Ministry of Agriculture, Natural Resources and Environment

Agricultural Research Institute

The implementation of biobeds in Cyprus

Omirou M.D.,[1*] Dalias P.,[2] Costa C.,[2] Alexandrou E.,[3] Papastephanou C.,[3] Ehaliotis C.,[4] Karpouzas D.[5]

[1] Agricultural Research Institute, Lab. of Agricultural Microbiology, Lefkosia, Cyprus
[2] Agricultural Research Institute, Soil Science Section, Lefkosia, Cyprus
[3] cp FOODLAB LTD, Lefkosia, Cyprus
[4] Agricultural University of Athens, Department of Natural Resources and Agricultural Engineering, Lab. of Soils and Agricultural Chemistry, Athens, Greece
[5] University of Thessaly, Department of Biochemistry -Biotechnology, Larisa, Greece

* tel: +35722403146, Email. michalis.omirou@arinet.ari.gov.cy

Why biobeds are important for citrus industry?

- Citrus is a major crop in Cyprus
  - 130 000 tn total production
  - 70 000 tn are exported to EU and Third countries
  - Intensive agricultural practices since they are infected by various insects like Med fly, thrips etc
  - Major losses are observed during post harvest transport from specific fungi such as *Peniccilium.*
- EU directive 2009/128, "Establishing a framework for Community action to achieve the sustainable use of pesticides"
- Therefore our purpose is to provide:
  - Policy makers with measures to mitigate point pesticide pollution
  - Farmers with effective tools for pesticide handling.

"Evaluation of biobeds for the decontamination of wastewaters of agricultural origin – BIOBEDS"

- The project is financially supported by Research Promotion Foundation of the Republic of Cyprus
- Partners
  - cp FOODLAB LTD, Cyprus
  - Agricultural Research Institute, Cyprus
  - Agricultural University of Athens, Greece
  - University of Thessaly, Greece
- **The objective:** establish an effective biobed system with focus on citrus plantations
- **Biomixtures**: will consist of soil/straw / different composted materials derived from local agricultural wastes
  - Evaluate degradation potential of the different biomixtures
  - Investigate the role of microorganisms in pesticide degradation

Experiment 1: Pesticides degradation

- 5 different biomixtures consisting of soil/straw/ various different composts
- Soil/straw(b6) and soil (b7) as controls
- 6 pesticides that are used in citrus industry
  - 3 insectisides (a-cypermethrin, chlorpyrifos, deltamethrin) applied at 20μg/g of biomixture.
  - 3 fungicides (SOPP, thiabendazole, imazalil) applied at 35μg/g biomixture.
- Experimental design: CRM with 3 replicates
- Moisture content 60% WHC
- Temperature 25^{o}C

