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Geological CO_2 affects microbial respiration rates in Stavešinci mofette soils

Geološki CO_2 vpliva na mikrobno dihanje v tleh na območju mofete Stavešinci

Irena Maček[1*], Urška Videmšek[1], Damijana Kastelec[1], David Stopar[2], Dominik Vodnik[1]
[1] University of Ljubljana, Biotechnical Faculty, Department of Agronomy, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia, *Corresponding author: irena.macek@bf.uni-lj.si.
[2] University of Ljubljana, Biotechnical Faculty, Department of Food Science and Technology, Večna pot 111, SI-1000 Ljubljana, Slovenia.

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, Videmšek & 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. 2000, 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, Videmšek & 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 ºC, 1 ml CO_2 h^{-1} equals 40 mg microbial C (Anderson & Domsch 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 Videmšek & al. 2009. Soil chemical properties for Location 1 are described by Maček 2004, Maček & al. 2005 and for Location 2 by Videmšek & 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_2O_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_2O_5 22 mg kg^{-1} (control), 44 mg kg^{-1} (high CO_2). Fresh samples were transported and stored at 4 ºC 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 1: Sample water content. Avg ± SE are shown (n = 4-6).
Tabela 1: Vsebnost vode v vzorcih. Prikazano je povprečje ± SN (n = 4–6).

	Soil water content (mass %)		
Sampling period	High CO_2	Medium CO_2	Low CO_2
March 2003	23.0 ± 3.1	22.3 ± 0.3	20.7 ± 0.7
April 2004	27.6 ± 0.3	no data	26.9 ± 0.6
June 2007	9.8 ± 1.2	9.8 ± 1.2	11.6 ± 2.0

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 CO_2 background all samples were pre-areated to equalize CO_2 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 CO_2 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 bottles was assumed to be the volume not occupied by soil or liquid (Lin & Brookes 1999).

The amount of produced CO_2 in the measuring bottle was calculated as:

$M_{CO2} = (C_g * (V_g + V_v * \alpha)) / m$

M_{CO2} = total CO_2 (ml g^{-1} soil), C_g = measured CO_2 concentration in the gas phase (%), V_g = volume of the gas phase (130 ml), V_v = volume of the liquid phase in the soil (ml), α = Bunsen coefficient for CO_2 = 0.758, m = dry weight of soil in the bottle.

For measurements performed at 22 ºC microbial biomass was calculated according to Anderson & Domsch (1978) where 1 ml CO_2 h^{-1} equals 40 mg microbial C.

Data analysis

Data of the microbial respiration at different CO_2 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 CO_2 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 (Pinheriro & 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 CO_2 concentrations (Kaligarič 2001, Vodnik & al. 2002, Maček & al. 2005, Pfanz & al. 2004, Pfanz & al. 2007). It has been shown that in the high CO_2 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 CO_2 exposed plants (Pfanz & al. 2004). All this should have an effect on microbial biomass and respiration.

As given in Fig. 1 glucose addition stimulated CO_2 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 CO_2 concentration. Different slopes of linear model lines indicate changes in microbial activities (Fig. 1). In 2003, SIR was significantly lower in high CO_2 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 CO_2 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 CO_2 soils, followed by medium and low (control) soils. In this year, significant difference was found between

