

## ***Interactive comment on “Methanotrophic activity and bacterial diversity in volcanic-geothermal soils at Pantelleria island (Italy)” by A. L. Gagliano et al.***

### **Anonymous Referee #2**

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Methanotrophic activity and bacterial diversity in volcanic-geothermal soils at Pantelleria island (Italy)

Gagliano et al.

#### General comments

The paper presented by the authors reports on diversity of methanotrophic bacteria in geothermal areas. They evidence the presence of more groups related closely or distantly to known methanotrophic bacteria or groups of bacteria. Overall the paper is well introduced and the results and discussion are quite well written. However methods must be better described (see specific comments), in particular all the part related to

C1318

gas handling. Moreover, the authors never specify the number of replicates, statistic is not reported neither in the text nor in graphs and Tables and this must be included and discussed.

#### Specific comments

##### Introduction:

There is a more recent balance you can cite, together with Etiope 2008, of volcanic methane in the context of global CH<sub>4</sub> budget by Stefanie Kirshke et al. 2013 Three decades of global methane sources and sinks Nature Geoscience 6, 813-823.

##### Materials and methods

###### 3.2 gas sampling and characterization

Soils gas samples: delete the “s” from soils.

The authors should explain here why are they doing this gas sampling along the profile and give more details on how are the probes done, length, for how long is the probe left on site, was it left on the site to equilibrate for how long? How much is the internal volume? It is not clear how do you sample the 20 ml of gas into the vial? I assume 20 ml are quite a big volume compared with the internal volume of the tube. Do you leave the syringe connected to the tube to equilibrate with the internal gas? Or do you use another system?

Is the area of probing a strong degassing area of you have mostly soil and slow degassing?

Please give more details on the gas measurement and sample handling for methanotrophic activity measurements. Do you take so many samples consequently in the same vial? Or you have replicates for each sampling time? How many replicates per treatment? How much gas do you sample each sampling event? I assume 1 ml loop needs at least 3 ml to flush it if the void is not previously made in the loop.

C1319

Extraction of DNA on dry soil is a well accepted procedure? To be sure to get all the DNA isn't the soil usually stored immediately after sampling as it is (fresh)? Couldn't the drying procedure let to lose some DNA? Can you cite some relevant literature which support soil drying or explain any previous test you have been doing to check this is ok?

Error of estimate should be reported for values presented in table 1 and the number of replicates analysed for each parameter should be specified.

The same needs to be done for Table 2, plus the units must be changes so to represent smaller numbers and to include the uncertainty of the estimate (st error or st deviation).

Error bars must be reported also for the points of graph 2 and 5 (in the legend please specify the number of replicates per each point). If possible report as asterisks also statistical differences among sampling times.

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Interactive comment on Biogeosciences Discuss., 11, 5147, 2014.

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