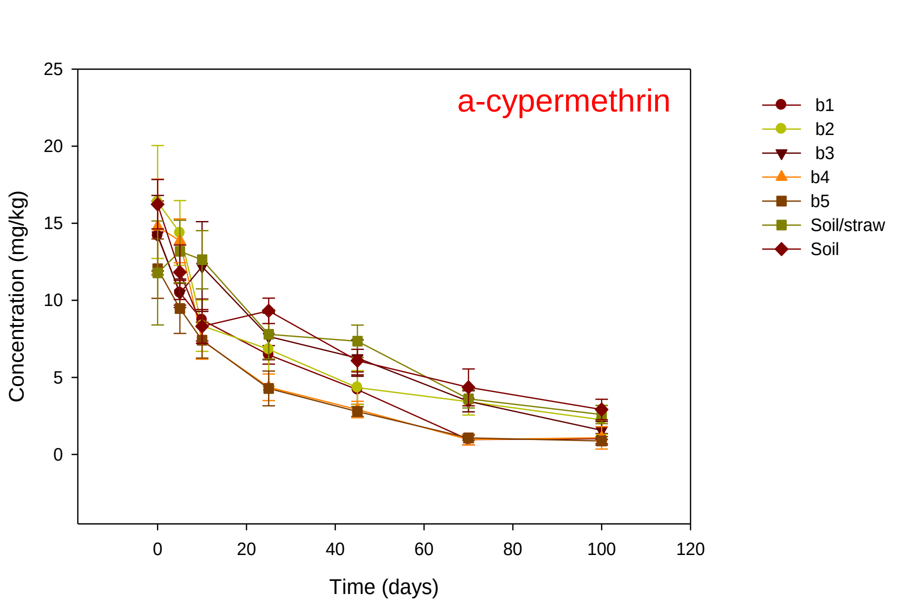
Volumetric content of biomixtures

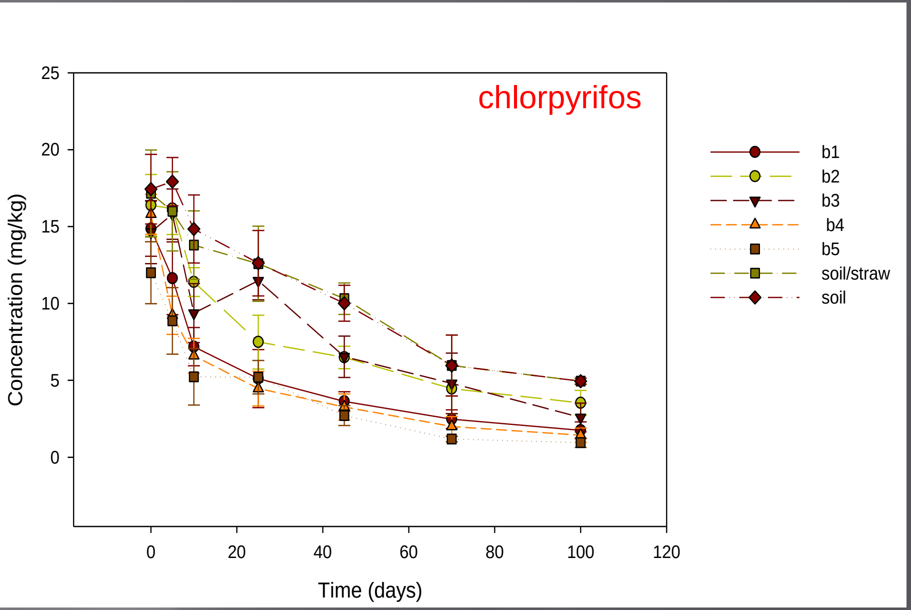
b1	composted olive branches	25%	soil	25%	straw	50%
b2	composted vines	25%	soil	25%	straw	50%
b3	composted grape marc	25%	soil	25%	straw	50%
b4	Composted wine seeds and skins	25%	soil	25%	straw	50%
b5	Composted wine seeds and skins	50%	soil	25%	straw	25%
b6	-	0%	soil	75%	straw	25%
b7	-	0%	soil	100%	straw	0%

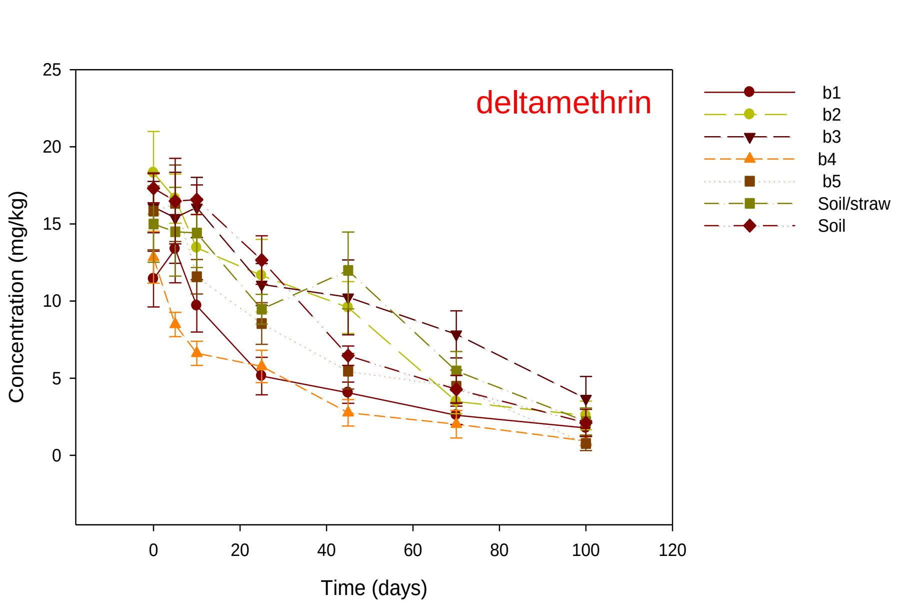
Physicochemical characteristics of biomixtures

	Total C (%)	Total N (%)	Exch K (mg/kg)	Avail P (mg/kg)	Total S (%)	Total Ca (%)	Relative Bulk Density (g/cm^3)
b1	5.29	0.61	459	136	0.23	1.26	0.24
b2	4.95	0.65	376	117	0.25	1.15	0.15
b3	5.75	0.72	980	191	0.14	1.14	0.60
b4	6.83	0.74	546	91	0.11	1.29	0.58
b5	16.54	1.30	1989	324	0.15	1.17	0.58
Soil/straw	10.19	0.37	242	109	0.08	1.32	-
Soil	0.65	0.054	123	18	0.02	1.11	-

