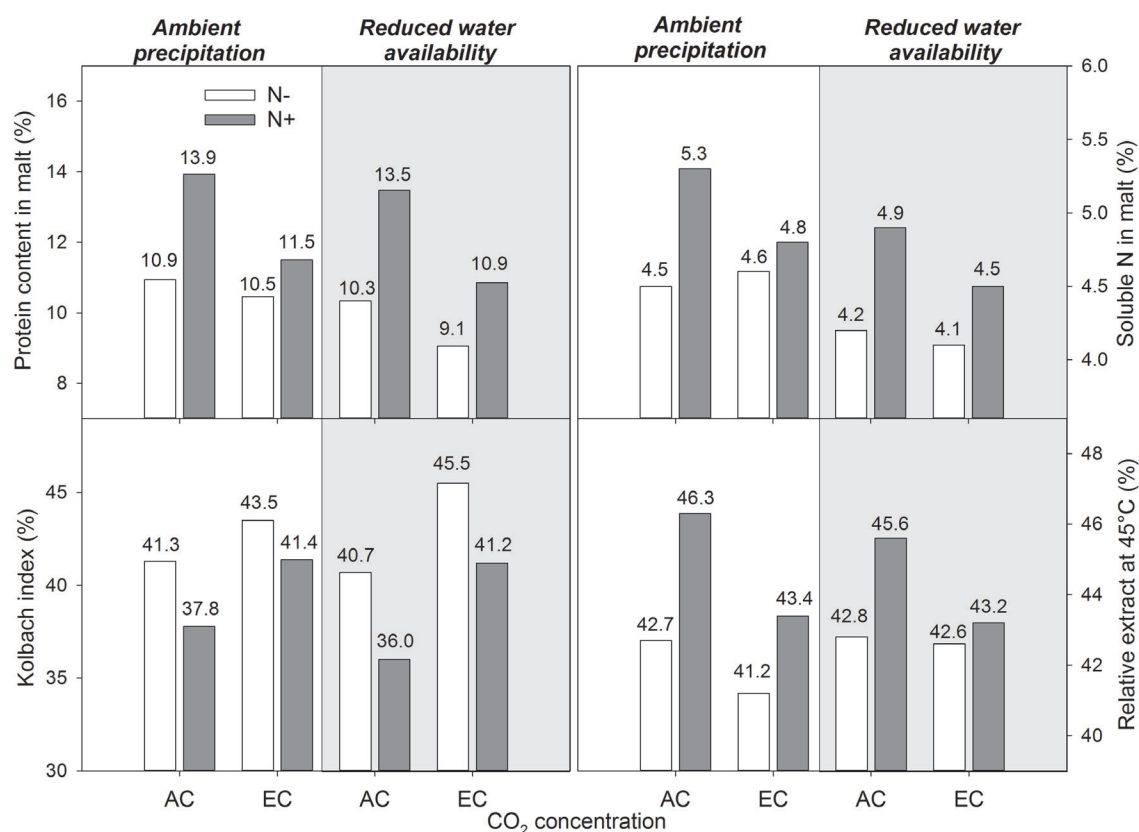


Figure 3 Effect of elevated CO_2 concentration (EC, $700 \mu\text{mol CO}_2 \text{ mol}^{-1}$) compared to ambient CO_2 concentration (AC, $400 \mu\text{mol CO}_2 \text{ mol}^{-1}$) and interactions with N nutrition (N+, 100 kg N ha^{-1} ; N-, 0 kg N ha^{-1}) or water availability (ambient precipitation and reduced water availability) on parameters related to proteolytic modification during the malting process.

negative impact of N nutrition and the friability decline by N nutrition was almost half under EC conditions compared to AC. The proportion of the whole unmodified and partly unmodified grains was significantly higher in N+ treatments and EC strongly alleviated this effect. The proportion of unmodified grains was also declined by reduced water availability. The decrease of friability and friability homogeneity with increasing N dose was also confirmed by Edney et al. (2012). Increasing friability under reduced water availability is contradictory to the results of Verma et al. (2003), but the reported effects of water availability on friability are small.

