Geological CO$_2$ affects microbial respiration rates in Stavešinci mofette soils

Geološki CO$_2$ vpliva na mikrobno dihanje v tleh na območju mofete Stavešinci

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Abstract: Substrate-induced respiration (SIR) was used to estimate microbial respiration and microbial biomass in soils from Stavešinci natural CO$_2$ spring (mofette) exposed to different geological CO$_2$ concentrations. SIR measurements clearly demonstrated higher microbial respiration and microbial biomass in control sites compared to high soil CO$_2$ sites. Sampling in two different locations and in three different years also confirmed long-term stability of this pattern, which was found for both locations and in different sampling periods.

Keywords: substrate-induced respiration, SIR, microbial respiration, microbial biomass, soil respiration, natural CO$_2$ springs, mofette

Introduction

Soil CO$_2$ concentrations are about 50-times higher than ambient atmospheric CO$_2$ concentration and often fluctuate due to soil compaction, waterlogging and/or vegetation (Bouma & Bryla 2000, Pfanz & al. 2004). Natural CO$_2$ springs (mofettes) are extreme ecosystems with soil CO$_2$ concentrations that can reach values above 80% (v/v) CO$_2$ in the upper 10-20 cm of soil at the most extreme sites (Vodnik & al. 2006). Most of the research at natural CO$_2$ springs in the past was focused on aboveground responses of vegetation (Raschi & al. 1997, Badiani & al. 1999, Vodnik & al. 2002, Pfanz & al. 2004, Pfanz & al. 2007). Much less work was done on the below ground responses of plants (Maček & al. 2005) or soil microorganisms (Maček 2004, Maček & al. 2008, Videček & al. 2009). Apart from the Stavešinci mofette, most of the reports on soil microbes come from the Haquanoa spring in New Zealand where arbuscular mycorrhizal (AM) fungi (Rillig & al. 2000) and mineralization (Ross & al. 2002, Ross & al. 2003) were studied. In most of these studies, however, mofettes were used as long-term natural model systems for studying effects of elevated atmospheric CO$_2$ on ecosystems. Thus sampling was done according to the atmospheric CO$_2$ concentrations, which are much more dependent on weather conditions and do not always reflect soil CO$_2$ concentrations and their direct effects on soil microflora. Soil CO$_2$ concentrations were taken into consideration in the studies of soil microorganisms first at the Slovenian mofette Stavešinci (Maček 2004, Maček & al. 2008, Videček & al. 2009).

Soil microbial biomass can be estimated by adding an easily available substrate (e.g. glucose) to the soil (substrate-induced respiration – SIR) (Jenkinson & Ladd 1981). Anderson & Domsch (1978) suggested that the initial maximal respiration rate induced by glucose was proportional to the size of the original soil microbial biomass. The method does not give an absolute value of the biomass, however, the results can be used for relative comparisons. The same authors also
report on highly significant correlation between fumigation-incubation technique and SIR for estimation of the microbial biomass. At 22 °C, 1 ml CO\(_2\) h\(^{-1}\) equals 40 mg microbial C (Anderson & Domshch 1978). In addition, Ross & al. (2000) report on positive correlation between SIR and atmospheric CO\(_2\) concentration up to 700 ppm at the New Zealand CO\(_2\) springs, however, no attempt was made to calculate microbial biomass C from the resultant CO\(_2\) values.

In this study substrate-induced respiration was used to estimate microbial biomass of soils exposed to different geological CO\(_2\) concentrations in Stavešinci mofette ecosystem. Soil samples were taken in three different CO\(_2\) regimes, defined as high, medium and low (control) geological CO\(_2\) and in three different years 2003, 2004 and 2007.

**Materials and methods**

**Site description and sampling**

The study was conducted in Stavešinci mofette, NE Slovenia (see Vodnik & al. 2006, Vodnik & al. 2009, for detailed site description). Briefly, the site is a flat post-agricultural area where very pure, cold CO\(_2\), without traces of sulphurous compounds, methane or carbon monoxide, is released into atmosphere through several vents. Atmospheric CO\(_2\) concentrations largely depend on weather and wind conditions due to the topography of the site, and range from 0.036 % to 1 % (v/v) at 0.5 m aboveground (Vodnik & al. 2006). On the other side, soil CO\(_2\) concentrations and CO\(_2\) effluxes are more stable variables for measuring exposure to geological CO\(_2\). Soil samples were taken from two separate locations (Location 1 and Location 2) ca. 40 m apart. Each sampling location covered an area of about 100 m\(^2\) with soil CO\(_2\) concentrations ranging from high to low (ambient/control) CO\(_2\) concentrations as measured by a portable gas analyzer (GA2000, Geotech, Germany) (Vodnik & al. 2006) and/or soil CO\(_2\) flux measurements (LI-6400-09 Soil CO\(_2\) flux chamber, LICOR, Lincoln, USA) (Vodnik & al. 2009). A good correlation between both methods has been confirmed before (Vodnik & al. 2009).