Results – Degradation study





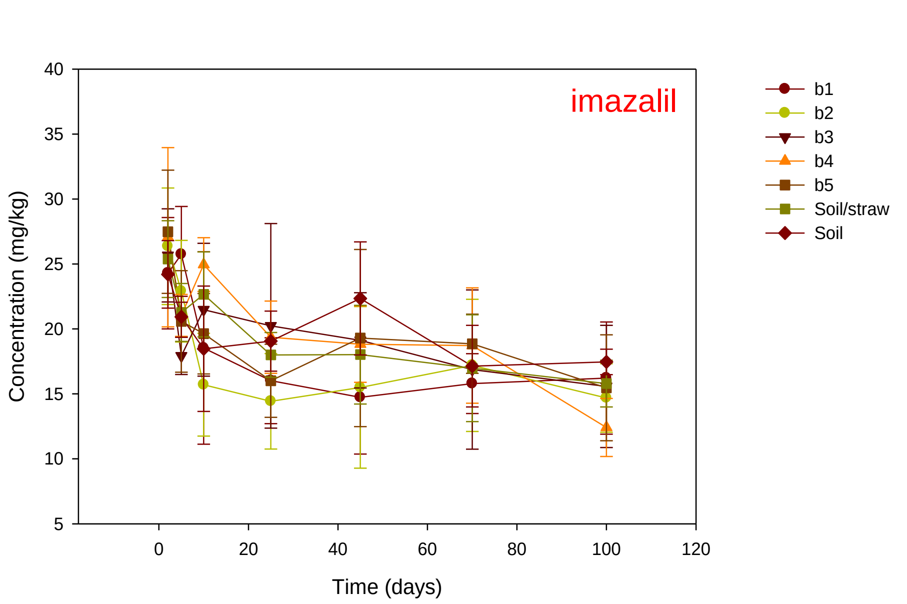


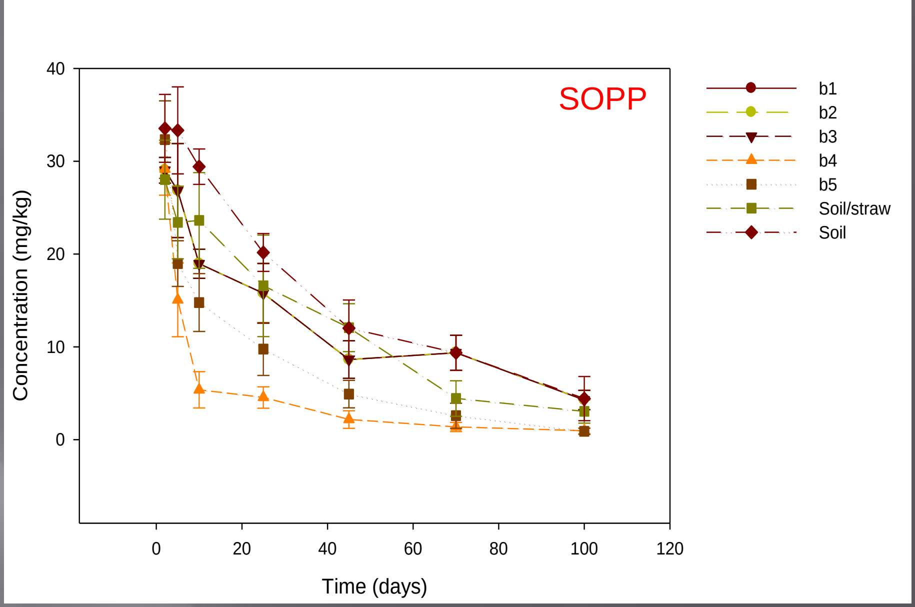
For all pesticides First Order Kinetics were utilized to calculate $T_{1/2}$ values