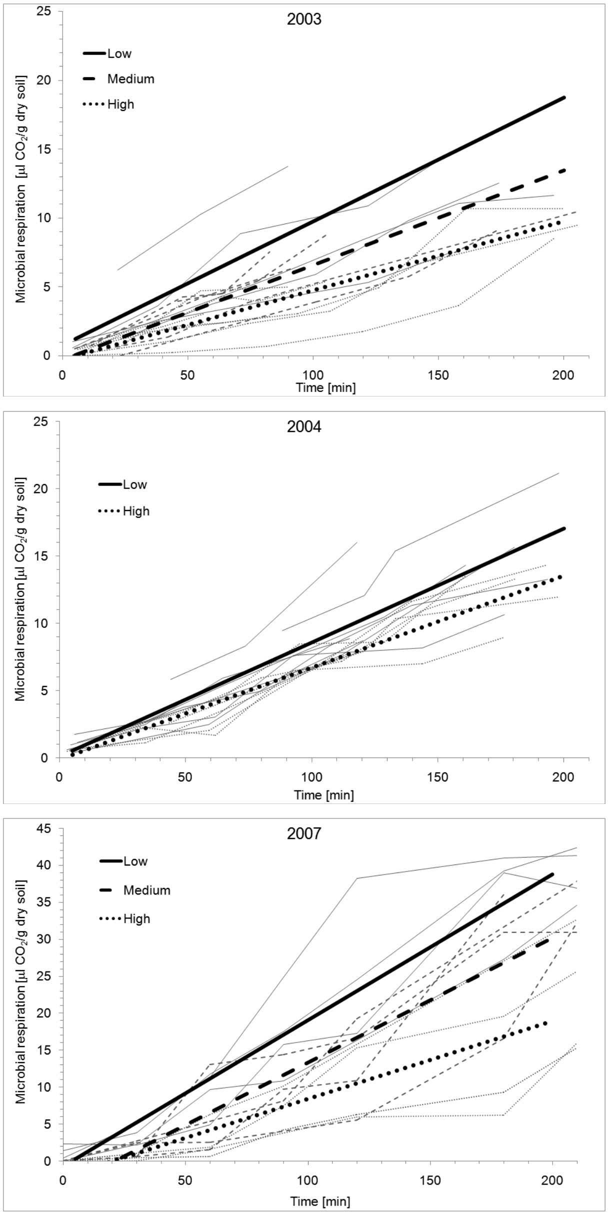

Fig. 1: Substrate induced microbial respiration (SIR), measurements of CO_2 production in soil samples from natural CO_2 springs in Stavešinci. Time course of microbial activity (respiration) after substrate addition measured on each sample (thin lines), linear model lines (thick lines); for low (full-lines), medium (dash-lines) and high (dot-lines) CO_2 concentrations.

Slika 1: S substratom inducirano mikrobno dihanje (SIR), meritve produkcije CO_2 v talnih vzorcih s 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.

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.

Year	**Parameter**		**95 % Confidence intervals**		
			Estimates	Lower limit	Upper limit
2007	Intercept	H	–0.2115	–0.4532	0.0303
		M	–0.3576	–1.0006	0.2854
		L	–0.0690	–0.6954	0.5574
	Slope	H	0.0106	0.0068	0.0143
		M	0.0169	0.0078	0.0260
		L	0.0197	0.0108	0.0287
2004	Intercept	H	–0.0094	–0.0732	0.0545
		L	0.0107	–0.1475	0.1689
	Slope	H	0.0068	0.0054	0.0082
		L	0.0085	0.0051	0.0118
2003	Intercept	H	–0.0271	–0.0880	0.0338
		M	–0.0283	–0.2035	0.1470
		L	0.0791	–0.0965	0.2547
	Slope	H	0.0050	0.0032	0.0068
		M	0.0069	0.0021	0.0116
		L	0.0090	0.0041	0.0138

high soil CO_2 and control ($p = 0.0009$) and also between high and medium soil CO_2 ($p = 0.0210$), but there was no difference between medium soil CO_2 and control. The estimated parameters of the linear mixed models with the 95 % confidence limits are presented in Tab. 2. Calculated microbial biomass is given in Tab. 3 (only for years 2003 and 2004). There is a clear increase in microbial biomass in both years with decreased geological CO_2 concentrations in the soil.

The effect of elevated atmospheric CO_2 on soil microbial respiration was reported before for the mofette areas in New Zealand (Ross & al. 2000), however, to the best of our knowledge no study reports on the effect of the extreme soil geological CO_2 enrichment on microbial biomass. Videmšek & al. (2009) have shown a shift in microbial community structure of CO_2-fixing bacteria in grassland soils from the Stavešinci mofette, depending on the soil CO_2 exposure. It has also been shown in the same mofette area that almost a complete turnover (β diversity) in community composition of symbiotic arbuscular mycorrizal fungi occurs, depending on soil abiotic factors (soil CO_2 exposure and hypoxia) (Maček & al. 2008).

For the Stavešinci mofette, SIR measurements and microbial biomass C estimation, clearly demonstrate higher microbial respiration and microbial biomass in control sites with low soil CO_2 concentration compared to high CO_2 samples (Fig. 1, Tab. 3). Differences between the years could be partially explained with the soil water content (Tab. 1). It is possible that due to higher

Table 3: Calculated microbial biomass.
Tabela 3: Ocenjena mikrobna biomasa.

	* Microbial biomass (µg g^{-1} dry soil)	
Year	2003	2004
High CO_2	115	162
Medium CO_2	159	no data
Low CO_2	231	205

* 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

Mikrobno dihanje in biomaso v talnih vzorcih lahko merimo z dodatkom lahko razgradljivega substrata npr. glukoze (s substratom inducirana respiracija – SIR). Respiratorni CO_2 merimo s plinsko kromatografijo. V naši raziskavi smo to metodo uporabili za oceno mikrobnega dihanja in mikrobne biomase v vzorcih z območja naravnih izvirov CO_2 (mofet) v Stavešincih (SV Slovenija), izpostavljenih različnim koncentracijam geološkega CO_2. Meritve kažejo na manjše dihanje in mikrobno biomaso v vzorcih, izpostavljenih veliki koncentraciji CO_2, v primerjavi s kontrolo. Z vzorčenjem na dveh različnih lokacijah znotraj območja vrelcev v Stavešincih in obenem v treh različnih letih (2003, 2004 in 2007) pa smo pokazali tudi dolgoročno stabilnost opaženega vzorca mikrobnega odziva, ki se je pojavil na obeh lokacijah in v vseh treh letih vzorčenja.

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