Efficacy of soil storage protocols for long term preservation of bacterial and fungal communities associated with crop plants

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BACKGROUND

- In recent years, a growing interest has emerged regarding the long-term storage of complex microbial communities, both for biodiversity preservation and biotechnological exploitation.
- Agricultural soil, due to high biodiversity and close relation with crops, represent a promising source of biocontrol agents, as well as plant growth promoting (PGP) microorganisms.
- Several storage protocols have been proposed, but their impact on the microbial component has been discordant, with different effects based on the considered pathosystem.

AIMS

- Determine the impact of different storage protocols on the consistency of microbial communities over one year of storage, as well as the interaction between the storage protocols and different pathosystems

METHODS

- Rhizosphere soil samples were collected from two vineyards near Castel Boglione (Asti, Italy; 44°43'57.5"N 8°23'00.7"E), with low and high esca disease incidence; in a kiwifruit vine orchard near Manta (Cuneo, Italy; 44°36'51.9"N 7°29'49.1"E), from plants with and without symptoms of kiwifruit vine decline syndrome (KVDS); and in a strawberry tunnel near Boves (Cuneo, Italy; 44°20'51.1"N 7°32'04.2"E), from a soil parcel treated with a solarization protocol and from a control soil parcel. Soil was sieved and mixed in order to uniform sample composition.
- **Molecular analyses**: DNA extraction was performed at sampling, after 6 months of storage and after 12 months of storage using the DNAeasy PowerSoil Pro kit (Qiagen, Hilden, Germany). Microbial composition of fungal and bacteria communities was characterized by means of **metabarcoding**. For fungi, the ITS2 subregion of the ITS region was investigated, using the ITS3_KYO2/ITS4ngs primer couple; for bacteria, the V3V4 subregion of the 16S region was investigated, using the Pro341f/Pro806r primer couple.
- **Vitality analyses**: soil aliquots were retrieved at sampling, after 6 months of storage and after 12 months of storage and resuspended in Phosphate Saline Buffer (PBS) for fungi and Ringer Solution for bacteria. Serial dilutions were plated on Dichloran Rose Bengal Chloramphenicol Agar (DRBCA) or Malt Extract Agar (MEA) for fungi and Tryptic Soy Agar (TSA), then colony forming units number was measured after 7 days of incubation
- **Functional analyses**: aliquots of soil suspensions were loaded in each well of BIOLOG EcoPlatesTM PM1 plates. Plates were incubated at 28°C in dark conditions. Absorbance at 590nm and 750nm was read at 24h intervals until the colour development in each well reached the plateau, then AWCD was calculated

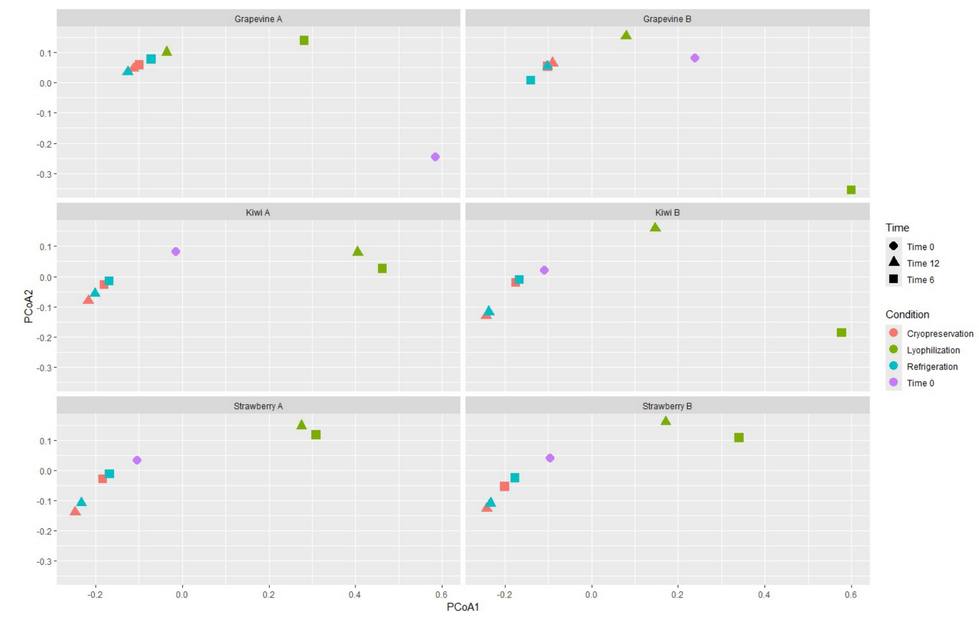

Figure 1: Principal Coordinate Analysis (PCoA) plot of the BIOLOG® data, as measured at harvest, after 6 months of storage and after 12 months of storage. Each colour indicates a different storage protocol, while different shapes indicate different timepoints.

