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Future climate alleviates stress impact on grassland productivity through altered antioxidant capacity

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ABSTRACT

Predicting future ecosystem functioning requires a mechanistic understanding of how plants cope with different stressors under future climate conditions with elevated CO_2 concentrations and warmer temperatures. Nonetheless, studies of stress responses under combined elevated CO_2 and warming remain scarce.

We assembled grassland communities in sunlit, climate-controlled greenhouses and subjected these to three stressors (drought, zinc toxicity, nitrogen limitation) and their combinations. Half of the communities were exposed to ambient climate conditions (current climate) and the other half were continuously kept at 3 °C above ambient temperatures and at 620 ppm CO_2 (future climate).

Across all stressors and their combinations, future climate-grown plants coped better with stress, i.e. above-ground biomass production was reduced less in future than in current climate. Among several tested potential biochemical and ecophysiological stress-relief mechanisms, we found three mutually non-exclusive mechanisms underpinning an improved stress protection under future climate conditions: (i) altered sugar metabolism; (ii) up-regulated levels of total antioxidant capacity and polyphenols; and (iii) more efficient use of ascorbate and glutathione as antioxidants.

1. Introduction

Wherever they grow, plants are frequently subjected to a large variety of environmental stressors. Nitrogen (N) and water are considered the most important limiting factors of plant productivity in the vast majority of terrestrial ecosystems (Schulze et al., 1987; Vitousek and Howarth, 1991). In addition to resource limitations, plant communities have to deal with anthropogenic stressors such as acid rain, or organic and inorganic pollutants like pesticides or heavy metals. Indeed, metal toxicity limits plant growth in many areas affected by mining or smelting activities, sewage sludge deposition or traffic (Kabata-Pendias and Pendias, 2001; Arienzo et al., 2004).

Predicting future plant responses to the ongoing increase in frequency and intensity of droughts (IPCC, 2007) requires a mechanistic understanding of how plants cope with drought stress under conditions of elevated atmospheric CO_2 concentrations and warmer temperatures. Besides climate related stressors, this also holds for future responses to natural stressors like nutrient limitation or anthropogenic stressors like heavy metal pollution, which is why multifactorial experiments are often called for (Norby and Luo, 2004; Mittler, 2006). Nonetheless, field studies of stress responses under combined elevated CO_2 and warming remain very scarce (Casella et al., 1996; Dukes et al., 2005; Mikkelsen et al., 2008; Bloor et al., 2010).

Single factor studies, on the other hand, have been frequently reported, especially the effects of elevated CO_2. The two

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Abbreviations: ANOVA, analysis of variance; ASC, ascorbate; D, drought stress; DOY, day of year; ET, evapotranspiration; GSH, glutathione; IS, insoluble sugars; MDA, malondialdehyde; N, nitrogen stress; PAR, photosynthetically active radiation; ROS, reactive oxygen species; SWC, soil water content; SD, standard deviation; SE, standard error; TAC, total antioxidant capacity; T_{air}, air temperature; U, unstressed; Z, zinc toxicity.

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fundamental responses of plants to rising atmospheric CO_2 concentrations are enhanced photosynthesis and reduced stomatal conductance. All other effects of elevated CO_2 on plants and ecosystems are derived from these changes (Long et al., 2004). Also the effect of single warming or elevated CO_2 on one environmental stressor has been reported in the literature. For example, elevated CO_2 can moderate water shortages during drought through its effect on stomatal conductance (Rogers et al., 1984), while warming can cause reduced water availability due to increased evapotranspiration (Loik et al., 2000). Future climate conditions may also affect the availability of N. Increased carbon (C) input under elevated CO_2 is found to decrease soil N availability through enhanced microbial immobilization (Luo et al., 2004; Hu et al., 2006), eventually leading to N limitation (Oren et al., 2001; Hungate et al., 2003; Luo et al., 2004). However, increasing soil temperatures could reverse this reduction in N availability, by enhancing net N mineralization (Loiseau and Soussana, 2000; Hovenden et al., 2008). Enhanced mineralization could also alter heavy metal availability if the metals are bound to soil organic matter (Antoniadis and Alloway, 2001). Hence, it is clear that a future climate can change the intensity of stress factors by decreasing or increasing the availabilities of resources and/or pollutants.