Among variants grown under conditions of sufficient water supply, there was no effect of N nutrition and EC on the content of β -glucans in the wort and on the viscosity of the wort (Figure 4). Both β -glucan content and viscosity of the wort increased under reduced water availability and AC. β -Glucan content was also moderately increased by reduced water availability under EC conditions, and without N nutrition (N-). The variant, grown under the combined effect of reduced water availability and EC, showed the lowest content of β -glucans in the wort.

3.5 Effect on haze of wort and total polyphenols in wort

The haze of wort was generally low across all variants within the experiment. The N+ variants exhibited lower values of the haze of wort measured by a hazemeter than the samples from N- variants (Figure 5). Reduced water availability slightly lowered the haze. EC conditions decreased the haze of wort in most variants. EC increased haze of wort only under sufficient water availability and N- treatment. Total polyphenols in wort were generally higher under reduced water availability and, for such conditions, the polyphenols in wort showed stable values irrespective of N nutrition or $[\text{CO}_2]$. On the other hand, the values of total polyphenols in wort exhibited higher variability under sufficient water availability with the highest value for the combination of N+ and EC treatments and the lowest value in the combination of N+ and AC.

The most frequent cause of haze formation in beer is associated with protein-polyphenol interactions. Siebert et al. (1996) suggested that the formation of insoluble complexes from proteins and polyphenols is the main cause of haze. Moreover, a particular ratio of haze-active proteins and haze-active polyphenols has a strong influence on the degree of haze (Ye et al., 2016). However, in

