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A decade of research in mofette areas has given us new insights into adaptation of soil microorganisms to abiotic stress

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ABSTRACT

Natural CO₂ springs (mofettes) represent extreme ecosystems with severe exhalations of ambient temperature geological CO₂, inducing long-term soil hypoxia. In this paper an overview of mofette research in the fields of microbial ecology and biodiversity is presented, with a focus on the studies describing the impact of the changed soil gas regime on communities of arbuscular mycorrhizal fungi, archaea and bacteria. Along with the fast development of new, high-throughput molecular techniques driving the field of molecular ecology, mofettes enable new insights into the importance of the abiotic environmental factors in regulating soil biodiversity, and the community structure of these functionally important microbial groups.

Key words: natural CO₂ springs, hypoxia, abiotic environmental factors, carbon capture and storage – CCS, soil ecology, archaea, bacteria, Glomeromycota

IZVLEČEK

DESETLETJE RAZISKAV NA OBMOČJIH MOFET NAM JE OMOGOČILO NOVE VPOGLEDE V ADAPTACIJO MIKROORGANIZMOV NA ABIOTSKI STRES

Naravni izviri CO₂ ali mofete predstavljajo ekstremen ekosistem, kjer zaradi izhajanja geološkega plina v tleh prihaja do dolgoročnega pojava hipoksije. V preglednem članku so predstavljene raziskave z območij mofet s področja mikrobne ekologije, ki opisujejo vplive spremenjenih koncentracij talnih plinov na združbe arbuskularnih mikoriznih gliv, arhej in bakterij. Skupaj s hitrim razvojem novih molekularskih pristopov, predvsem novih generacij visokozmogljivega paralelnega sekvenciranja, ki poganjajo področje molekularne ekologije, mofete omogočajo raziskovanje vpliva abiotskih dejavnikov okolja na biodiverzitetu in strukturo združb teh funkcionalno pomembnih skupin talnih mikrobov.

Ključne besede: naravni izviri CO₂, hipoksija, abiotski dejavniki okolja, zajemanje in skladiščenje CO₂, ekologija tal, arheje, bakterije, Glomeromycota

1 INTRODUCTION

Natural CO₂ springs, or mofettes, are extreme ecosystems where ambient temperature geological CO₂ reaches the surface, resulting in a severe and relatively constant change in concentrations of soil gases. CO₂ vents are present in areas with tectonic activities in many locations worldwide (Pfanzen et al., 2004), while in Slovenia they are in the north-eastern part of the country close to Gornja

Radgona. Several CO₂ vents in this area represent Stavešinci mofette system where a soil gas regime has been well described, both spatially and temporally (Vodnik et al., 2006, 2009). In addition, also other soil parameters (e.g. soil chemistry, soil water content) (Vodnik et al., 2006, Vodnik et al., 2009) and plant eco-physiological responses have been well characterized in more than ten scientific

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papers (e.g. Kaligarič, 2001, Vodnik et al., 2002a, 2002b, Pfanž et al., 2004, Maček et al., 2005, Pfanž et al., 2007). An important but often neglected feature in practically all mofette sites is the CO₂ induced soil hypoxia (reduced O₂ concentration) that affects all the present biota in this ecosystem (Maček et al., 2005, Maček et al., 2011, Šibanc et al., under review). Hypoxia is a common but usually transient abiotic stress factor that is also present in many other terrestrial ecosystems, e.g. flooded or compacted soils (Perata et al., 2011). Mofette systems, however, enable new insights into microbial responses and adaptations to long-term changes in the soil abiotic

environment. This represents a new research direction, driven by the rapid development of the new molecular tools progressively used in research of molecular and microbial ecology.

In this paper we present an overview of the mofette research performed over the last decade with a focus on the studies describing the impact of the changed soil gas regime on soil microorganisms, their communities, and biodiversity. This includes several groups of organisms, focusing mainly on the arbuscular mycorrhizal fungi, bacteria and archaea.



Figure 1: A meadow within the Stavešinci mofette area (NE Slovenia) where different groups of soil microorganisms (fungi, bacteria and archaea) have been studied. A decreased growth of vegetation can be seen in the centre of the CO₂ vents (in front, left side) with the highest concentrations of geological CO₂ in the soil.

