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Short communication

Recovery of paddy soil methanotrophs from long term drought

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ABSTRACT

Air-dried paddy soils stored for 1–18 years were used to examine the resistance of methanotrophs to drought. Older air-dried soils representing longer-lasting drought events reduced methanotrophic diversity, and adversely affected methane oxidation rate after re-wetting. In early incubations the type II methanotrophs are outperformed by the less abundant type I.

Wetland rice paddies are characterized by alternating drainage and flooding cycles. Therefore, aerobic proteobacterial methanotrophs from rice paddies are exposed to fluctuating substrate availability through changing water levels (Ratering and Conrad, 1998; Conrad and Frenzel, 2002). Moreover, after drainage, methanotrophs face a desiccation stress. However, they are proven to form different resting stages (i.e. exospores, *Azotobacter*-type cysts, and lipoidal cysts), enabling them to persist for extended periods during unfavourable conditions (Whittenbury et al., 1970). Heat and desiccation resistant exospores are formed by *Methylosinus* species, which together with *Methylocystis*, are grouped as type II methanotrophs. *Methylocystis* may form desiccation resistant lipoidal cysts. *Azotobacter*-type cysts are formed by some type I methanotrophs (*Methylobacter*), and are resistant to desiccation (Whittenbury et al., 1970) while other type I (*Methylocaldum*, *Methylosarcina*, *Methylomonas*, *Methylococcus*) form cysts that are similar to those of *Methylobacter*, but are not as desiccation resistant (Whittenbury et al., 1970; Bodrossy et al., 1997; Wise et al., 2001). To induce germination, an exposure to a sufficiently high methane and oxygen concentration is necessary (Higgins et al., 1981; Rothfuss et al., 1997). Furthermore, a heat stress is thought to trigger the transformation from dormant to active states too (Whittenbury et al., 1970; Ho and Frenzel, 2012). Desiccation in rice paddies is a recurring event as part of rice agriculture practice which may affect the methanotrophic community and activity. Previously, studies show that disturbances led to reduced diversity and evenness in methanotrophic communities (Bodelier et al., 2000; Wertz et al., 2007). However, disturbed communities were still able to oxidize methane in similar ranges as in the undisturbed controls, and can even overcompensate for community changes caused by the disturbance (Ho et al., 2011).

In contrast to previous studies, we determined the recovery of the methanotroph diversity and the methanotrophic activity in a natural setting after a long-term drought spanning over two decades. Simulating drought, we used sieved (2 mm) air-dried paddy soils that were stored sealed in water-proof plastic containers for varying periods (Fig. 1). These soils were stored under ambient temperature. The recovery of methanotrophic activity and community composition of the rewetted soils were monitored using gas flux measurements and a diagnostic microarray, respectively, over 80 days. Due to the post-hoc nature of this experiment, no data on the initial community composition in the different soils is available. However, samples were taken in an area of 100 m radius in the lowlands of the rivers Po and Sesia (Vercelli, Italy). These paddy fields with a circumneutral pH are planted to wetland rice for at least one century (Lüke et al., 2010) and methanotrophic

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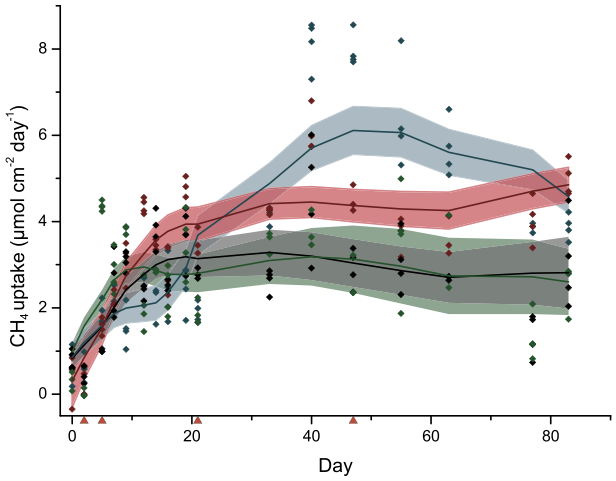

Fig. 1. Effect of the length of desiccation on methane uptake rates. Individual measurements and smoothed (Fast fourier transformation; n = 3) average with 95% confidence interval. Green, black, red and blue denote the soils from 1993, 1998, 2006 and 2010. The soil microcosms were prepared, incubated, and the flux measurement were performed as described previously (Ho et al., 2011). The red arrow heads indicate time points (2, 5, 21, and 47 days) where duplicate microcosms were sacrificed. Nucleic acid was extracted as described in detail before (Krause et al., 2010), and subjected to a *pmoA* specific diagnostic microarray analysis as described elsewhere with minor modifications (Bodrossy et al., 2003; Ho et al., 2011).

communities at this location did not show any large scale spatial pattern (Krause et al., 2009). Therefore, we assumed that the quantitative methanotroph community were largely similar at the different sampling times and were dominated by species possessing the particulate form of the methane monooxygenase as previously shown (Reim et al., 2012).

