Re-inoculation of a soil-borne methanotrophic mixed culture: Testing the CH_4 mitigation potential in different soil types

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Background

- Soil-borne methane oxidizing micro-organisms act as a terrestrial methane (CH_4) sink and are potentially useful in decreasing global CH_4 emissions.
- Understanding the ecophysiology of methanotrophs is crucial for a thorough description of global carbon cycling and for the successful application of methanotrophic micro-organisms in CH_4 mitigation strategies.
- The aims of the study were (**a**) to test different soil sites for CH_4 oxidation performance at field and laboratory scales, (**b**) to isolate an effective methanotrophic mixed culture (MMC), (**c**) to describe the influence of different carbon sources on this MMC and (**d**) to evaluate if the MMC once re-inoculated to the soils can alter the CH_4 balance of soils.

Material and Methods

Here, we report the *in situ* balance of soils from abandoned landfills (**L**), meadows (**M**) and wetlands (**W**) (all located in Tyrol/Austria), their capacities to produce and oxidize CH_4 at *laboratory-scale* and the isolation of a soil-borne methanotrophic-heterotrophic mixed culture (MMC) that was used for carbon (C_1 and C_2) feeding experiments and re-inoculation trials. CH_4 flux rates were determined employing a static chamber technique (Hofmann *et al*. 2016). For potential methane oxidation (PMO) analysis, soil was weighed into flasks that were closed, gassed with 1% (v/v) CH_4 and 2.5% (v/v) CO_2 and incubated at 25°C for 24 h. For isolation of a MMC, soil samples were inoculated to liquid dilute nitrate mineral salts (DNMS) medium (Dedysh and Dunfield 2017). Growth and PMO of the MMC in response to different carbon sources (C_1 and C_2) were studied. MMC was also re-inoculated into all soils and success was evaluated by PMO measurements. DNA from cultures and soil samples was extracted by using the NucleoSpin Soil Kit (Macherey-Nagel, Germany). Microbial abundances (total bacteria, type I and type II MOB) were quantified by droplet-digital PCR (ddPCR), following the protocol provided in Praeg *et al.* (2021), and 16S amplicon sequencing was performed to characterize the methanotrophic and accompanying microorganisms.

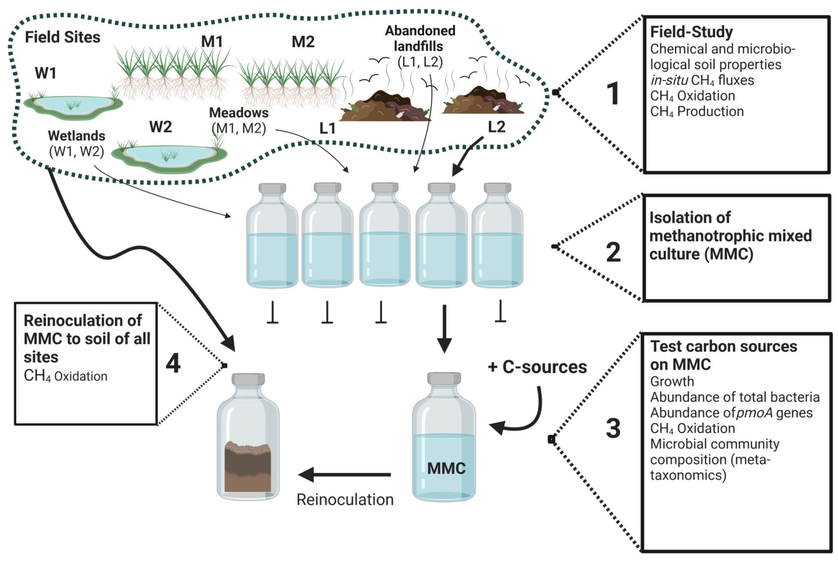

Fig. 1: Schematic representation of the experimental set-up: field-related investigations (**1**), the isolation procedure for gaining a methanotrophic-heterotrophic mixed culture (MMC) (**2**), testing of different carbon sources (**3**) and re-inoculation of the MMC to all soils (**4**).

Results & Discussion

❖*In-situ* gas measurements showed net CH_4 balances of +/- 0 in the meadows and landfills, whereas the **wetlands** had clearly positive CH_4 balances, ranging from +5.0 to +100 µmol CH_4 m^{-2} h^{-1}.

❖MMCs were isolated from the soils (**Fig.2B**) (from all sites) - one derived from a **landfill** led to the **strongest and most stable mixed culture** and was used for all further experiments.