Slika 1: Travišče znotraj območja mofet v Stavešincih (SV Slovenija), kjer so potekale obstoječe raziskave različnih skupin talnih mikroorganizmov (gliv, bakterij in arhej). V središču vrelcev CO₂ (levo spredaj) je vidna slabša rast vegetacije na mestih, kjer je izpostavitve geološkemu CO₂ v tleh največja.

2 WHY MOFETTE RESEARCH MATTERS?

In the beginning of the 1990s the first reports about the possibilities of using mofettes in environmental and biological studies were published using Italian CO₂ springs (e.g. Miglietta et al., 1993, Raschi et al., 1997). Following the initial use – primarily for research of the vegetation and plant above-ground responses to elevated, atmospheric CO₂ concentrations in the range of those predicted by climate change models (e.g. Raschi et al., 1997) –

a second feature, the importance of high soil CO₂ concentrations and CO₂ induced hypoxia in mofette soils and its impact on soil biota, was observed (Maček et al., 2005). Mofettes were consistently shown to be very specific ecosystems with extremely high concentrations of CO₂ present in the soil air and high CO₂ efflux from soil surface (Vodnik et al., 2006, 2009). This is also one of the reasons why, in the last few years, the focus of

mofette research has shifted to the use of different mofette sites as model ecosystems for studies of plant and soil microbial responses to potential CO₂ leakage from underground carbon capture and storage (CCS) systems (Lal, 2008, Krüger et al., 2011, Noble et al., 2012, Frerichs et al., 2013). CCS is the process of capturing CO₂ from large point sources and depositing it underground. It is proposed as one of the possible measures for storing waste CO₂. Thus, in the 20 years of mofette research, the focus of the studies in different fields of applied sciences has moved from the initial studies of plant ecophysiological responses to elevated CO₂ in the atmosphere as a long-term natural analogue to other above ground fumigation systems (e.g. FACE – Free Air Carbon dioxide Enrichment experiments), to measuring plant (Maček et al., 2005) and microbial responses (e.g. Maček et al., 2009, Videmšek et al., 2009, Krüger et al., 2011, Maček et al., 2011, Frerichs et al., 2013, Šibanc et al., under review) to high soil CO₂ concentrations and CO₂ induced hypoxia. Only recently, the first reports on soil fauna responses to CO₂ induced soil hypoxia were also published, with a description of the new Collembola species, specific for mofette sites (Russell et al., 2011).

Geological CO₂ in mofette areas induces changes in several abiotic soil factors, including acidification (Jamnik, 2005), higher concentrations of nutrients due to reduced mineralization rates (Maček et al., 2009), and hypoxia. The latter has been consistently shown as a major abiotic factor affecting soil microbes (Maček et al. 2009, Maček et al., 2011, 2013, Šibanc et al., 2012, Šibanc et al., under review). Hypoxia is not only limited to mofette sites, but is a wider phenomenon and a common transient property of soils that often

appears in waterlogged and flooded areas or due to soil compaction. In a special issue of *New Phytologist* (*New Phytologist* 190, 2011) on ‘Plant anaerobiosis’ several mechanisms involved in plant response to flooding stress, the effects floods may have on patterns of plant distribution and biodiversity, and the devastating impact on crop growth are described (Perata et al., 2011). Interestingly, no reports on the response of plant symbiotic arbuscular mycorrhizal fungi or any other rhizosphere organisms to hypoxia were considered in this issue, though rhizosphere organisms represent an important ecosystem component affecting plant performance in practically all natural environments.

This however indicates a general rule, since reports on soil hypoxia impacts on rhizosphere and soil biota are scarce, inconsistent and often neglected. Thus, since the first rhizosphere study conducted within the Stavešinci mofette field, focusing on the research of high CO₂ concentrations and hypoxia on root respiration (Maček et al., 2005), hypoxia was chosen as the our stress of choice for further investigations: it is present in many natural ecosystems (Perata et al., 2011) and in addition, mofettes provide an unique example of plant and soil communities subject to well characterized (Vodnik et al. 2006, 2009), localized, long-term selection pressure (Maček et al., 2011). This represents a relatively rare opportunity for research of the different aspects of soil ecology and the driving forces of soil diversity in natural ecosystems, and therefore sheds some light on an important research issue that needs immediate attention in order to better understand soil biodiversity and its ecological functions.