Upper 10 cm of soil was sampled in Location 1 in March 2003 (\(n = 4-5\) sampling points) and in April 2004 (\(n = 6-8\) sampling points) in high CO\(_2\) (73.6 % ± 2.7 v/v), medium (9.3 % ± 0.6 v/v) and low CO\(_2\) (0.4 % ± 0.03 v/v) exposure. Location 2 soil was sampled in July 2007 (\(n = 4\) sampling points) for high CO\(_2\) (228.0 ± 50.4 µmol m\(^{-2}\) s\(^{-1}\)), medium (42.4 ± 11.3 µmol m\(^{-2}\) s\(^{-1}\)) and low CO\(_2\) flux (21.1 ± 7.3 µmol m\(^{-2}\) s\(^{-1}\)), see also Videnšek & al. 2009. Soil chemical properties for Location 1 are described by Maček 2004, Maček & al. 2005 and for Location 2 by Videnšek & al. 2009. In brief, the values for Location 1; pH 5.4 (control), 3.8 (high CO\(_2\)); organic matter 3.2 % (control), 3.8 % (high CO\(_2\)); total N 0.26 % (control), 0.32 (high CO\(_2\)); available P\(_2\)O\(_5\) 48 mg kg\(^{-1}\) (control), 265 mg kg\(^{-1}\) (high CO\(_2\)) and for Location 2; pH 5.7 (control), 4.9 (high CO\(_2\)); organic matter 3.3 % (control), 3.9 % (high CO\(_2\)); total N 0.32 % (control), 0.36 (high CO\(_2\)); available P\(_2\)O\(_5\) 22 mg kg\(^{-1}\) (control), 44 mg kg\(^{-1}\) (high CO\(_2\)). Fresh samples were transported and stored at 4 °C and all the measurements were performed within two days after sampling. Before measurements soil was thoroughly mixed and all visible plant particles were removed.

**Soil water content**

Soil water content was determined by drying soil samples over night at 110 °C and weighing.

<table>
<thead>
<tr>
<th>Sampling period</th>
<th>High CO(_2)</th>
<th>Medium CO(_2)</th>
<th>Low CO(_2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>March 2003</td>
<td>23.0 ± 3.1</td>
<td>22.3 ± 0.3</td>
<td>20.7 ± 0.7</td>
</tr>
<tr>
<td>April 2004</td>
<td>27.6 ± 0.3</td>
<td>no data</td>
<td>26.9 ± 0.6</td>
</tr>
<tr>
<td>June 2007</td>
<td>9.8 ± 1.2</td>
<td>9.8 ± 1.2</td>
<td>11.6 ± 2.0</td>
</tr>
</tbody>
</table>

Table 1: Sample water content. Avg ± SE are shown (\(n = 4-6\)).
**Substrate-induced respiration (SIR)**

Respiration rates were estimated by incubating 30 g of soil in 130-ml bottles sealed with rubber seals at room temperature (22 °C), for the 2003 and 2004 measurements, and at 28 °C in July 2007. All samples had equal dry weight. In order to avoid geological CO₂ background all samples were pre-areated to equalize CO₂ concentrations to ambient concentrations. For SIR measurements the samples were amended with 25 mg glucose g⁻¹ dry soil and thoroughly mixed. Basal respiration was taken as the respiration rate of soils not amended with glucose and was subtracted from the SIR value. The concentrations of CO₂ in the headspace of the bottles were measured by gas chromatography, using a Becker Packard model 417 (Delft, Netherlands) gas chromatograph (GC), with thermal conductivity detector temperature 100 °C, 1.8-m column (2 mm inside diameter) packed with Prapak QS 180 cm column at 50 °C, injector temperature 100 °C, caring gas (He) flow 20 ml min⁻¹ and Hewlett Packard 3392A integrator. Samples (2.5 ml) of headspace gas were taken with a gas-tight syringe and injected into the gas chromatograph. Since the pH of the aqueous phase was < 6.5, the effective gas headspace of the bottle was assumed to be the volume not occupied by soil or liquid (Lin & Brookes 1999).

The amount of produced CO₂ in the measuring bottle was calculated as:

\[ M_{CO_2} = \left( C_g \times \left( V_g + V_v \times \alpha\right)\right) / m \]

\[ M_{CO_2} = \text{total CO}_2 (\text{ml g}^{-1} \text{ soil}), C_g = \text{measured CO}_2 \text{ concentration in the gas phase (%), } V_g = \text{volume of the gas phase (130 ml), } V_v = \text{volume of the liquid phase in the soil (ml), } \alpha = \text{Bunsen coefficient for } \text{CO}_2 = 0.758, m = \text{dry weight of soil in the bottle.} \]

For measurements performed at 22 °C microbial biomass was calculated according to Anderson & Domsch (1978) where 1 ml CO₂ h⁻¹ equals 40 mg microbial C.

