

FUNGICIDES DEGRADATION IN AN ORGANIC BIOMIXTURE: IMPACT ON MICROBIAL BIODIVERSITY

¹Laura Coppola, ²Francesca Comitini, ¹Cristiano Casucci, ²Vesna Milanovic, ¹Elga Monaci, ¹Maria Marozzi, ²Manuela Taccari,
²Maurizio Ciani, ¹Costantino Vischetti

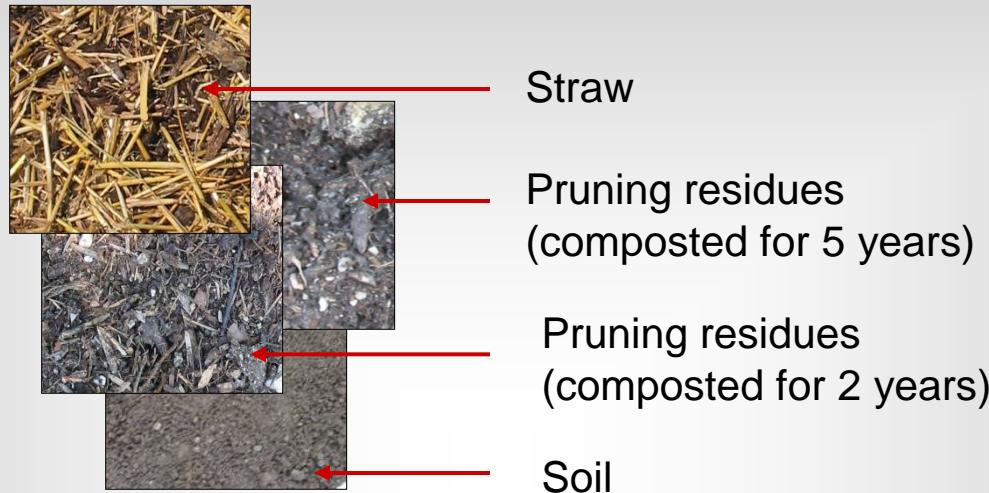


¹Dipartimento di Scienze Ambientali e delle Produzioni Vegetali, Università Politecnica delle Marche, via Brecce Bianche, 60131 Ancona

²Dipartimento di Scienze Alimentari, Agro-Ingegneristiche, Fisiche, Economico-Agrarie e del Territorio, Università Politecnica delle Marche, via Brecce Bianche, 60131 Ancona

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Different substrates were tested to develop a system to mitigate water point-source contamination adapted to Italian conditions



A laboratory experiment was carried out to test a mixture of compost and straw:

- in biodegrading different mixture of fungicides usually applied in italian vineyards
- the effect of fungicide application on microbial community

Substrate



40% wheat straw



60% pruning residues

+

Cutted to obtain fractions of 3-6 cm

60% W.H.C.

Stored under a controlled condition:

T = 24°C

Moisture = 60% W.H.C.



Density = 0,41g/cm³

pH = 8,04

Organic Carbon = 33,1%

Non Treated

Treated

Control Samples

Treated Samples

Fungicides used



Formulates	Active Ingredients	% a.i.	Chemical Group	Formula
FORUM MZ	Dimethomorph (DM)	9,0%	morpholine	
RIDOMIL GOLD COMBI	Metalexyl (MX)	5%	Phenylamide	
TOPAS	Penconazole (PC)	10,2%	triazole	
QUADRIS MAX	Azoxystrobin (AZ)	22,9%	Strobilurin	
SWITCH	Cyprodinil (CY)	37,5%	Anilinopyrimidine	
	Fludioxonil (FL)	25,0%	Phenylpyrrole	

Treated Samples

Formulate were diluting in deionised water



Active ingredient concentrations were estimated as those residual in the tank at the end of the field treatments