3 MOFETTE RESEARCH INTO SOIL MICROBIAL DIVERSITY

There is a limited understanding of the importance of abiotic factors in regulating biodiversity and structure of many functionally important microbial communities in soil. Understanding the significance of the soil biota and the feedback between above- and belowground communities may be critical for designing sustainable production systems in the future, and for using the ecosystem services they can provide effectively (Gianinazzi et al., 2010, Mace et al., 2012). Soils

represent a dynamic and complex system that requires intense, complex, and logistically difficult sampling strategies in order to get sufficient information that lead to solid conclusions on the biodiversity and the ecological drivers of this diversity. In the last few decades the development of the DNA- and RNA-based methods has increased our knowledge on soil microbial diversity and function with a big boost because of recent development in the high-throughput

sequencing methods (e.g. massively parallel pyrosequencing) (e.g. Schloss, 2009, Lemos et al., 2011). Thus, the fast development of the new molecular methods, especially in the fields of metagenetics, metagenomics and

metatranscriptomics, now give us a much better tool to study microbial diversity and its functions in practically all environments, including soils and extreme ecosystems like mofettes (Table 1).

Table 1: A list of studies on the different aspects of microbial biology and diversity in mofette soils.

Preglednica 1: Seznam študij s področja raziskav mikrobiologije in biodiverzitete talnih mikroorganizmov na območjih mofet.

Microbial group	Gene region and/or methodology used	Mofette location	Study
AM fungi	16S rRNA gene, T-RFLP, pyrosequencing (Roche 454 FLX), clone libraries	Stavešinci, SI, Bossoleto, IT, Cheb basin, CZ	Maček et al. (2013), Šibanc et al. (2013)
AM fungi	Plant root colonization, soil glomalin concentration	Stavešinci, SI	Maček et al. (2012)
AM fungi	16S rRNA gene, RFLP, clone libraries, plant root colonization	Stavešinci, SI	Maček et al. (2011)
Soil yeasts	26S rRNA D1/D2 domain, sequencing, isolation and culture techniques	Stavešinci, SI	Šibanc et al. (2012)
AM fungi	Plant root colonization, soil glomalin concentration	Hakanoa, New Zealand	Rillig et al. (2000)
Soil microbes	Cell number (qPCR) and activity measurements, <i>nirK</i> genes DGGE fingerprinting	Larcher See, DE	Krüger et al. (2009, 2011)
Soil archaea and bacteria	16S rRNA gene, DGGE, activity measurements	Larcher See, DE	Frerichs et al. (2013)
Soil archaea and bacteria	16S rRNA gene, T-RFLP, clone libraries	Stavešinci, SI	Šibanc et al. (under review)
CO ₂ -fixing bacteria	<i>cbbI</i> genes, RFLP	Stavešinci, SI	Videmšek et al. (2009)
Soil microbes	Substrate induced respiration (SIR)	Stavešinci, SI	Maček et al. (2009)
Soil microbes	Lipid biomarkers and ¹³ C analyses, cell numbers (qPCR), biomass, and activity measurements	Latera Caldera, IT	Beaubien et al. (2008), Oppermann et al. (2010)
Soil microbes	16S-23S spacer region, ITS region, Automated Ribosomal Intergenic Spacer Analysis (ARISA), qPCR, PLFA, enzyme analyses	Mammoth Mountain, U.S.A.	McFarland et al. (2013)

3.1 CASE STUDY 1 – ARBUSCULAR MYCORRHIZAL FUNGI

In terrestrial ecosystems, symbiotic associations between plant roots and mycorrhizal fungi are near ubiquitous, with 90 % of all plant species forming mycorrhizas (Smith and Read, 2008). The vast majority of all terrestrial plants receive inorganic nutrients indirectly from symbiotic associations with arbuscular mycorrhizal (AM) fungi (ph. Glomeromycota) (Fig. 2), via efficient exploration of the soil by fungal hyphae, and not by a direct uptake from the soil by plant roots (Smith and Read, 2008, Hodge et al., 2010). In exchange, the plants supply up to 20 % of photosynthates to the fungi as the only energy source of the fungus (ca five billion tonnes carbon per year) (Bago et al., 2000). The nutrient exchange within plant root cells mainly takes place at the fungus-plant symbiotic interface formed around the finely branched fungal arbuscules (Parniske, 2008). Yet, despite its ecological importance, astonishingly little is known about their ecological and physiological responses to hypoxia (Maček et al., 2011).