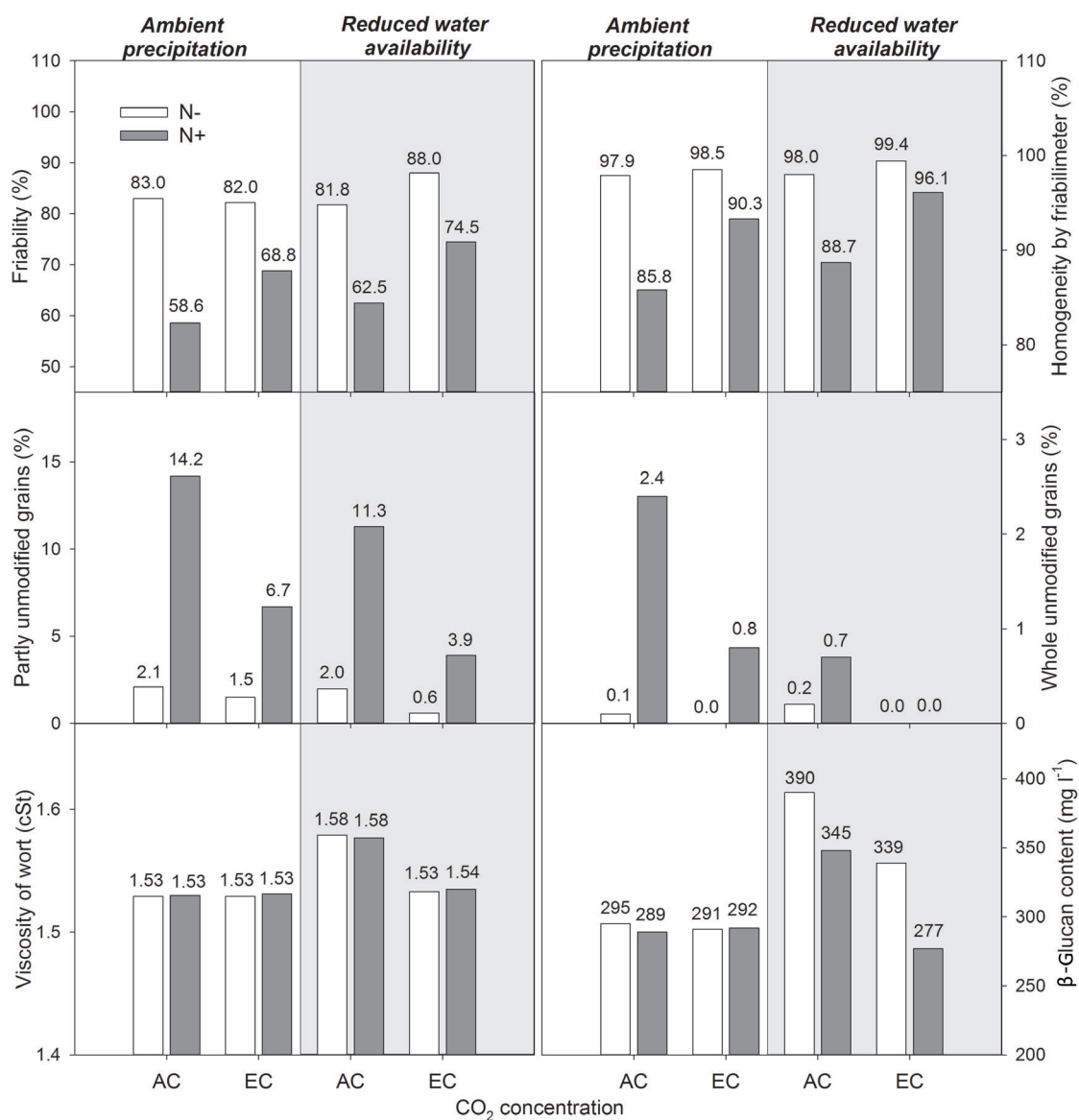


Figure 4 Effect of elevated CO_2 concentration (EC, $700 \mu\text{mol CO}_2 \text{ mol}^{-1}$) compared to ambient CO_2 concentration (AC, $400 \mu\text{mol CO}_2 \text{ mol}^{-1}$) and interactions with N nutrition (N+, 100 kg N ha^{-1} ; N-, 0 kg N ha^{-1}) or water availability (ambient precipitation and reduced water availability) on parameters related to cytolitic modification during the malting process.

our study the haze was in negative relation to both, i.e. the protein content which increased with N+ treatment and total polyphenols, which increased with reduced water availability.

3.6 Effect on the total malting quality

National, as well as international organisations, create various systems for the overall malting quality assessment – malting quality indexes (Molina-Cano et al. 1986; Schildbach, 1987). The aim of malting quality indexes is to transfer obtained data into more explicit and comprehensible form mainly for the basic orientation of malting experts and malting barley growers and

breeders. In the Czech Republic, the Malting Quality Index (MQI, Psota and Kosař, 2002) was created in cooperation with Czech malt producers. MQI is mainly used for testing barley varieties. Apart from being used as the parameter integrating a number of malting quality indicators, it is useful for comparing the effects of environmental factors or crop management practices.

MQI, which summarizes the effect of eight malting quality parameters (N content in non-malted grain, malt extract, relative extract at 45°C , Kolbach index, diastatic power, apparent final attenuation, friability, β -glucan content in wort) was in our study affected mainly by N nutrition (Figure 5). N nutrition reduced MQI by ap-

proximately 2.5 points. A lower effect of N nutrition on MQI was discovered only under reduced water availability and EC conditions. In this case, MQI was reduced by 1.4 points only. EC enhanced MQI by less than 1 point. The higher effect of EC was again confirmed for the combination of reduced water availability and N+ treatment where EC increased MQI by 2.1 points. Generally, water availability had the lowest effect on MQI. It is worth mentioning that the effect of reduced water availability was discovered only for the combination of EC conditions and N+ treatment (increase by 1.4 points).