1° treatment

$$PC = 2,2 \text{ mg kg}^{-1}$$

$$DM = 22,6 \text{ mg kg}^{-1}$$

2° treatment

$$PC = 1,3 \text{ mg kg}^{-1}$$

$$MX = 8,4 \text{ mg kg}^{-1}$$

3° treatment

$$PC = 1,1 \text{ mg kg}^{-1}$$

$$MX = 8,4 \text{ mg kg}^{-1}$$

4° treatment

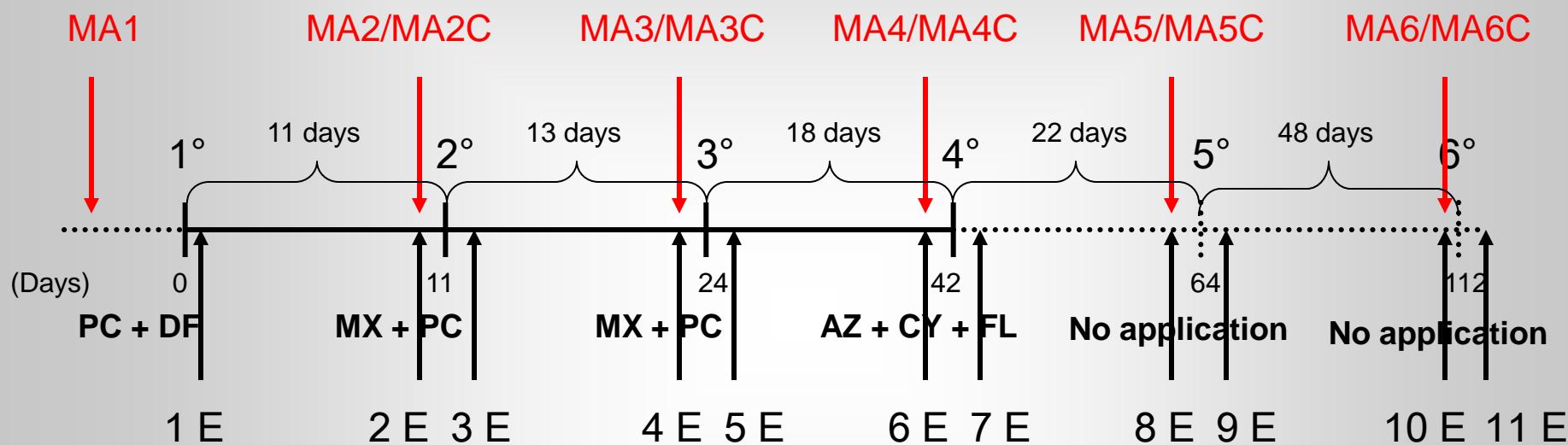
$$AZ = 50,0 \text{ mg kg}^{-1}$$

$$FL = 20,0 \text{ mg kg}^{-1}$$

$$CY = 30,0 \text{ mg kg}^{-1}$$

Experimental Set-up

Microbial analysis (MA): sub-samples were collected 1 day before each pesticide treatment



Chemical Analysis (E): sub-samples were collected 1 day before and 1 day after each treatment

