

Dear Editor,

Thank you for your advises on Major Revisions, we provided all the answers to your questions and to your suggestions.

Please find our answers to the major concerns.

*1.The title does not reflect what you have done in this study.*

We agree with you that the title must be more focused on methanotrophy and diversity of methanotrophs. Accordingly the title was modified into: **Methanotrophic activity and diversity of methanotrophs in volcanic-geothermal soils at Pantelleria island (Italy).**

However TTGE/DGGE and similar fingerprinting analysis (such as T-RFLP, ARISA etc) describing the structure of bacterial communities, do give informations on bacterial diversity. Thus, although composition of the soils of Favara Grande was not described in this manuscript at a taxonomic level, we still retain useful the TTGE analysis because it shows the presence of a detectable bacterial community in all the three soils and allows comparisons among them. The TTGE figure is now placed into supplementary materials and the TTGE-related results were removed from the abstract. The results of TTGE are thus only mentioned in the discussion.

*2.The presentation of results is not well organized. It can be done as follows:  
(1)Introduce the geological settings and relevant sampling information (Fig. 1).*

This has been done

*(2)Present soil physical-chemical properties (New Table 1 by combining original Table 1 and original Table 3. Note: methane oxidation activity could be removed from Table 1, while the information about pmoA could be kept)*

Please see answer to the specific comment (8).

*(3)Show methane oxidation activity (New Fig.2 ). This figure could be made by data from Table 1 and original figure 2.*

Ok this has been done.

*(4)The effort to isolate methanotrophs that are likely responsible for in situ methane oxidation (New Fig. 3). However, the methanotrophic isolate appeared not exactly what the authors had expected for. This implied that there existed bias during cultivation. Therefore, molecular approaches were employed to investigate diversity of methanotrophic communities by phylogenetic tree.*

*(5)The genetic diversity of pmoA genes (New Fig. 4)*

Points (4) and (5) need to be clarified together. The molecular approach was employed to preliminarily assess the actual presence of methanotrophic bacteria, which was expected on the basis of the methane consumption previously detected. Then the construction of the *pmoA* library from FAV2 was finalised to shed light on the diversity of methanotrophs in the most active site. The *pmoA* PCR analysis allowed us to assess the diversity of methanotrophic bacteria, independently of their actual cultivability. Such molecular analyses preceded (also chronologically) the isolation attempts, and were not carried out as a makeshift solution to overcome cultural biases; on the contrary, the expected positive results obtained from PCR analyses encouraged us to proceed in cultivation attempts on the FAV2 samples collected in the second sampling campaign (no specific bacterial species, however was expected to be isolated). For all these reasons the molecular approach must be placed before the cultural one and the request of the editor cannot be satisfied.

*(6) In discussion. The authors need to discuss how relevant the isolate Pan1 to in situ methane oxidation activity and the implication of pmoA genes from culture-independent techniques.*

The role of the isolate Pan1 has been hypothesized in the discussion section, lines 624 and lines 668-674.

Specific comments.

*(1)Page 1 Line 21 to Line 25. Please delete bacterial diversity and focus this study on microbial methane oxidation. It is a bit abrupt that TTGE was included, and indeed the authors showed no more information of bacterial diversity other than a TTGE fingerprint. This part must be removed in this manuscript, or placed as a very minor section for bacterial diversity in supplementary materials.*

See answer to the comment n.1

*(2)I agree with the reply that it is very hard to manipulate soil pH and investigate its effect on methane oxidation activity. But, please discuss the possibility of methane oxidation under low pH conditions.*

Observations on methane oxidation at low pH have been made in different parts of the MS although the most active soils of Pantelleria are only slightly acidic. See in particular lines 555 – 570, 655 – 661; 668-674.

*In addition, please discuss the possibility of better cultivation strategy for methanotrophs by addition of rare earth metals. (Pol et al., 2014 EM)*

This aspect has been better addressed in the discussion section. See lines 631-661.

*(3)The geological settings could be placed as a subsection within the Material and method.*

OK, done

*(4)In table 1. Please put the a,b,c,d as the not at the bottom of the table, but not in the main message of Table 1. In addition, primer information could be specified in the note, without reference to Table 2.*

OK, done

*(5)I side with reviewers for scientific units. In table 1, please change ng g hour-1 to nmol g hour-1; in addition, Please change methane flux unit as nmol methane per gram soil throughout the manuscript.*

Ok, done

*(6)Table 2 could be placed as supplementary table or simply deleted, because 16S rRNA gene diversity was not main theme in this study (341F/534R pair and fD1/rD1 pair could then be removed; and M13F and M13R could be briefly described in the materials and methods section*

OK, done

*(7)Table 3. It is not common to define ppm by using umol mol-1 because it usually refers to volume/volume. Therefore, it could be revised asµL L-1 throughout the manuscript. In addition, It seems that the authors can simply use percentage. For example, oxygen concentration was actually 14.38%, 4.10% and 2.45% (FAV1 site) at depths of 13cm, 25cm and 50 cm, respectively.*

ppm (ppb, ppt ...) and % are not univocal units because it has always to be specified if they are expressed by weight or by volume. Furthermore they are not units referable to the International System. In geochemical studies it is widely diffused to express ppm by volume as  $\mu\text{mol mol}^{-1}$  and ppm by weight as  $\mu\text{g g}^{-1}$  or  $\text{g kg}^{-1}$ , which are unequivocal SI units. We never found gas concentrations expressed as  $\mu\text{L L}^{-1}$ . Nevertheless, to avoid large numbers we expressed the gas concentration in table 3 in  $\text{mmol mol}^{-1}$ .

(8) *Table 1 and Table 3 can be combined. However, methane consumption data could be shown as a figure (Fig. 2a) and placed side by side with Fig. 2 (original figure 2 could be termed fig. 2b).*

The tables cannot be simply merged. The soil gas concentrations of table 3 refer to different depths (13, 25 and 50 cm) none of which correspond to the soil sampling depth (0-3 cm) in table 1. We therefore prefer to maintain two separate tables. Instead the data of methane oxidation activity has been eliminated from the table and inserted in figure 2

(9) *Figure 3 can be deleted or moved to supplementary part.*

OK done

(10) *Figure 4 should be placed after Figure 5.*

This cannot be done because of the reasons explained in the answers to general points n.4 and 5.

*And please specify Methylocystis sp. strain Pan1. in growth curve refers to isolate Pan1 in phylogenetic tree.*

OK, done