Geophysical Research Abstracts
Vol. 18, EGU2016-5185, 2016
EGU General Assembly 2016

Preliminary evaluation of the use of soil bacterial 16S rDNA DNA markers in sediment fingerprinting in two small endorheic lagoons in southern Spain

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Recently, several studies have shown the effect of soil management on the soil microbial community in olive orchards, how this might differ due to a combination of management and soil type, and how these can be identified using DNA markers (Landa et al., 2014). Using DNA markers of soil bacteria seems to have the potential to detect differences in soil properties between different areas (Joe-Strack and Petticrew, 2012), particularly in those that by their location and characteristics might not present differences in other chemical or geochemical soil properties. This presentation describes the preliminary results of an exploratory survey to evaluate the potential of soil bacteria community composition in determining the origin of the sediment in two small endorheic lagoons in southern Spain.

Two lagoons (Zoñar and Dulce) in southern Spain with a small contributing area (877 and 263 ha respectively) were selected for this study. These lagoons were chosen because of their environmental relevance and increasing siltation problems. The dominant land use in most of their contributing catchments is rain-fed olive tree cultivation. In May 2015, two small subcatchments within each of the lagoon's contributing area were sampled. At each sampling point, a composite sample was collected of three subsamples taken within a 5 m radiusa. We differentiated between 0-20 and 20-40 cm soil depth. Additionally, in both lagoons samples were taken from the sedimentation of the stream draining the subcatchment into the lagoon shores, at 0-20 –cm depth. Prior to each sampling each of the the two subcatchments were explored for indications of different properties or management that could help divide it into different "homogeneous" units, including: soil management, visual indications of erosion symptoms (e.g. rills, soil mounds around olive trees), colour, and landscape position. As a result, the subcatchment in each lagoon was divided into three areas (referred to as 1, 2 and 3). The bulk community of DNA was extracted from 250 mg of soil samples (three replicates per sample) using the procedure described in Landa et al. (2014). The bacterial 16S rRNA gene V1-V2 hypervariable regions were amplified in polymerase chain reaction (PCR). The sequencing procedure was performed according to the manufacturer's recommendations using MiSeq Reagent Kit v2 for 300 cycles on MiSeq desktop sequencer.

The raw dataset for each sample consisted of the number of counts for each of the 6640 operational taxonomic units (OTU) analyzed. All the screening and analysis was performed independently for each lagoon. Given the large number of OTUs, a first screening was made discarding any OTU that did not presented at least five samples with counts >20 for that OTU. This lowered the number of OTUs to 205 in Dulce and 217 in Zoñar. Because of the limited number of samples, we did not perform independent analysis for each soil depth. All the analyses were performed twice; one with the original number of counts and another with the normalized number of counts. We screened the OTU following a 4-step method to determine those with the best ability to discriminate among the three potential source areas. These steps were: 1) eliminate OTUs with no readings or very few, that could be experimental noise; 2) keep only OTUs that are different among source areas; 3) eliminate OTUs that range outside of feasible solutions to explain average values found in sediment; and 4) eliminate OTUs with the largest variability. Afterwards, several over-determined mixing models were solved considering different combinations of OTUs using limSolve (Soetaert et al., 2014) in R.

Preliminary results show that 0.2 to 0.6 % of the searched OTUs (i.e. 14 to 42) had the potential for use in the mixing models after the four-step screening process. The results indicate a large variability in the number of counts among the samples from different areas within the subcatchments ranging, on average, from 49 to 127 % in Dulce and from 80 to 117 % in Zóñar. These rangesare within values reported for other soil chemical and

physical properties, although the higher values are above the most commonly reported CVs which tend to be in the range from 30 to 80 %. Some groups, that are relatively stable to the normalization process, can provide enough information for solving a mixing model, although the specific groups vary between the two catchments as expected from previous studies. Overall, all the models for Zóñar tended to provide similar results with low contributions from source areas 1 and 2, and a much larger contribution from source area 3. For this solution, the mixing model was able to replicate the values of all the OTUs included in the model. The predicted values for Dulce were not as stable. The model with 10 OTUs were similar with a very low contribution from source area 2, a moderate contribution from source area 3 and a maximum contribution from source area 1. However, these values differed from those with only three OTUs, and they also differed between themselves when the normalized and non-normalized values were used. This solution also seemed to replicate the averaged measured values of most of the OTU′s included in the model.

These preliminary results demonstrate the potential of soil bacterial 16S rDNA in sediment fingerprinting studies, although some questions need to be addressed in more detail, including: the temporal evolution of the distribution of the bacterial markers with soil depth; the implications of selective transport by runoff; and the relatively large variability of counts among samples from the same area. We are currently repeating the sampling in one of the subcatchments to provide some insight into these issues.
Key words: sediment, fingerprinting, soil, microbial, DNA, lagoon

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