AM fungi are a functionally important microbial group with poorly understood community ecology (Helgason and Fitter, 2009). Different studies suggest that where an extreme environmental stress occurs in soils, there are a small number of AM fungal lineages that are better able to tolerate those conditions, which results in unique, adapted populations (Helgason and Fitter, 2009, Dumbrell et al., 2010, Maček et al., 2011). AM fungi form an extensive mycelial network in soil and therefore will be subject to strong selection pressures from the abiotic soil environment (e.g. Dumbrell et al., 2010, Maček et al., 2011). However, reports on molecular community analyses and diversity studies of AM fungi in extreme ecosystems are still very scarce (e.g. Appoloni et al., 2008, Maček et al., 2011). In the last 15 years several molecular techniques have been developed, typically

targeting different regions of ribosomal rRNA genes that allow identification of the fungal endophytes within roots and soil (e.g. Helgason et al., 1998, Dumbrell et al., 2011). Only recently, some reports on using high-throughput sequencing techniques on the characterization of natural AM fungal communities were published (Öpik et al., 2009, Dumbrell et al., 2011). The newly developed methodology now allows us sufficient sampling intensity within different habitats to answer numerous ecological questions about this important group of soil fungi. However, to the best of our knowledge – apart from our research on mofettes (e.g. Maček et al. 2011, 2013) – there are no other studies on the direct effect of soil hypoxia on AM fungal communities (Table 1). Within the Stavešinci mofette area, studies on AM fungal root colonization (Maček et al., 2011, 2012), the concentration of glomalin-related soil protein, produced by AM fungi (Maček et al., 2012) and the structure of AM fungal communities (Maček et al., 2011, Maček et al., 2013, Šibanc et al., 2013) were conducted, investigating CO₂/hypoxia related responses of this fungal group. Maček et al. (2011) report on significant levels of AM fungal community turnover (beta diversity) between soil types and the numerical dominance of specific AM fungal taxa in hypoxic soils. This work strongly suggests that direct environmental selection acting on AM fungi is a major factor regulating AM fungal communities and their phylogeographic patterns. Consequently, some AM fungi are more strongly associated with local variations in the soil environment than with their host plant's distribution (Maček et al., 2011). There are more reports to follow this initial study of AM fungi in mofette areas, including the ones involving high-throughput sequencing techniques (Roche 454 FLX) (Maček et al., 2013, Šibanc et al., 2013), thus allowing more intensive sampling and more detailed analyses of the mofette AM fungal communities.

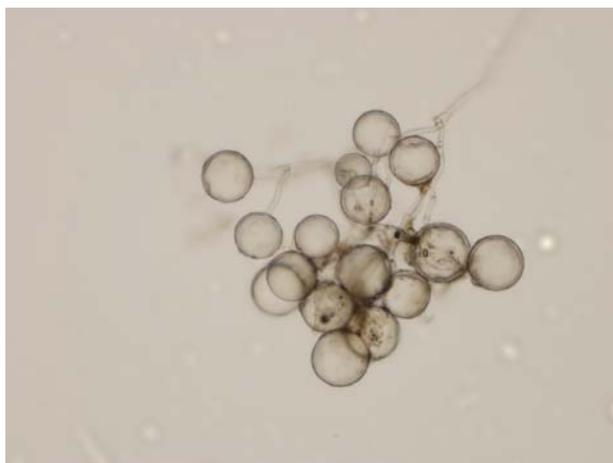


Figure 2: A cluster of AM fungal spores in a sporocarp, isolated from a glasshouse pot culture initiated with AM fungal inoculum from a location exposed to high geological CO₂ concentration (>60 % CO₂) within a Stavešinci mofette area. The isolate represents a potentially new AM fungal species. Photos taken by Olympus Provis AX70 microscope and digital camera.

Slika 2: Grozd spor AM gliv znotraj glivnega sporokarpa. Spore so bile izolirane iz lončne kulture iz rastlinjaka, inokulirane z vzorcem tal z AM glivami, vzorčenim iz območja velike koncentracije geološkega CO₂ znotraj območja mofet v Stavešincih. Izolat predstavlja potencialno še neopisano vrsto AM gliv. Posneto z mikroskopom Olympus Provis AX70 in digitalno kamero.

