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### Root respiration response to high CO<sub>2</sub> concentrations in plants from natural CO<sub>2</sub> springs

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#### Abstract

Many plant species are able to grow at very high carbon dioxide (CO<sub>2</sub>) concentrations at the sites of the natural geothermal CO<sub>2</sub> enrichment (natural CO<sub>2</sub> springs). It is to expect that in such an environment the increased concentrations of soil CO<sub>2</sub> could directly affect root function, e.g. respiration, which could be of crucial importance for plant performance. In our study, root respiration of different grasses growing at the natural carbon dioxide spring Stavešinci (NE, Slovenia) was studied. By using liquid phase measurements (Clark-type oxygen electrodes) and potassium hydrogencarbonate addition, high concentrations of dissolved inorganic carbon species were obtained in the system, leading to high concentrations of soluble CO<sub>2</sub> (CO<sub>2(aq)</sub>), thus simulating a high CO<sub>2</sub> environment. For all species measured (*Alopecurus pratensis* L., *Dactylis glomerata* L., *Echinochloa crus-galli* (L.) PB., *Holcus lanatus* L., *Phleum pratense* L., *Poa pratensis* L. and *Setaria pumila* (Poir.) Roem. & Schult.) a relatively low sensitivity of root respiration to increased CO<sub>2(aq)</sub> was observed. A significant inhibition (ranging from 16 to 54%) was measured at 8.3 mM CO<sub>2(aq)</sub> in the assay buffer solution. When root respiration of plants from different CO<sub>2</sub> exposures, i.e. locations at the mofette, was compared at ambient CO<sub>2</sub> concentration, no consistent differences were observed.

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#### 1. Introduction

Plants growing near natural CO<sub>2</sub> springs (NCDS) are exposed to an extreme environment where soil and

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atmospheric CO<sub>2</sub> concentrations can reach very high values. The concentrations of soil CO<sub>2</sub> in the Stavešinci CO<sub>2</sub> spring area (NE Slovenia) appear in a wide range, from practically normal (below 1% CO<sub>2</sub>) up to 100% CO<sub>2</sub>, as measured several times directly in the CO<sub>2</sub> releasing cracks (for details on the site see Turk et al., 2002; Vodnik et al., 2002b; Pfanz et al., 2004). Although severely inhibited in growth, the plants of

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different species are able to sustain soil  $CO_2$  concentrations in the range of ten-percentages, what was proved by measurements of gaseous regime in the rooting horizon of individuals growing very close to gas releasing vents (Pfanz et al., 2004).

Since early ninties plant functioning under natural  $CO_2$  enrichment has been studied in many respects (Raschi et al., 1997; Badiani et al., 2000); still, very few studies included plant roots into their research in any aspect (Rillig et al., 2000). Beside some reports indicating that shoot respiration could be increased under natural  $CO_2$  enrichment (*Quercus ilex* and *Quercus pubescens*, Tognetti et al., 1998) it is not clear what is the response of mitochondrial respiration of natural  $CO_2$  spring plant species. Practically no work has been done on root respiration under such conditions, although roots are most probably the first target of the increased  $CO_2$  action.

On the other hand, it holds true that also global change oriented research has not focused on the effects of elevated CO<sub>2</sub> on root respiration to an extent found in shoot respiration studies (see reviews by Amthor, 1991; Drake et al., 1999) or even photosynthetic studies. Studies of root respiratory response under elevated (i.e. double atmospheric) CO2 concentrations have yielded inconsistent and species-specific results. There are reports on inhibition of respiration in the range of CO<sub>2</sub> concentrations normally found in soil (Nobel and Palta, 1989; Palta and Nobel, 1989; Qi et al., 1994) and others, suggesting small or even no effect of CO2 concentration on root respiration (Bouma et al., 1997; Burton et al., 1997). Rarely respiratory response of plant tissues under very high CO<sub>2</sub> (percentage concentrations) has been studied (Palet et al., 1991).

In the present research, we studied root respiration of several species that grow in a high CO<sub>2</sub> environment at the natural CO<sub>2</sub> spring Stavešinci. The main goal of the study was to assess general sensitivity of root respiration to the increased CO<sub>2</sub> concentration. In the three species (*Dactylis glomerata, Echinochloa crusgalli* and *Setaria pumila*), the response of root respiration to high CO<sub>2</sub> concentration was measured at three different concentrations of dissolved inorganic carbon (DIC) species and thus  $CO_{2(aq)}$  in the assay buffer solution. Additionally, we were interested in how the prevailing CO<sub>2</sub> regime during the growth at the NCDS affects root respiration of the plants. For this purpose, root respiration was measured on several plant species; seeded *E. crus-galli* and *S. pumila* and naturally grown *Alopecurus pratensis*, *D. glomerata*, *Holcus lanatus*, *Phleum pratense* and *Poa pratensis* under ambient and highly elevated CO<sub>2</sub> concentration.

#### 2. Material and methods

Two different approaches were used in our studies. In the first part of the study, the response of root respiration under different concentrations of  $CO_{2(aq)}$ (DIC) was measured in young growth chamber grown seedlings, descendants of NCDS plants. In the second part, we measured root respiration of different plant species, sampled at the site with natural geothermal CO<sub>2</sub> enrichment (carbon dioxide spring Stavešinci, NE Slovenia). In this case, the measurements were carried out at two concentrations of  $CO_{2(aq)}$ , simulating  $CO_2$ conditions for normal, non-enriched soil and the soil well enriched in  $CO_2$ .

# 2.1. Root respiration of growth chamber grown grasses under different concentrations of $CO_{2(aq)}$ (DIC) during the measurements

Root respiration of the three species E. crus-galli, S. pumila and D. glomerata was measured under different concentrations of CO<sub>2(aq)</sub> (DIC) in 2003. The seeds of these grasses, which were collected at the natural CO<sub>2</sub> spring area in the previous year, were surface sterilized by 10% hypochloride (6% active chlorine) and seeded into petri dishes (35 cm diameter) which were half filled with autoclaved quartz sand to which 150 ml of Hoagland nutrient solution (Millner and Kitt, 1992) was added. Plants were grown in growth chambers (14 h illumination period, PAR 450  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>, at 23 °C during light and 18 °C during dark period) for three weeks. Just before root respiration measurements were conducted, the sand was gently washed away from the roots with distilled water and roots were excised from the rest of the plant. Excess water was blotted from the roots and samples of 50-80 mg (Matamala and Schlesinger, 2000; Staddon et al., 2003) were taken for respiratory measurements. Root respiration was measured using liquid-phase oxygen electrodes (Hansatech, Norfolk, England) at constant temperature 20 °C, in a Hoagland's nutrient solution, buffered with 100 mM MES (morpholinoethanesulfonic acid, Sigma) and 100 mM HEPES

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