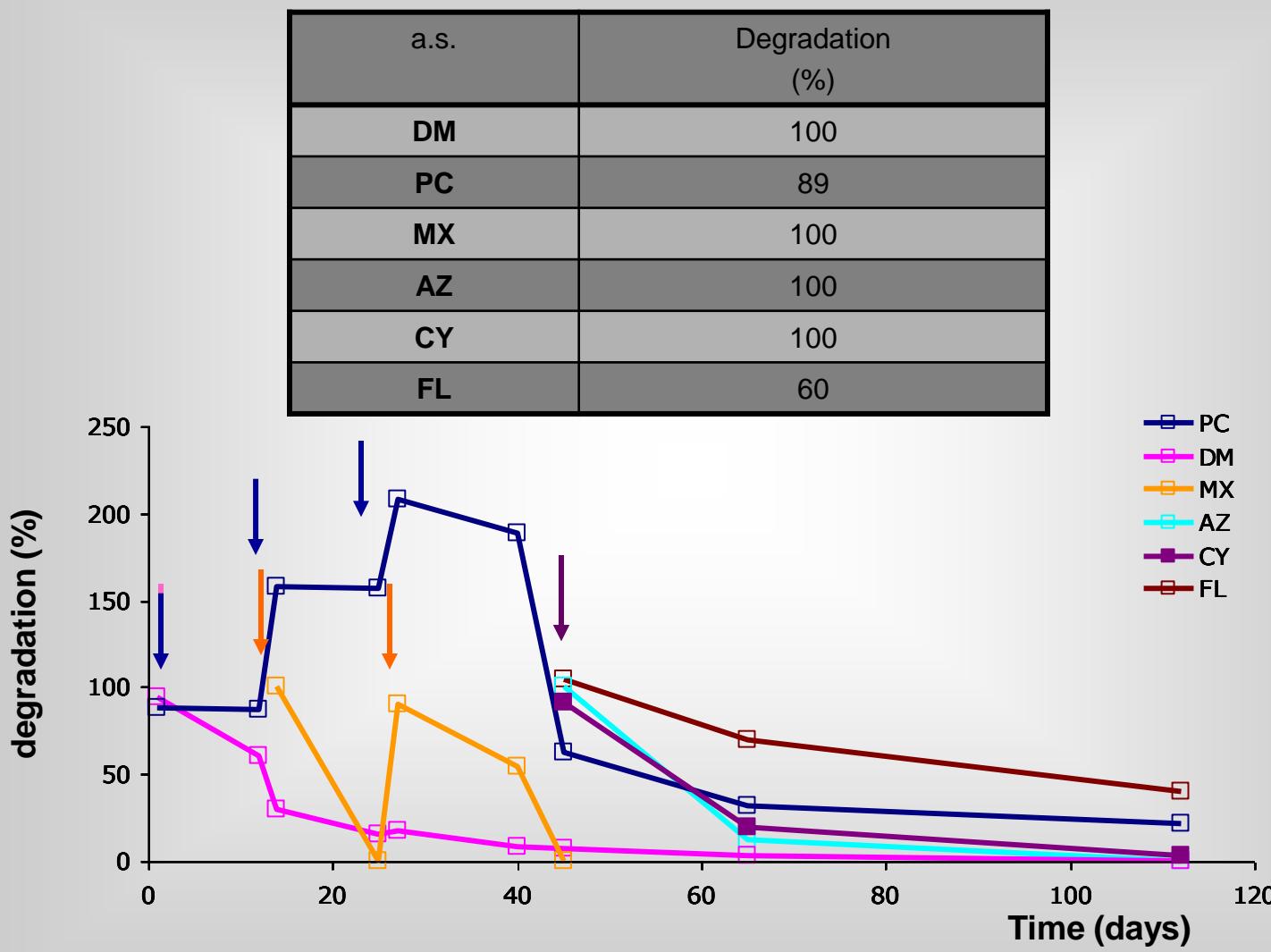
Control Samples

2 Replicates

Treated Samples

3 Replicates

Degradation



Biomixture is capable of degrading the complex mixture of fungicides which have been applied at multiple steps.



Biomixture is characterized by high organic carbon pool that may have a positive influence on microbial activity. Thus, fungicide degradation may be the result of a great microbial activity in the organic substrates.