3.2 CASE STUDY 2 – SOIL ARCHAEA, BACTERIA and FUNGI

Soil is the most biologically diverse environment on Earth, with a biodiversity which can often be several orders of magnitude greater than that present aboveground (Heywood, 1995). A large portion of this diversity involves the greatly unknown diversity of different prokaryotic organisms, bacteria, and archaea. Up to now only a few studies of soil microorganisms from mofette areas were conducted (Table 1). In the Slovenian Stavešinci mofette soils Videmšek et al. (2009) examined the abundance and diversity of *cbhL* genes, encoding for the large subunit of RubisCO in CO₂-fixing bacteria. In this same area Maček et al. (2009) reported on reduced levels of substrate induced respiration (SIR), indicating reduced microbial biomass and activity in high geological CO₂ exposed soil. However, apart from the Slovenian Stavešinci mofette, at least two other mofette areas in Europe and one in U.S.A. have been involved in studies of microbial responses to geological CO₂ exhalations.

First, a terrestrial CO₂ vent located at the Laacher See, Germany was used by the group of Krüger et al. (2009, 2011) as a model ecosystem for investigating the impact of potential leakage from

carbon capture and storage systems (CCS) on the surrounding environment. They reported on lower bacterial cell numbers, higher levels of bacterial non-isoprenoidal tetraethers lipids (most likely derived from anaerobic bacteria), and higher archaeal cell numbers at the vent compared to the control site. The investigation of archaeal and bacterial communities, based on potential sulphate reduction rates, methane production, and a lipid biomarkers study, showed a shift towards anaerobic and acidophilic species in high CO₂ sites. Moreover, recently a study employing molecular markers (community fingerprinting technique – denaturing gradient gel electrophoresis – DGGE) was used to identify the shifts in the communities of archaea and bacteria among geological CO₂ impacted and control soil samples in the mofette field near Laacher See (Frerichs et al., 2013). The study of the abundance of several functional and group-specific gene markers revealed differences in the composition of the mofette soil microbial communities, for example a decrease of Geobacteraceae and an increase in sulphate-reducing taxa in the vent core, reaching moderately elevated (up to 30%) soil CO₂ concentrations.

Second, within the Latera Caldera mofette in the volcanic district in Central Italy, Beaubien et al. (2008) reported on decreasing trends in adenosine triphosphate (ATP) biomass, bacterial cell counts, and the higher activity of strictly anaerobic, sulphate-reducing bacteria and methanogenic archaea in the centre of the CO₂ vent compared to the transit zone and background, while H₂ dependant methanogenesis was absent and aerobic methane oxidation was negatively correlated with increased CO₂. In addition to this study in the same mofette area, Oppermann et al. (2010) found CO₂-utilising methanogenic archaea, Geobacteraceae, and sulphate-reducing bacteria mainly at the CO₂ vent, only minor quantities were found at the reference site. Also, their results suggest a shift in the microbial community towards anaerobic and acidophilic microorganisms as a consequence of the long-term exposure of the soil environment to high geological CO₂ concentrations.

A very recent report comes from the Mammoth Mountain, a dormant volcano from eastern

California (U.S.A.), and an area known for geological CO₂ induced tree mortality (McFarland et al., 2013). The authors of the study assessed the soil microbial community response to CO₂ disturbance that resulted in localised tree kill. As a result to reduced soil carbon availability soil microbial biomass decreased, which was linked to the loss of soil fungi. In contrast, archaeal populations responded positively to the CO₂ disturbance, presumably due to reduced competition of bacteria and fungi.

To our knowledge, however, there is no published data on the overall community structure or diversity of bacteria, and archaea in mofette areas based on clone libraries, and especially so in the most extreme locations (with the soil CO₂ concentrations well above 60 %). In these sites, however, CO₂ induced hypoxia could strongly affect microbial communities (Šibanc et al., under review).

4 CONCLUSIONS

All these studies are important not only for their use in the research of impacts of elevated atmospheric CO₂ concentrations on plants and possible leakage of CO₂ in CCS systems and related impacts on biota, but also from a biotechnological and ecological perspective. Extreme environments have previously served as a rich source of potentially useful organisms in different fields of applied biotechnology and agronomy (e.g. new antibiotics discovery, isolates in commercial inoculums of AM fungi). Little is known about what kind of organisms actually live in these habitats and even less about their

ecological function. Moreover, as major shifts in microbial community composition have significant implications for ecosystem functioning (e.g. changes in carbon cycling driven by changes in methanogenic archaea populations), understanding their response to long-term environmental changes is of crucial ecological importance. Thus, a full phylogenetic characterisation of fungal, archaeal, and bacterial communities, their taxonomy, and an investigation into the processes regulating their diversity and community structure has yet to be reported (Maček et al., 2013, Šibanc et al., 2012, 2013, Šibanc et al., under review).

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