4 Conclusion

Within the multifactorial field experiment conducted in Open Top Chambers, we were able to evaluate not only the separate effects of EC, N nutrition and reduced water availability on malting quality of spring barley but also their mutual interactions. The results showed a dominant effect of N nutrition which reduced the total malting quality (MQI) particularly by increased protein content

in grain and malt, reduced extract and Kolbach index. EC, however, substantially alleviated the negative impact of N nutrition and also slightly increased malting quality in treatments without N fertilisation. EC increased starch and reduced protein contents in grain, which resulted in a higher extract. Although the alleviation effect of EC on these parameters was considerable, the effect on MQI was less pronounced under ambient precipitation. Reduced water availability generally showed a positive effect on malting quality parameters, and there was also an apparent interaction with EC. Thus, the best malting quality parameters were achieved for the combination of reduced water availability and EC while under AC, the effect of water availability was rather small.

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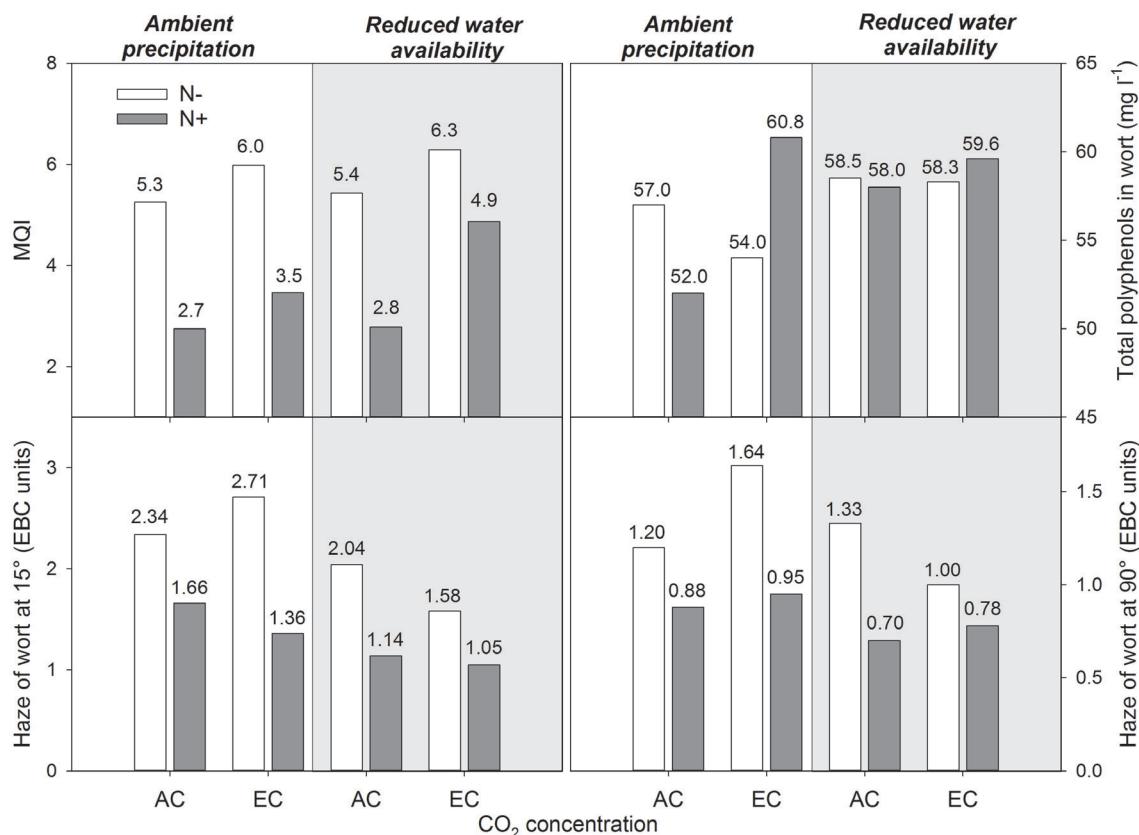


Figure 5 Effect of elevated CO₂ concentration (EC, 700 μmol CO₂ mol⁻¹) compared to ambient CO₂ concentration (AC, 400 μmol CO₂ mol⁻¹) and interactions with N nutrition (N+, 100 kg N ha⁻¹; N-, 0 kg N ha⁻¹) or water availability (ambient precipitation and reduced water availability) on total polyphenols, haze of wort and total malting quality (Malting Quality Index).

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