Regardless of a possible impact on the intensity of a stressor, a changing climate could also affect the plant's protective capacity and, as a consequence, their growth responses to stress. As a result of stress-induced perturbations in plant metabolism, levels of reactive oxygen species (ROS) generally increase (Mittler, 2002). The production of ROS during stress results from imbalances in pathways such as photorespiration, from the photosynthetic apparatus and from mitochondrial respiration. In addition, environmental stress has been shown to trigger the active production of ROS by NADPH oxidases (Knight and Knight, 2001). Reactive oxygen species can act as signals for the activation of stress response and defence pathways, but they can also be harmful for biological structures and processes, and can lead to DNA, amino acid and protein oxidation and lipid peroxidation (Asada, 1999). To circumvent the deleterious effects of ROS, plants have evolved robust antioxidant defensive systems to minimize free radicals damage (Mittler et al., 2004). Interestingly, oxidative stress and antioxidant systems may be altered in a future climate. Yet, the effect of combined elevated CO_2 and warming on stress-relief mechanisms has received little attention (Aranjuelo et al., 2008). Single elevated CO_2 can diminish intrinsic oxidative stress through decreased ROS formation, resulting from an enhanced use of reductant for assimilation in photosynthesis and a reduced photorespiration (Halliwell and Gutteridge, 1989; Schwanz and Polle, 1998). Consequently, a down-regulation of the protective mechanisms by elevated CO_2 has been reported in several studies (Schwanz and Polle, 1998; Vurro et al., 2009). Another observed effect of elevated CO_2 occurs prior to any photooxidative process and operates through an enhanced thermal dissipation of excessive energy (nonphotochemical quenching), resulting in an improved photoprotection (Aranjuelo et al., 2008). Furthermore, also moderate warming can contribute to alleviating oxidative stress through an enhancement of metabolic reactions (Han et al., 2009).

Grasslands cover 15% of the European land area and are an important food source for livestock (Ciais et al., 2010). An understanding of potential stress-induced reductions in biomass production is thus relevant, both now and under changing climate conditions. In the present study we have determined how grassland communities respond to a variety of stressors in a future climate at both the cellular and the community level. Hence, this study responds to the need for research that relates molecular information to whole plant processes (Chaves et al., 2003). The experimental set-up contained realistically assembled grassland communities that were grown under a current and a projected future climate, and subjected to drought, N limitation, Zn toxicity and their combinations. We hypothesize that combined elevated CO_2 and warming ameliorates plant protection against stress and that this increased protection mitigates the decline in grassland productivity in response to stress.

2. Materials and methods

2.1. Experimental set-up

The study was performed on assembled grassland communities at the Drie Eiken Campus, University of Antwerp, Wilrijk, Belgium (51°09′ N, 04°24′ E, 10 asl). Average annual precipitation at this location is 776 mm, average annual air temperature is around 10.8 °C. The experimental set-up consisted of six sunlit, south facing climate-controlled chambers. The interior surface area was 1.5 m × 1.5 m, the height at the north side 1.5 m and at the south side 1.2 m. The top of the chambers consisted of a colourless polycarbonate plate (4 mm thick), whereas the sides were made of polyethylene film (200 μm thick), both UV transparent. Three of the six chambers tracked the current climate with current air temperature (T_{air}) and CO_2 concentration, while the other three chambers were exposed to a future climate scenario with 3 °C warming and a target CO_2 concentration of 620. We further refer to these climate scenarios as 'current' and 'future climate', respectively, although it is recognized that future climate conditions as predicted by the IPCC also include other aspects such as changes in precipitation and wind patterns, more frequent extreme events and increased emissions of aerosols, methane and nitrous oxide (IPCC, 2007). The CO_2 concentration was measured and regulated with a CO_2 control group with an infrared analyser (WMA-4, PPSystems, Hitchin, UK). In the current climate chambers the concentration was 394 ± 34 ppm (SD) while in the future climate chambers it was 625 ± 53 ppm (SD). Every half hour, T_{air} was monitored with a temperature sensor (Siemens, type QFA66, Germany) and photosynthetically active radiation was measured with a quantum sensor (SDEC, type JYP1000, France). During the experiment monthly average T_{air} was 17.1, 16.4 and 18.4 °C in May, June and July 2008, respectively. In the current climate chambers T_{air} was on average 0.6 ± 1.6 °C (SD) lower than outside and the future climate chambers were 2.5 ± 1.6 °C (SD) warmer than outside. Total monthly irrigation equalled 61.5, 64.4 and 85.1 mm in May, June and July, respectively. Irrigation was calculated from the monthly rainfall over the period 1995–2005 and corrected for differences in evapotranspiration (ET) inside and outside the chambers. To this end, De Boeck et al. (2006) calculated ET inside current climate chambers from changes in soil water content (SWC) and the amount of administered water, and the outside ET with Hamon's equation (Haith and Shoemaker, 1987) based on day length, vapour pressure and T_{air}.

2.2. Plant communities

This study was part of a larger experiment that consisted of 30 randomly placed grassland communities per chamber. Each community was composed of six species, selected from three functional groups which were equally represented: two grass species (*Poa pratensis* L. and *Lolium perenne* L.), two N-fixing dicots (*Medicago lupulina* L. and *Lotus corniculatus* L.), and two non-N-fixing dicots (*Rumex acetosa* L. and *Plantago lanceolata* L.). The communities contained 18 individuals (three per species) planted in a hexagonal grid with a 4.5 cm interspace, with interspecific interactions maximized by avoiding clumping. Communities were established early May 2008 (day of year (DOY) 134–137) by transplanting 5-week-old seedlings to PVC containers (tubes, 24 cm inner diameter and 40 cm height, closed with a lid at the bottom) filled with sandy soil