Microbial analysis



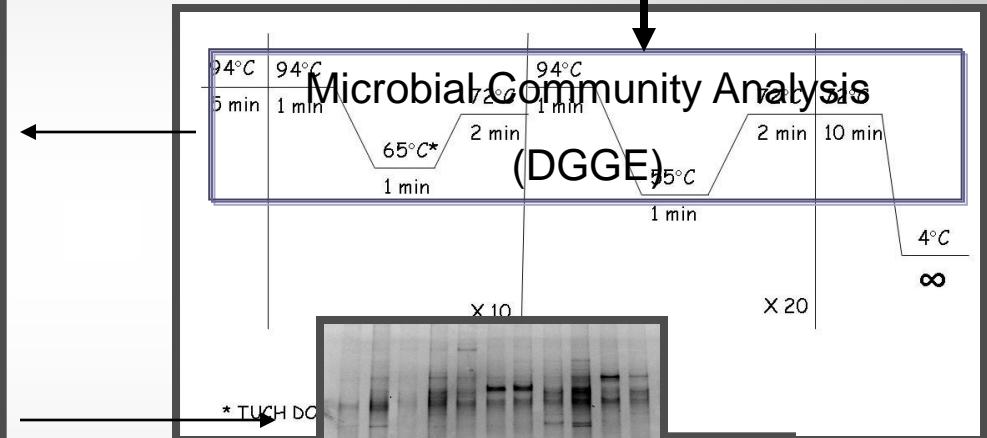
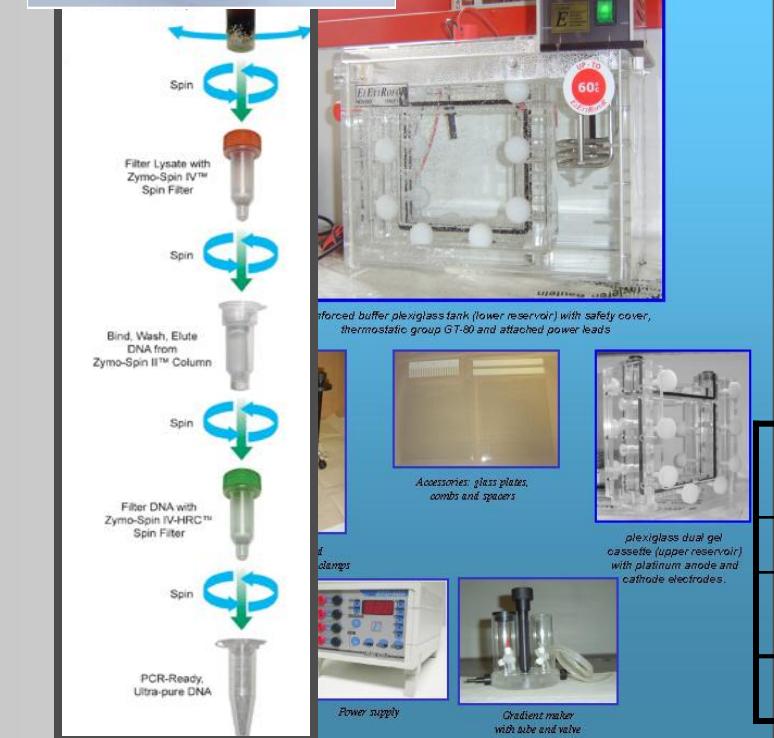
on
Kit)

DNA Quantification

QUANTO-DROP

Independent microbial analysis (PCR)

