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Resilience of (seed bank) aerobic methanotrophs and methanotrophic activity to desiccation and heat stress

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ABSTRACT

In seasonally changing environments, aerobic methanotrophs are exposed to elevated temperatures and drought. Prior exposure to adverse conditions (site history) may leave an imprint on the methanotrophic community composition in the form of a seed bank. While the significance of a microbial seed bank is established, the potential of this seemingly preserved community following emergence from inactive states and its sensitivity to adverse conditions are still lacking. We used a paddy soil representing an environment experiencing recurring desiccation and heat stress as per agricultural practice, and two lake sediments with sporadic/limited or no exposure to desiccation and heat stress as model systems. In a microcosm study, we induced drought combined with a heat treatment by air-drying the samples at ambient (25 °C) and elevated (75 °C) temperatures, designated as mild and severe stress, respectively. Fresh soil/sediment were used as reference. Upon rewetting, we followed the recovery of the methane uptake rate, and the population dynamics was monitored using qPCR assays and a diagnostic microarray analysis. Remarkably, methane uptake rates were not adversely affected even after severe stress, and activity recovered to levels comparable to the fresh soil/sediment incubations after 40 days. In particular, the alphaproteobacterial methanotrophs (*Methylosinus-Methylocystis* group) exponentially increased in population size upon rewetting. Interestingly, the qPCR and microarray analyses revealed that some gammaproteobacterial methanotrophs (e.g. *Methylocaldum*- and *Methylosarcina*-related methanotrophs) increased in relative abundance after the desiccation and heat stress, indicating the unexpected resistance of this subgroup to the stress treatment. Although the initial and recovering communities were significantly different, population abundance recovered over time. The shift in the trajectory of the recovering communities suggests that repeated exposure to adverse factors will change the seed bank's composition. Overall, the indigenous (seed bank) methanotroph populations showed remarkable recovery from the induced stress.

1. Introduction

Microorganisms require water to sustain cell activity. However, some microorganisms are able to withstand prolonged desiccation by reverting to a reversible resting state (i.e. dormancy) at the onset of drought, contributing to the microbial seed bank. Dormancy enables cells to persist for extended periods of desiccation, resource scarcity, and in the face of other environmental stressors, and likely, to confer protection against predation (Murase and Frenzel, 2008; Lennon and Jones, 2011; Murase et al., 2011). Hence, dormant cells can be present in overwhelming numbers, even exceeding the abundance of active cells, particularly in the soil environment (Lennon and Jones, 2011). From the dormant reservoir, physiologically inert microorganisms can become metabolically active and grow. It has been suggested that among the methanotrophs, the seed bank population become relevant during the recovery from disturbances and fluctuating environmental conditions (Ho et al., 2011, 2013a; Krause et al., 2012). Hence, the microbial seed bank has potentially an important role in ecosystem functioning by determining future community dynamics and activity, and shaping the microbial composition in an ever changing environment.

Elevated temperatures above ambient levels and drought events (water stress) induce physiological responses in soil microbial

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communities. While an increase in temperature alone may induce growth of microorganisms, a drought event coupled to temperature rise adversely affects microbial abundance, causing a significant decrease in major soil microbial phyla (Sheik et al., 2011). Hence, a drought event constraints the microbial population size besides shifting the community composition (Sheik et al., 2011; Angel and Conrad, 2013). Numerous studies determined general processes (e.g. microbial respiration) or shifts in broad microbial phyla containing physiologically distinct species as response variables to drought and/or temperature rise (Schimel et al., 1999; Luo et al., 2001; Sheik et al., 2011; Evan and Wallenstein, 2012). Here, we target the response of a unique group of microorganisms catalyzing a well-defined process (methane oxidation). In contrast to processes catalyzed by a broad group of microorganisms, aerobic methane oxidation is restricted to microorganisms belonging to a few narrow phylogenetic group within *Proteobacteria* and *Verrucomicrobia*. The effect of disturbances on microbial community composition and activity was more effectively captured by targeting microorganisms catalyzing distinct processes as shown before for the aerobic methane-oxidizers (Levine et al., 2011; Bodelier et al., 2013) and ammonia-oxidizers (Placella and Firestone, 2013).

Canonical aerobic proteobacterial methanotrophs belong to the *Gammaproteobacteria* (*Methylococcaccea* and *Methylothermaceae*, provisionally grouped into type Ia/Ib), and *Alphaproteobacteria* (*Methylocystaceae* and *Beijerinckiaceae*, provisionally grouped into type II). Studies on pure cultures have shown that representatives of these two phylogenetically and biochemically distinct subgroups form different resting stages with those of *Methylocystaceae* (*Methylocystis* and *Methylosinus*) being more resistant to desiccation and heat stress (Whittenbury et al., 1970; Semrau et al., 2010). Based on their ecological characteristics, gammaproteobacterial and alphaproteobacterial methanotrophs are thought to adopt different life strategies (Ho et al., 2013a), and their global distribution can be habitat-specific (Knief, 2015). The *pmoA* encodes for the beta-subunit of the particulate methane monooxygenase, the key enzyme present in essentially all methanotrophs; *Methylocella* and *Methyloferula* possess only the soluble form of the methane monooxygenase (Dedysh et al., 2000; Vorobev et al., 2011). Hence, the *pmoA* gene captures a wide inventory of the aerobic methanotrophs. This, together with the congruency of the 16S rRNA and *pmoA* gene phylogenies (Kolb et al., 2003; Lüke and Frenzel, 2011; Knief, 2015) makes the *pmoA* gene suitable for culture-independent studies.