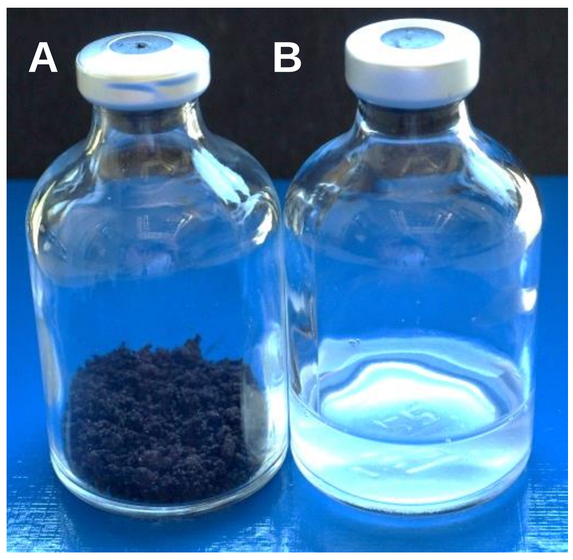

Fig. 2: (**A**) Determination of the potential methane oxidation capacity (PMO) of the soils and (**B**) cultivation of a stable methanotrophic (mixed) culture.

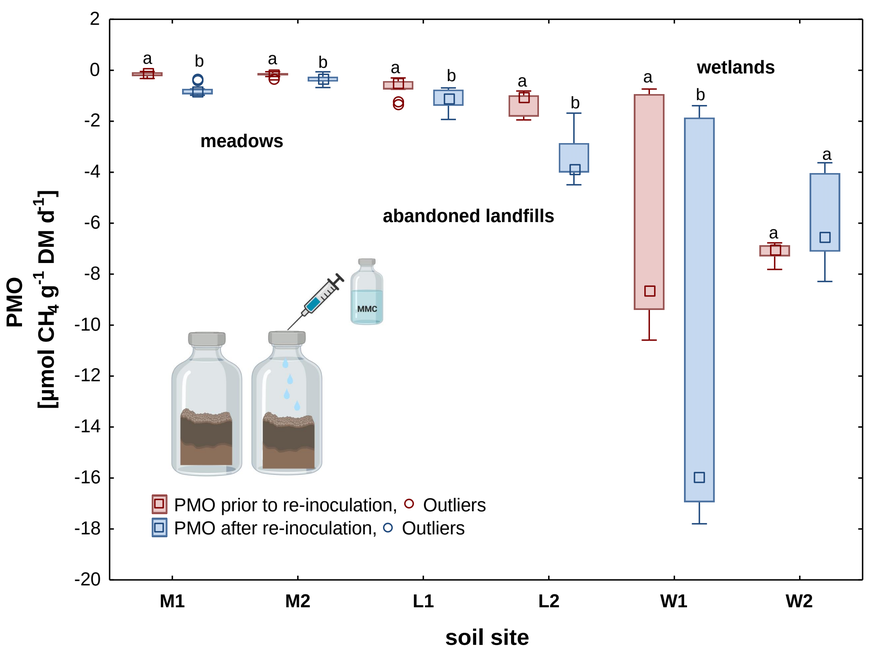

Fig. 3: Comparison of potential CH_4 oxidation (PMO) capacity (µmol g^{-1} DM d^{-1}) after 24 h, before and after re-inoculation with a methanotrophic mixed culture (MMC) (n=9). The letters given refer to significant differences before (prior to) and after re-inoculation per site ($p<0.05$, independent *t*-test). M1, M2, meadows; L1, L2, abandoned landfills; W1, W2, wetlands. DM, dry matter.

❖Inoculation of soils with the MMC distinctly and soil-specifically enforced the PMO, except for the wetland W2 (**Fig.3**).

❖The relative **increase** in PMO was **most pronounced** in **landfill** L2 and reached a 3-times higher CH_4 oxidation capacity (**Fig.3**).

❖Besides the methanotrophic community, consisting of methanotrophs of **type I and II**, also a diverse **heterotrophic community** was present within the MMC (**Fig.4**).

❖Applied **carbon sources** significantly influenced both heterotrophic and methanotrophic members of the MMC and distinctly **changed the community composition** and the abundance of MOB (**Fig.4** and **5**).

