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Soil DNA metabarcoding: evaluating the efficiency of multiplex primer sets in recovering the soil invertebrate's community as soil quality Indicators

DNA metabarcoding del suolo: valutazione dell'efficienza di alcuni set di primer nell'investigare la comunità invertebrata del suolo come indicatori della qualità dei suoli

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Introduction

DNA metabarcoding, a promising new technology, involves the direct extraction of DNA from soil samples, PCR amplification of the extracted DNA with specific primers, followed by libraries preparation with sample-specific tags and sequencing through Next Generation Sequencing technologies (Hamilton et al., 2009).

In the present study, two combinations of primers sets targeting the mitochondrial *cox1* gene were tested in order to evaluate their efficiency in characterizing meso- and meio-faunal soil communities.

Materials and Methods

Three different types of soil samples (organic farm, conventional farm and forest), representing three main types of land-use in the study area, were collected in replicate in two farms from stable meadows and barley, during three different seasons (*i.e.*, spring, summer and autumn). The farms are located in the municipalities of Albairate (MI) and Cisliano (MI).

Soil samples were homogenized, grounded and the DNA was extracted from each replicate by Nucleospin® soil kit (Macherey-Nagel, Düren - Germany).

Fragments of the *cox1* gene (~300 to 650 bp) were amplified using the four pairs of primers (1= COIF2 - COIR2; 2= LCOI490 - HCO2198; 3= mlCOIintf - JgHCO2198; 4= Foldf-foldr), two combinations of tested primers were: A=primers 1+2+3+4, B=primers 1+3+4. Samples were randomly selected for applying the primers combination resulting in 29 samples for A and 26 samples for B combination, respectively.

Libraries were assembled pooling PCR products, according with the primers combinations (*A* and *B*), in equimolar concentrations and sequenced on a paired 2X250 bp run on Miseq platform (Illumina, San Diego, CA, USA). Raw sequences were processed rebuilding full amplicon fragments via pair overlapping and analyzed using Qiime platform. In order to identify the obtained Operational Taxonomic Units (OTUs), a *cox1*-based reference database was built. The *cox1* dataset contains our target taxa (*i.e.*, phyla belonging to Animalia) with the inclusion of Archea, Bacteria and Fungi representatives in order to detect cross-amplifications between the used primers with non-target taxa. A descriptive statistic was performed to determine the frequencies and percentages of OTUs in samples and sites

Conclusions

The major result of this preliminary study is that the set of primers, used to assembly PCRs, is a crucial factor affecting the capability to analyze efficiently the desired group of interest. The primer combination B could represent a promising method to evaluate the soil invertebrate's communities.

Results

A total of 13,506,930 raw reads were obtained, 79.9% of the reads were lost after filtering due to their low quality resulting in a total number of 2,713,429 high-quality reads (mean = 50,247 ± 3,477 sequences/sample). The 2,713,429 reads were assigned to 194,668 OTUs, 67% of them were unspecific in the set A and only 21% were unspecific in the set B (Fig. 1). Regarding the OTUs assignment, ~63% of the reads obtained from the set B and ~22% of those from set A were assigned to the Animal kingdom. Focusing on our target groups, the invertebrates inhabiting soil (Fig. 2), the set B had a higher percentage of sequences assigned to Arthropods (41.6 %), Annelida (5.9 %), while in the case of set A the percentage of Arthropoda was 10.9 % and Annelida 5.4 % of the sequences. The set B could recover a percentage of Arthopods four times higher than set *A* (41.6% and 10.9% in set B and set A, respectively). Also higher percentages of sequences in the set B were assigned to Nematodes and other Metazoans like Rotifera and Tardigrades comparing with the set A (0.8% and 1% in set B; 0.01% and 0.3% in set A, respectively).

Separating the obtained data according to the sampling site, the results confirmed that the primer set is a main factor affecting the recovered data, since the percentages of assigned OTUs to Arthropodes and Other groups of interest of Animalia were higher in the set B regardless the sites. For example, the percentage of sequences assigned to Arthropodes in set B was four times higher than that of the set A in the Organic and Conventional sites.

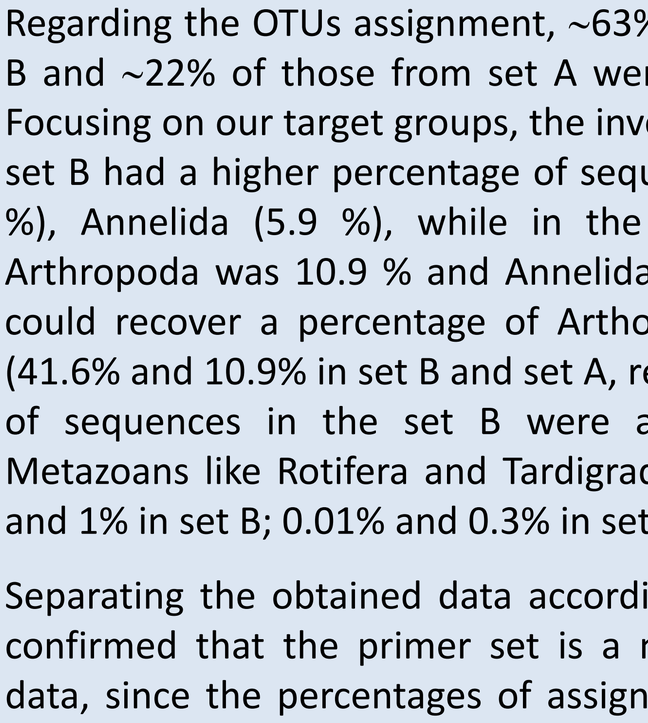

Fig. 1

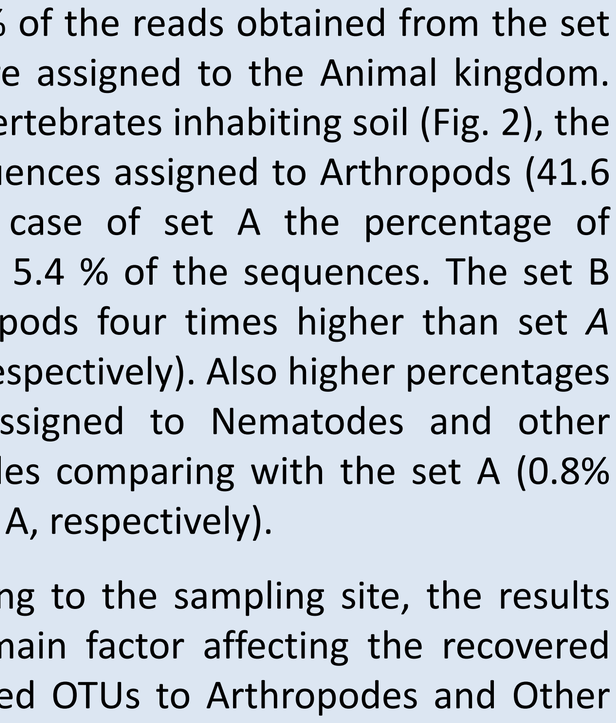

Fig. 2

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