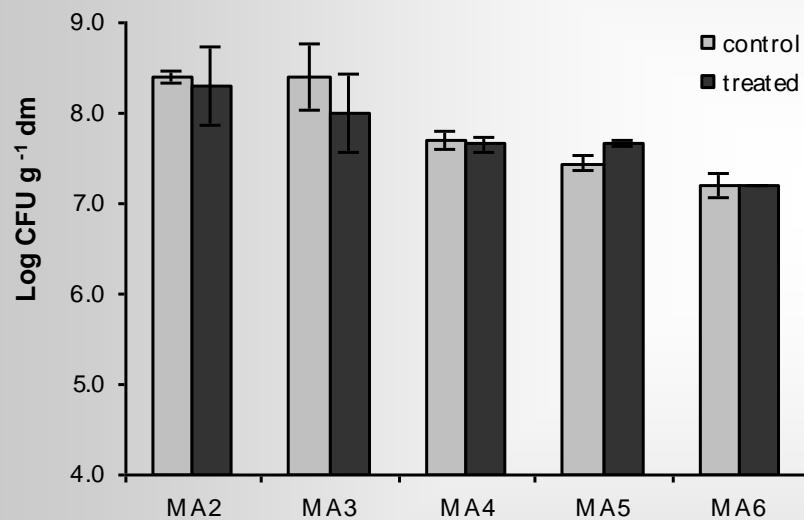
DNA Amplification



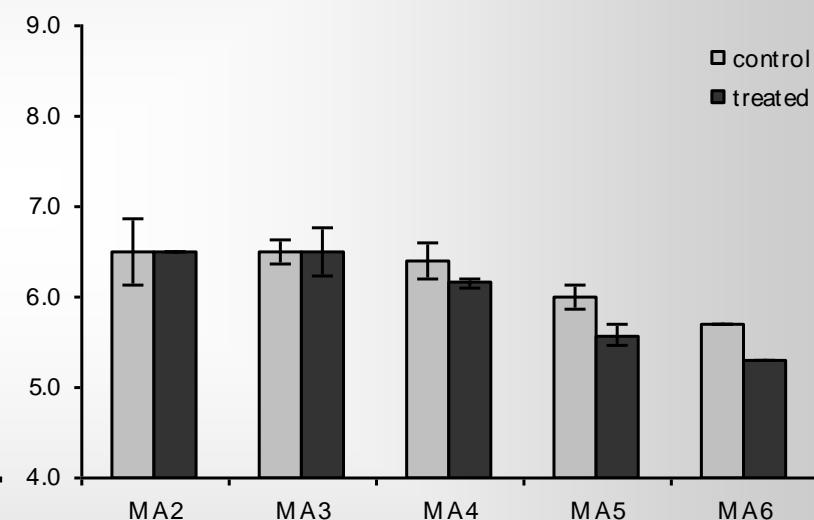
P 1	5'-CGCCCGCCGCGCGCGGGCGGGGGCGGGGGC ACGGGGGGCCTACGGGAGGCAGCAG-3'
P 2	5'-ATTACCGCGGCTGCTGG-3'
NL 1	5'-CGCCCGCCGCGCGCGGGCGGGGGCGGGGGC CATATCAATAAGCGGAGGAAAAG-3'
LS 2	5'-ATTCCCAAACAACTCGACTC-3'