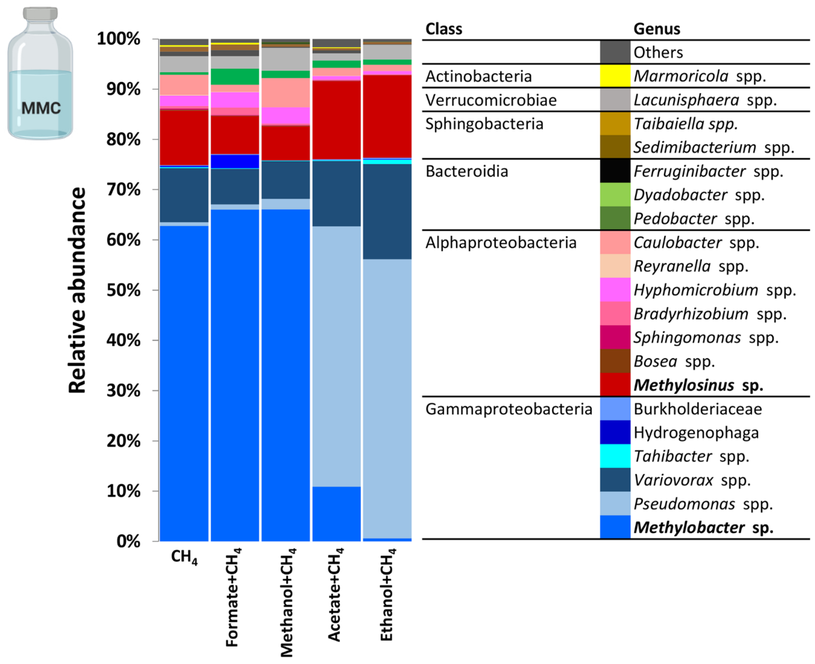

Fig. 4: Effects of different C sources in addition to CH_4 on the microbial community composition (relative abundances of microbial taxa) presented at the highest resolvable tax level. Taxa with a relative abundance <2% are summarized as 'Others'. Bars represent means (n=3).

❖All substrate additions caused increases of total bacteria compared to the controls (CH_4 supply only), with significant increases in the case of methanol and ethanol amendments (**Fig.5**).

❖While formate and methanol (**C_1 supply**) led to significant increases in MOB type I, the **C_2 sources** (acetate and ethanol) significantly decreased the abundance of type I. In contrast, type II methanotrophs were increased by the addition of C_2 sources (**Fig.4** and **5**).

❖Studying mixed cultures carries the risk of working with an undifferentiated culture but offers the advantage of promoting near-natural conditions compared with pure cultures and allows feedbacks between heterotrophic and methanotrophic microorganisms.

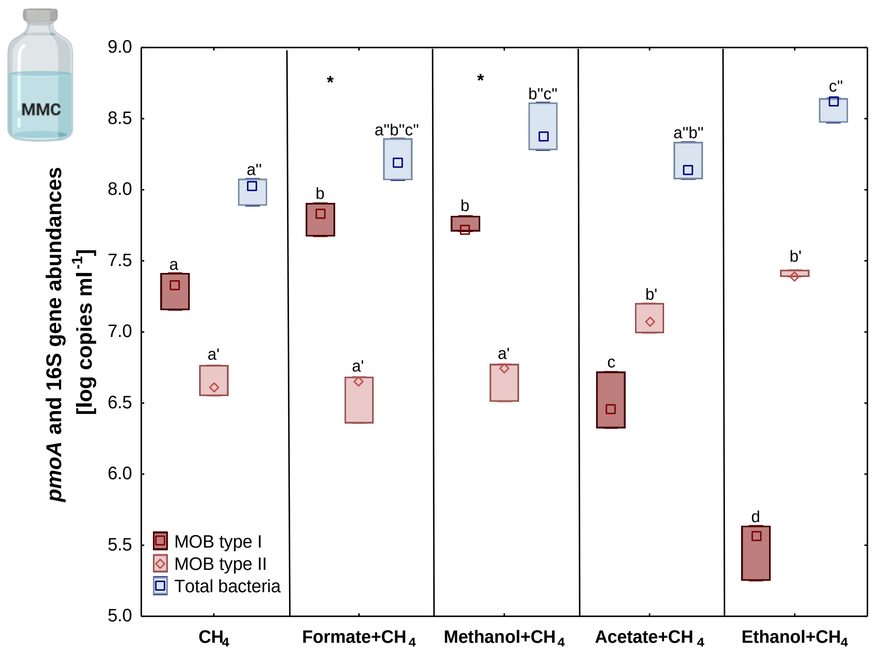

Fig. 5: Effects of different C sources in addition to CH_4 on abundances of methane oxidizing bacteria (MOB) and total bacteria (all n=3). Data are shown in log copies ml^{-1} culture suspension, small squares show median values, and boxes indicate 25-75%. Small letters (e.g.: a, a', a'') indicate significant differences between MOB type I, MOB type II, and total bacteria, respectively ($p<0.05$, Bonferroni post-hoc test), and asterisks (*) show differences between the sum of MOB compared to the control (CH_4) approach ($p<0.05$, independent *t*-test).

Conclusion & Outlook

- Working toward natural and soil-borne methanotrophic mixed consortia provides promising grounds for future mitigation of CH_4 emissions from soils.
- The success of re-inoculating the mixed-methanotrophic culture (MMC) to soils varied depending on soil type. However, our results showed that a stable MMC has the potential to positively influence the CH_4 balance of soils.
- The re-inoculation stood its test under laboratory conditions and was a first step toward field trials. Further investigations will be necessary to prove its suitability and efficiency *in situ* and for long-term application.

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References

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