**Data analysis**

Data of the microbial respiration at different CO₂ levels were analysed for each year/location separately. Because of the longitudinal nature of the data (each sample was measured consequently several times during a time interval and the intervals between the measurements differ for different samples) the linear mixed models with restricted maximum likelihood method were used for the estimation of the parameters. Time and CO₂ exposure group (high, medium, low) and their interaction were included in the model as fixed effects and soil sample with its time dependence were included in the model as random effect. The compound symmetry structure of the within samples random effect covariance was used in the model (Pinheiro & Bates, 2000). The calculations were done with the statistical package R (R Development Core Team, 2009).

**Results and discussion**

Microbial soil biomass is dependent on quantity and quality of soil organic matter (Zak & al. 1993, Cheng 1999), which in turn depends on plant production. Both, plant roots and above ground vegetation are directly affected by high soil CO₂ concentrations (Kaligarić 2001, Vodnik & al. 2002, Macék & al. 2005, Pfanz & al. 2004, Pfanz & al. 2007). It has been shown that in the high CO₂ exposed mofette plants content of N is lower and C/N ratio in plant tissues is higher, compared to control (Pfanz & al. 2004). In addition, lower concentrations of several other elements (P, K, S, and Zn) have been reported for high geological CO₂ exposed plants (Pfanz & al. 2004). All this should have an effect on microbial biomass and respiration.

As given in Fig. 1 glucose addition stimulated CO₂ release from all soil samples, indicating that soil microorganisms were activated by the addition of the respiratory substrate. The respiration data show linear (p < 0.0001) increase of the CO₂ concentration. Different slopes of linear model lines indicate changes in microbial activities (Fig.1). In 2003, SIR was significantly lower in high CO₂ soils, compared to control soils (p = 0.0118). A similar trend was found in 2004, however there was no significant difference between high and low CO₂ soils. Similar to findings from the previous two years also microbial respiration measured in 2007 in samples from the second mofette (Location 2) showed the lowest values in high CO₂ soils, followed by medium and low (control) soils. In this year, significant difference was found between
Fig. 1: Substrate induced microbial respiration (SIR), measurements of CO$_2$ production in soil samples from natural CO$_2$ springs in Stavešinci. Time course of microbial activity (respiration) after substrate addition measured on each sample (thin lines), linear model lines (thick lines); for low (full-lines), medium (dash-lines) and high (dot-lines) CO$_2$ concentrations.

Slika 1: S substratom inducirano mikrobno dihanje (SIR), meritev produkce CO$_2$ v talnih vzorcih področja naravnih izvirov CO$_2$ v Stavešincih. Časovna odvisnost mikrobne aktivnosti (dihanja) po dodatku substrata na posameznem vzorcu (tanke črte), premice linearnih modelov (debelejše črte); prikazano za majhne (polna linija), srednje (črtkana linija) in velike (pikčasta linija) koncentracije CO$_2$. 
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Table 2: The estimated parameters of the linear mixed models with the 95 % confidence limits.  
Tabela 2: Ocene parmetrov linearnih mešanih modelov s 95 % intervali zaupanja.

<table>
<thead>
<tr>
<th>Year</th>
<th>Parameter</th>
<th>95 % Confidence intervals</th>
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<td></td>
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<td>Estimates</td>
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<td>2007</td>
<td>Intercept</td>
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Table 3: Calculated microbial biomass.  
Tabela 3: Ocenjena mikrobna biomasa.

<table>
<thead>
<tr>
<th>Year</th>
<th>High CO\textsubscript{2}</th>
<th>Medium CO\textsubscript{2}</th>
<th>Low CO\textsubscript{2}</th>
</tr>
</thead>
<tbody>
<tr>
<td>2003</td>
<td>115</td>
<td>159</td>
<td>231</td>
</tr>
<tr>
<td>2004</td>
<td>162</td>
<td>no data</td>
<td>205</td>
</tr>
</tbody>
</table>

* Measured 2 h following glucose addition.
water content in 2004 the respiratory substrate glucose, introduced into the sample in a solid form, could not distribute evenly (formation of clumps during mixing of soil) and thus was not available to all the potential users. In the study on the evaluation of the SIR method by Lin & Brookes (1999) glucose was added both in solid or liquid form, however, similar patterns of CO$_2$ evolution were found for both protocols. In addition, it was concluded in the same study, that no correction for CO$_2$ dissolved in the soil solution was needed for the soils below pH 6.5, which is also the case for Stavešinci soil. Higher absolute values of the microbial respiration measured in 2007 are probably due to higher incubation temperatures during the SIR experiment. Nevertheless, the same pattern in microbial respiration response to geological CO$_2$ as in the previous two years was observed. It is interesting to note that in 2007 samples originated from the second mofette (Location 2), which is about 40 m distant from the Location 1 (sampling in 2003 and 2004) with different soil properties and less extreme CO$_2$ regime (see the Methods section). The values for microbial biomass for the years 2003 and 2004 (Tab. 3) are in the range of those found for other grasslands (Habekost & al. 2008).

Conclusions

According to the results of this study we conclude that high concentrations of geological soil CO$_2$ decrease substrate induced microbial respiration and microbial biomass. This pattern of microbial activity was stable and was not affected by different soil properties, different sampling periods, temperature of incubation, or soil water content.

Povzetek


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