Prior exposure to environmental conditions (site history) is thought to leave a legacy on the microbial community composition (Fierer et al., 2003; Evan and Wallenstein, 2012; Allison et al., 2013; Meisner et al., 2013) in the form of a seed bank (Eller et al., 2005; Lennon and Jones, 2011), which in turn, reflects the resilience of the contemporary community to future recurring conditions. Therefore, depending on site history, the (seed bank) methanotroph communities indigenous to different environments potentially show varying degrees of resistance and resilience to desiccation and heat stress. However, it is unclear (i) how the resting stages' resistance may translate into community shifts and population dynamics, and (ii) how representative pure cultures are for the diverse and uncultivated majority in soils and sediments in their response to desiccation and heat stress. Here, we aim to address these questions with respect to site history, on how past events may influence contemporary methanotrophic community response and activity. This is in contrast to our previous studies investigating the resilience of paddy soil methanotrophs to a long-term drought (Collet et al., 2015), recurring desiccation-rewetting events (Ho et al., 2016), and a heat shock (Ho and Frenzel, 2012). We hypothesize that prior exposure to drought will allow a methanotrophic community and activity to recover from the stress event more effectively, indicated by a less divergent microbial composition and resilience of activity during recovery following stress. Based on a seminal study (Whittenbury et al., 1970), we anticipate that the dormant methanotrophs are inducible by an exposure to desiccation and heat stress, and return to ambient temperature, after which they contribute to methane oxidation. To test our hypotheses, we exposed a paddy soil experiencing recurring desiccation and heat stress as per agriculture practice, and two lake sediments with sporadic/limited (Lake Neusiedl) or no exposure (Lake Constance) to desiccation and a heat treatment at ambient (25 °C for seven days) and elevated (75 °C for five days) temperatures, respectively designated as mild and severe stress. We tested the upper limits of heat-resistance (75 °C) to induce the formation of resting cells as shown before in Whittenbury et al. (1970). Methane uptake rates were monitored over 40 days following rewetting of sediments/soil. Recovery and population dynamics of the methanotrophic community were followed using group-specific quantitative PCR assays and a diagnostic microarray analysis targeting the *pmoA* gene.

2. Methodology

2.1. Soil and experimental set up

The paddy soil was sampled in May 2010 in a rice field at the CRA Agricultural Research Council, Rice Research Unit (Vercelli, Italy). The soil parameters and agricultural practice in the rice field have been described before (Krüger et al., 2001). The Lake Constance, Germany (N 47°43′16.92″; E 09°10′45.08″) and Lake Neusiedl, Austria (N 47°55′57.04″; E 16°45′23.83″) sediments were sampled in April and May 2010, respectively. Sediments were collected from the littoral of both lakes. Lake Constance is an alpine deep lake with seasonal water level fluctuations, and the sampling site is regarded as permanently inundated, with a recorded temperature of around 8 °C during the sampling period (Frenzel et al., 1990; Rahalkar et al., 2009; Zintz et al., 2009). Lake Neusiedl is a steppe shallow lake with an average depth of 1.1 m, with a recorded temperature range of 15 °C – 20 °C at the time of sampling (Mahringer, 1970; Herzig, 1995). Fluctuations in the water level and drying out events have been recorded in Lake Neusiedl. The most recent drying out event occurred around 150a ago (Mahringer, 1970). Hence, these sites represent environments with annually recurring (paddy soil), sporadic/limited (Lake Neusiedl sediment), and no exposure (Lake Constance sediment) to desiccation events.

The pH of the samples were within a narrow range (7.4–7.9). A portion of each sample was air-dried continuously in an oven at 25 °C (for seven days) and 75 °C (for five days), representing mild and severe stress, respectively. More than 94% gravimetric water loss was recorded already after 16 h of air-drying at 25 °C. Fresh and air-dried samples were filled in sterile Petri dishes. Incubation with un-treated (fresh) soil/sediments served as a reference. For the air-dried samples, autoclaved distilled water was added to bring the gravimetric water content to levels of the fresh samples (Lake Constance sediment: 53.3%; Lake Neusiedl sediment: 52.7%; Vercelli paddy soil: 17.3%) hence, the gravimetric water content for a sample in all incubations regardless of treatment was consistent. Gravimetric water loss was compensated for using autoclaved distilled water weekly. To ensure a standardized incubation condition, these microcosms were incubated in the dark in gas tight jars at 25 °C under an atmosphere of 10 vol% methane in air. As the samples originated from high methane-emitting environments (i.e. lake sediments and paddy soil; Frenzel et al., 1992; Shrestha et al., 2008), a higher methane concentration than ambient level was supplied during the incubation (Ho et al., 2011, 2015). The atmosphere in the gas tight jar was replenished every other day to avoid