Experimental set up and methanotrophic activity was monitored as described in detail before (Ho et al., 2011). Methane uptake in all soils was similar until after 3 weeks incubation where the younger (stored since 2006, and 2010) and older (stored since 1993, and 1998) soils diverged (Fig. 1). Henceforth, methane uptake was significantly higher in the younger soils (two-sided KS-test, average days 19–83, $P \leq 0.05$). This may be a result of an increase in cell-specific activity (Steenbergh et al., 2010), but is more likely, growth in population size as shown before in the same incubation set up (Ho et al., 2011). Consistent with the methane uptake rates, we detected an increase in the hybridization signal intensity for the different methanotroph subgroups (mainly type Ib; Fig. 3). In particular, type II specific probes (Mcy413; McyM309; NMsiT-271) (Table 1, Fig. 3) showed relatively higher hybridisation signal intensity earlier (5 days) during the incubation, indicating their ability to form drought resistant spores/cysts which can take 7–15 days to germinate (Higgins et al., 1981). Later during the incubation (21–47 days), type II specific probes showed an overall increase in the hybridization signal intensity, suggesting of a growing community. This was further supported by the increase in the hybridization signal intensity of type II *pmoA* transcripts at 47 days (probe Mcy413; McyM309) (Fig. 3).

On the contrary, type I specific probes showed lower hybridisation signal intensity early (2 days) following incubation, suggesting that their resting stages were less resistant to drought. However, later on (5 days), type I methanotrophs represented by *Methylobacter* (probes Mb_SL#3-300; Mb380) and *Methylosarcina* (probe Mmb562) showed *pmoA* gene expression in all soils except the oldest, indicating the initiation of methane oxidation (Fig. 3). It is possible that the presence of *Methylobacter* after 5 days in the 1993 and 2006 soils were masked by the high abundance of *Methylosinus*

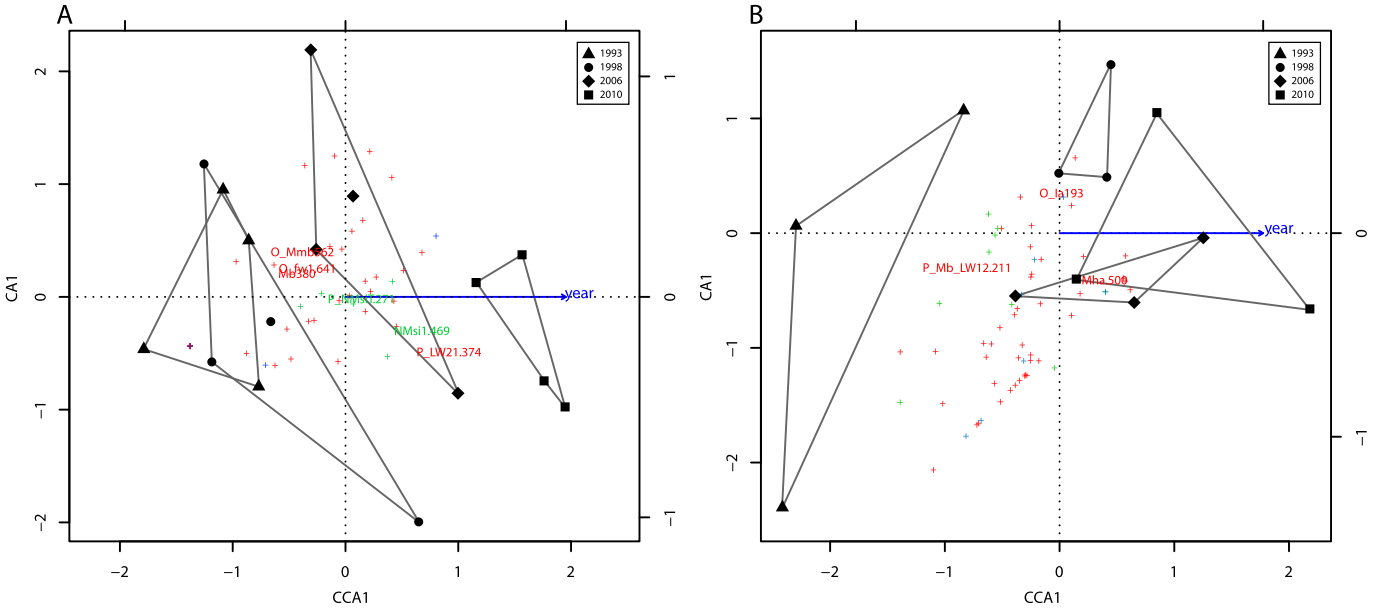

Fig. 2. Canonical correspondence analysis (CCA) of the methanotrophic community based on the microarray analysis of *pmoA* gene (A), and gene transcript (B). Probe selection was based on the subset defined by Krause et al. (Krause et al., 2012) corrected by the removal of probes redundant for the samples analysed and addition of highly indicative probes (Dufrene and Legendre, 1997). The samples (n = 4) are shown as black symbols and joined by the hulls visualizing the different soil ages as indicated by the year of collection (▲ 1993, ● 1998, ◆ 2006, ■ 2010). The differences between the different aged soils are significant on the community level ($P < 0.01$) while on transcript level only a tendency is shown ($P < 0.1$, analysis of similarity, Bray–Curtis dissimilarity). The CCA analysis is conditioned against the incubation time with soil age as constraint. The coloured crosses mark the different probes. Probes which are indicative for one of the soils with a high probability ($P < 0.05$) by indicator value analysis using the labdsv package in the R software environment are shown with their full name and discussed in the text. Red, green, and blue indicate the specificity of the probes for type I, type II, and other *pmoA* sequences, respectively. The relative hybridization signals for the probes used in the CCA are given in Fig. 3.