Supplementary Table S1. Materials and Methods for DNA extraction, PCR amplification, cloning and Sanger sequencing of soil samples from 6 black truffle (*Tuber melanosporum*) plantations in Teruel, Spain

Soil DNA was extracted from 0.25g sieved soil (fresh wt.) using the PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA) following the manufacturer's instructions. Genomic DNA was PCR-amplified with fungal specific primers ITS1F and ITS4, following these cycling conditions: 5 cycles of denaturation at 94ºC for 30 s, annealing at 55ºC for 30 s, and extension at 72ºC for 1 min, followed by 33 cycles of denaturation at 94ºC for 30 s, annealing at 48ºC for 30 s and extension at 72ºC for 1 min, with a final extension at 72ºC. Ethidium-bromide-stained PCR-products were visualized after electrophoresis by UV-transillumination.

PCR products were gel-purified and ligated into pGEM–T easy vector (Promega, Madison, WI, USA). Positive transformants from each of the clone libraries were screened for the presence of the insert by colony PCR under the same reaction conditions as described above. Plasmids were cleaned by Wizard Plus Miniprep DNA Purification System (Promega, Madison, WI, USA) and digested by *Eco*RI enzyme to group clones before DNA sequencing. Both strands were sequenced separately using vector specific primers T7 and SP6 using Big Dye Terminator v3.1 Cycle Sequencing Kit on a 3130x1 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

Sequences were assembled and edited using Sequencher 4.0 (Gene Codes, Ann Arbor, Michigan, USA). Chimeric sequences were removed from the data set.

The data were clustered using the CAP3 assembler (Huang & Madan, 1999). Each cluster was considered to be an operational taxonomic unit (OTU) at 97% sequence similarity over ≥90% of the alignment length. The resulting OTUs (including singlets) were used as query for subsequent BLAST searches using NCBI-BLASTn (Altschul *et al.,* 1997) against the non-redundant GenBank database (Benson *et al.,* 2011) and a custom-curated database, which contained all fully identified fungal ITS sequences screened from INSD (International Nucleotide Sequence Database, with sequences from GenBank, EMBL, DDBJ) and UNITE databases (Abarenkov *et al.,* 2010). OTUs at 97% sequence similarity that could not be identified using custom-curated databases were queried by BLAST search against GenBank to search for similarity to the nearest relative in the NCBI database.