RESULTS

Figure 3: PCoA biplot of fungal and bacterial communities. Individual points represent samples, which are associated with a storage protocol (colour) and sampling timepoint (shape). The upper plots are related to the fungal communities, while the lower plots are associated with the bacterial communities. The arrows in each plot are feature vectors and indicate the features with the highest weight on the point distribution in the space defined by the selected Principal Coordinates (PCs), with different colours indicating different taxonomies. Due to space constraints, only the data associated with the healthy plants (for grapevine and kiwifruit) and the untreated plants (for strawberry) are presented.

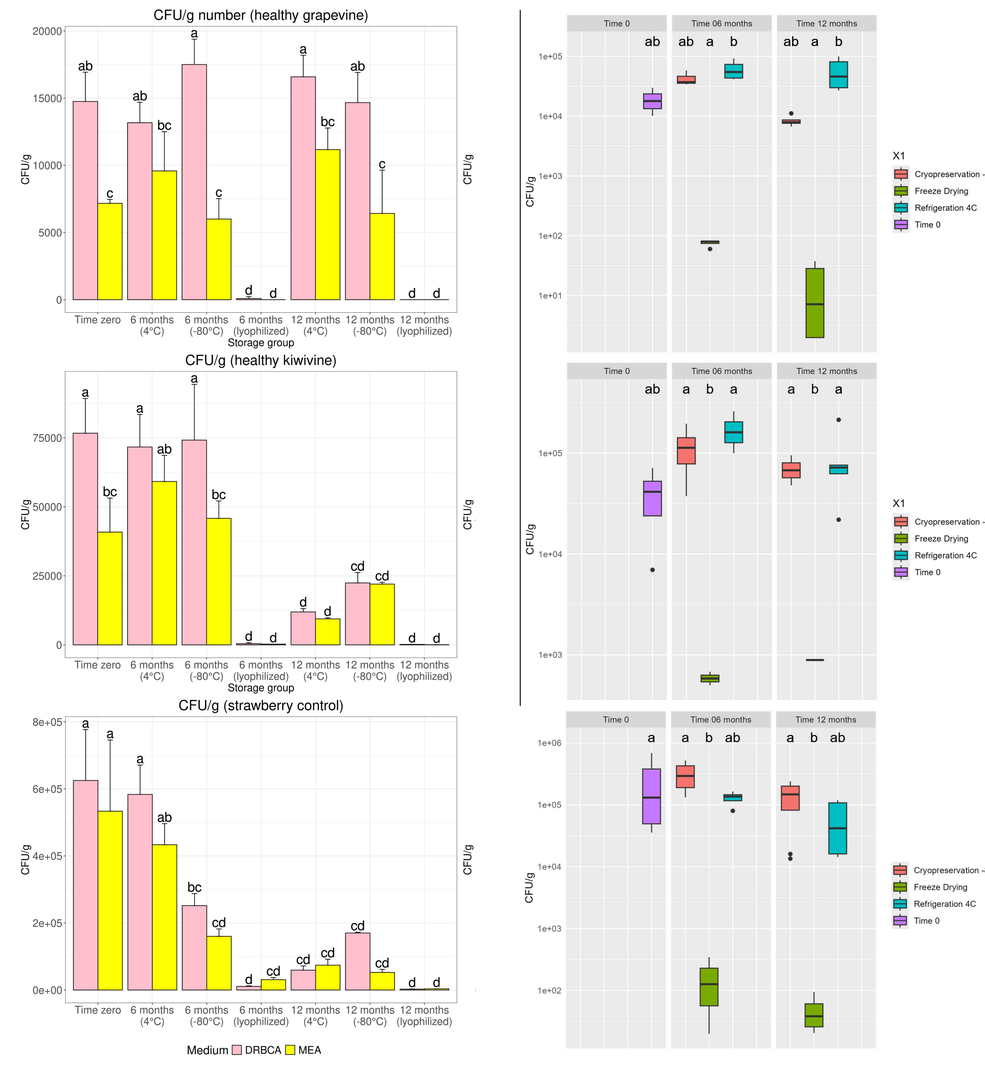

Figure 2: Colony forming units (CFU) barplot (for fungi) and box and tail plot (for bacteria). In both plots, different letters indicate the presence of statistically significant differences based on ANOVA test followed by a Tukey's HSD test, with significance threshold set at 0.05. In the fungal plot, bar colour is associated with growth medium, while in the bacterial plot colour is associated with the storage protocol. Due to space constraints, only the data associated with the healthy plants (for grapevine and kiwifruit) and the untreated plants (for strawberry) are presented.

DISCUSSION

- For microbial composition, vitality and metabolic functions, presence of **statistically significant effects** of both the **length of storage** and the **storage protocols** on the bacterial and fungal communities, but with different impact based on both the considered pathosystem and the health condition of the pathosystem.
- Selected protocols presented at best a partial efficacy, with **storage at -80°C** proving the **best** for a majority of considered matrixes
- The observations of this work suggest that in order to adequately preserve overall microbial community composition several **different storage protocols** should be used in **parallel**

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