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

## **Anonymous Referee #1**

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Review of Gagliano et al. Biogeosciences Discuss. 11, 1-32 (2014)

### General comments

The article describes an investigation of the methane oxidation potential of the geothermal soils of Pantelleria. The article is overall well written. The geochemical part including site description, chemical-physical characterization, gas analysis and measurements of soil methanotrophic activity are well performed. The culture-independent (molecular) approach and especially the culture-dependent approach look a bit more preliminary.

### Specific comments

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P. 2, line 9, p.3 line 16 and p. 3: I do not understand what the units 'kta-1' and 'ta-1' refer to. P. 4, lines 23-29: The verrucomicrobial methanotrophs use the Rubisco pathway for carbon fixation (Khadem et al., 2011, J. Bacteriol. 193: 4438-4446). They do not possess the RuMP and serine pathways. Paragraph 3.3: Why were the samples air dried? This may result in loss of activity. Why did the authors not vary the pH for activity measurements? Paragraphs 3.6, 4.6 and last part of the Discussion section: The isolation of verrucomicrobial methanotrophs is not trivial, they show hardly growth on agar plates (previously the floating filter technique was used). Not much variation was included concerning, amount of inoculum, medium composition, pH (lowering the pH to 5 would be interesting), temperatures, incubation time. Recently it was shown that *M. fumariolicum* was dependent on addition of rare earth metals (e.g. cerium, lanthanum) to the medium (Pol et al. 2014, Environ. Microbiol. DOI: 10.1111/1462-2920.12249). The growth curve reported in Fig. 5 shows an increase in OD from 0.06 to 0.09, which means not even a doubling. As a rule of thumb: a consumption of 8 mmol of methane should result in an increase in OD to 2.0. Please explain. I would like to see a good growth curve from which also the doubling time could be calculated. The purity of the *Brevibacillus agri* strain (Laursen et al. 2007) is questionable and growth on methane is not well documented in this article. Apparently the isolate obtained in this study did not use methane. Paragraph 4.3: The TTGE profiles show 4 comparable very dominant bands in samples FAV2 and FAV3. Why were these bands not sequenced in order to find out which bacterial species they represent?

Paragraph 4.4 & 4.5: A total of 12 samples showed a *pmoA* PCR product of the right size. However only one clone library was produced and from this only 16 clones were sequenced.

Technical corrections: Methane consumption rates are reported in different units. This should be uniform, and would like to suggest to use 'nmol' rather than 'ng'. P.5, line 18: replace 'cultural' with 'culture-dependent' Fig. 5 & p. 7, line 25, p.14, line 25 and other places: What is 'umol.mol-1'?

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