Culture - Depending Analysis

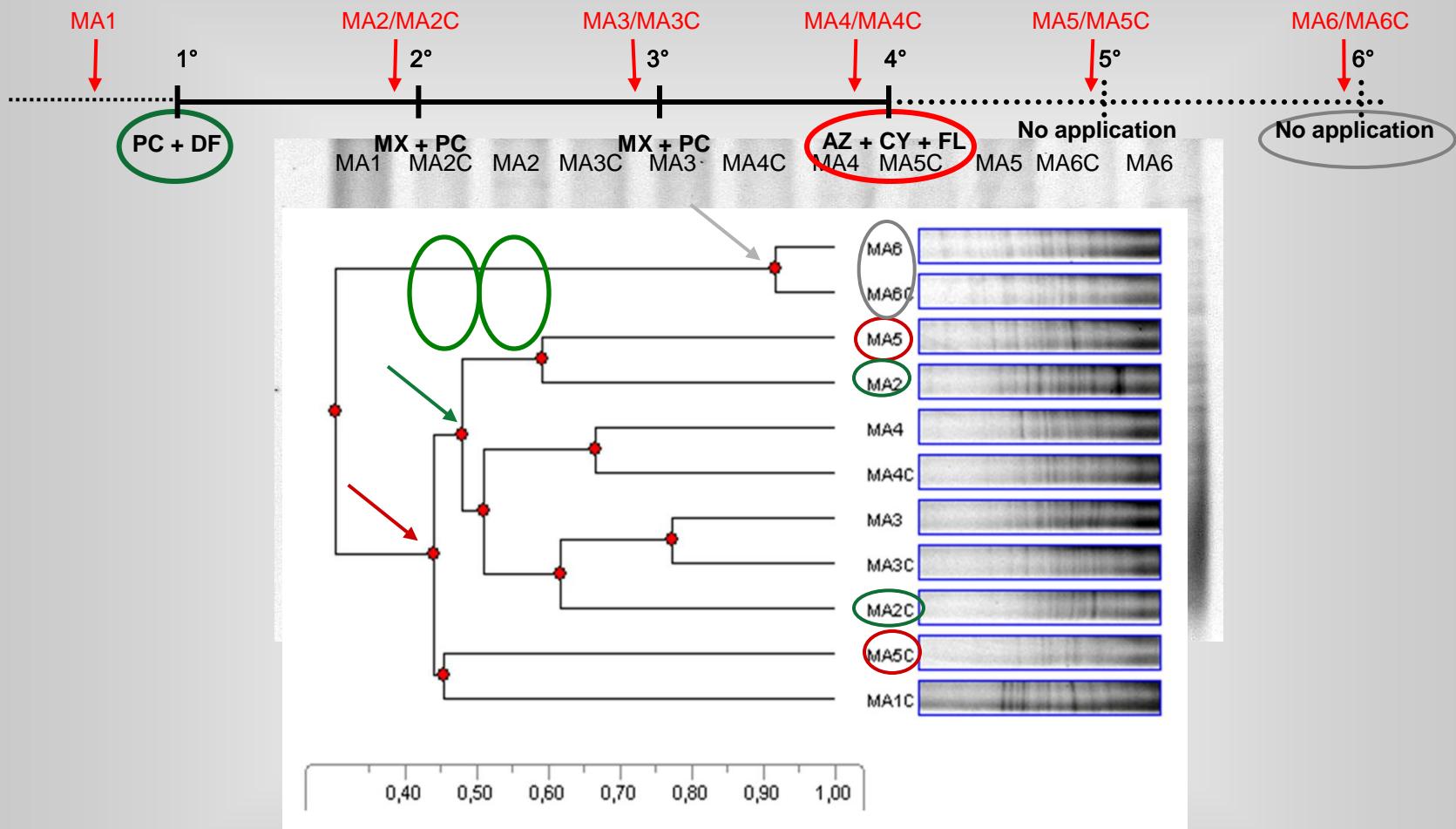
Bacteria



Fungi

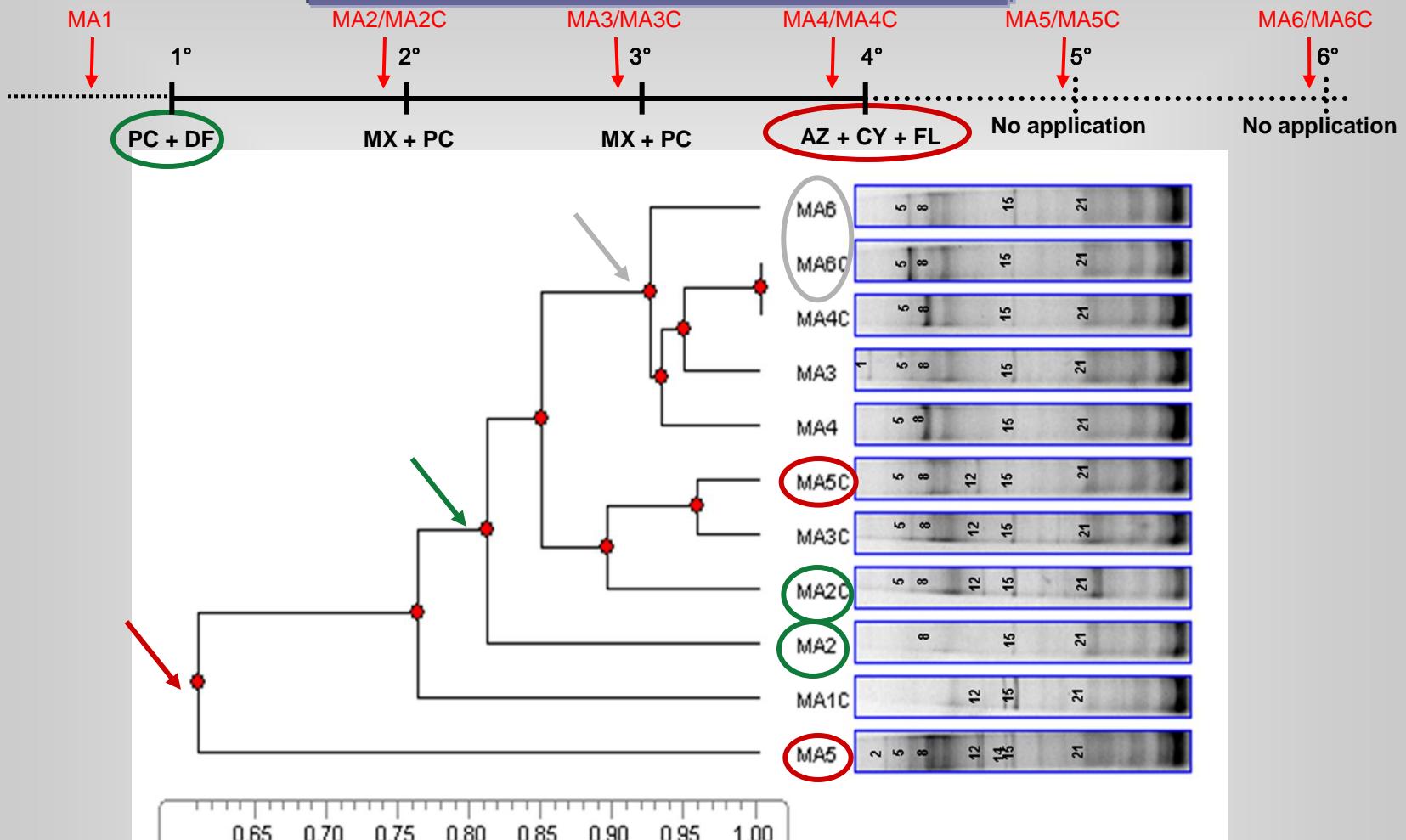


UPGMA*Bacteria



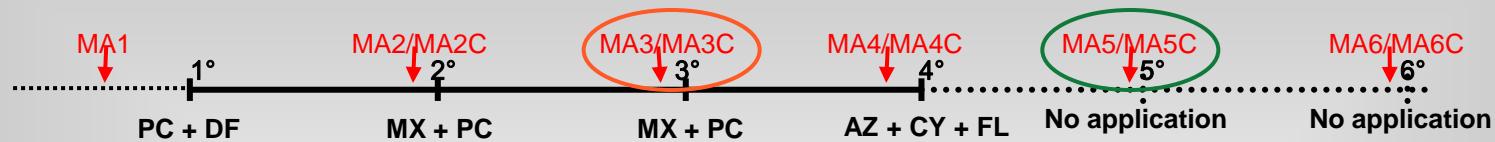
*Unweighted Pair Group Method with Arithmetic Mean

UPGMAE Fungigi



*Unweighted Pair Group Method with Arithmetic Mean

Sequences of fungi



Band(s)	Size (bp)	Closest relative	% Identity	Source ^a	GenBank accession no.
1	182	<i>Filobasidiella neoformans</i>	100%	FJ914904	HM803178
2	188	<i>Cladosporium subtilissimum</i>	100%	EU583482	HM803176
5	187	<i>Saccharomyces cerevisiae</i>	100%	EU833231	HM803177
8	91	<i>Aspergillus oryzae</i>	94%	GQ382276	HM803181
12	117	<i>Aspergillus niger</i>	99%	GU395669	HM803180
14	135	<i>Issatchenka terricola</i>	100%	EU441898	HM803179
15	57	<i>Uncultured yeast isolate</i>	100%	EU834334	HM803183
21	198	<i>Ochrocladosporium elatum</i>	97%	AB100652	HM803182

^a Accession number of the sequence of the closest relative found by a BLAST search

Since identified strains belong to yeast flora and ascomycete filamentous fungi it was hypothesized that they could be involved in degradative activity in this specific ligno-cellulosic substrate.

Furthermore, it was interesting to note that the changes induced by contamination on the microbial community structure appeared to be only temporary.

Conclusion

This study encourages the use of this organic mixture for a biodepuration system in the Mediterranean area suggesting a possible recycling of exhausted organic material for agronomic purposes with a particular attention to recalcitrant pesticides

The temporary changes in the microbial community allow to use the material in the vineyard since no species modification for this specific culture have been detected at the end of the experimental time.