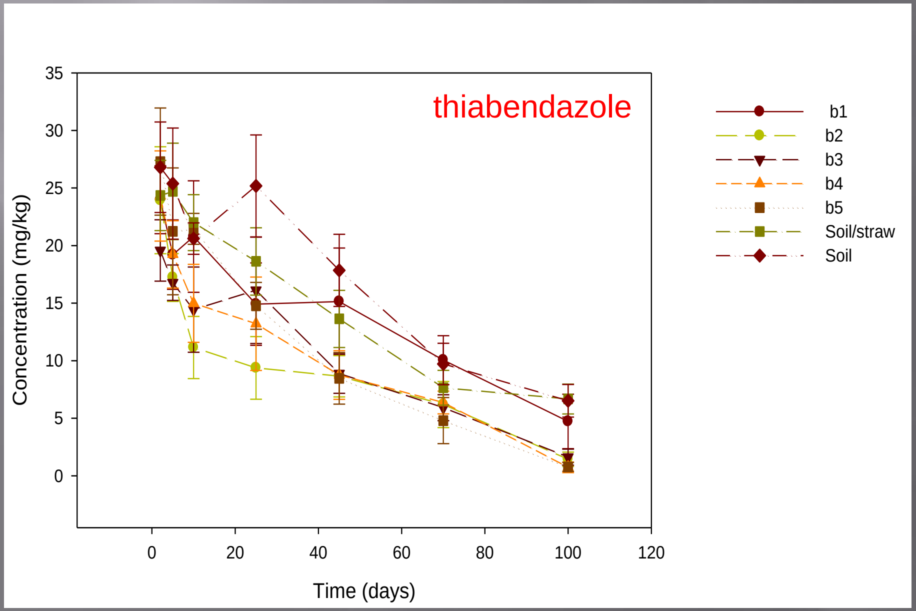
Degradation study - Instecticides

	t 1/2		
	Chlorpyrifos	Deltamethrin	a-cypermethrin
b1 (olive-tree prunings)	48.6d	53.1c	62.7d
b2 (grape-vine prunings)	36.6c	62.8d	57.5c
b3 (grape marc)	43.2d	36.3c	53.1c
b4 (winery by-products)	21.5a	32.8b	23.7a
b5 (winery by-products)	29.3b	22.2a	31.3b
Soil/straw	76.6f	61.3d	69.1e
Soil	67.6e	84.2e	83.6f

Results – Degradation study







Degradation study - fungicides

	t 1/2		
	SOPP	TBZ	Imazalil
b1 (olive-tree prunings)	34.5c	53.9 d	138.5 b
b2 (grape-vine prunings)	36.8c	28.7 ab	146.1 c
b3 (grape marc)	34.2c	40.5 c	186.3 d
b4 (winery by-products) →	4.9a	30.5 b →	115.1 a
b5 (winery by-products)	13.8b →	26.5 a	230.4 e
Soil/straw	32.2c	46.6 c	153.9 c
Soil	31.8c	57.2 e	300.2 f

Degradation study Results summary

- All pesticides showed increased dissipation in compost-containing biomixtures
- **Biomixtures b4** and **b5 (winery by-products)** showed the highest degradation capacity for most pesticides
- **Imazalil** was the most persistent chemical and **biomixture b4** showed the highest degradation capacity for this molecule
- **SOPP** was rapidly dissipated in all substrates that were examined including soil.

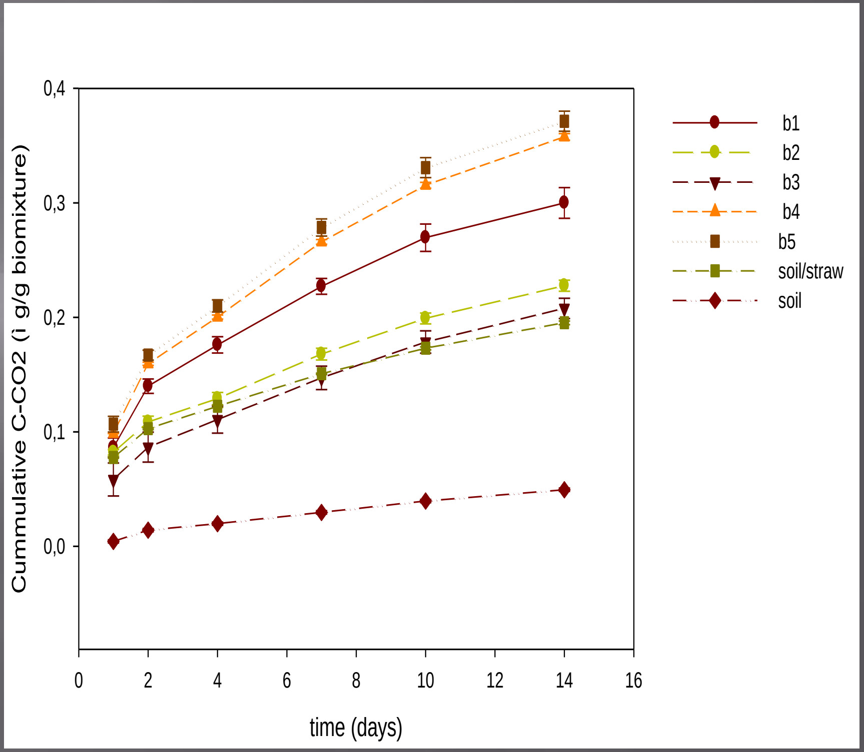
How degradation results are linked with microbial activity and community structure?

- Soil microbial activity
  - *Microbial respiration (NaOH traps)*

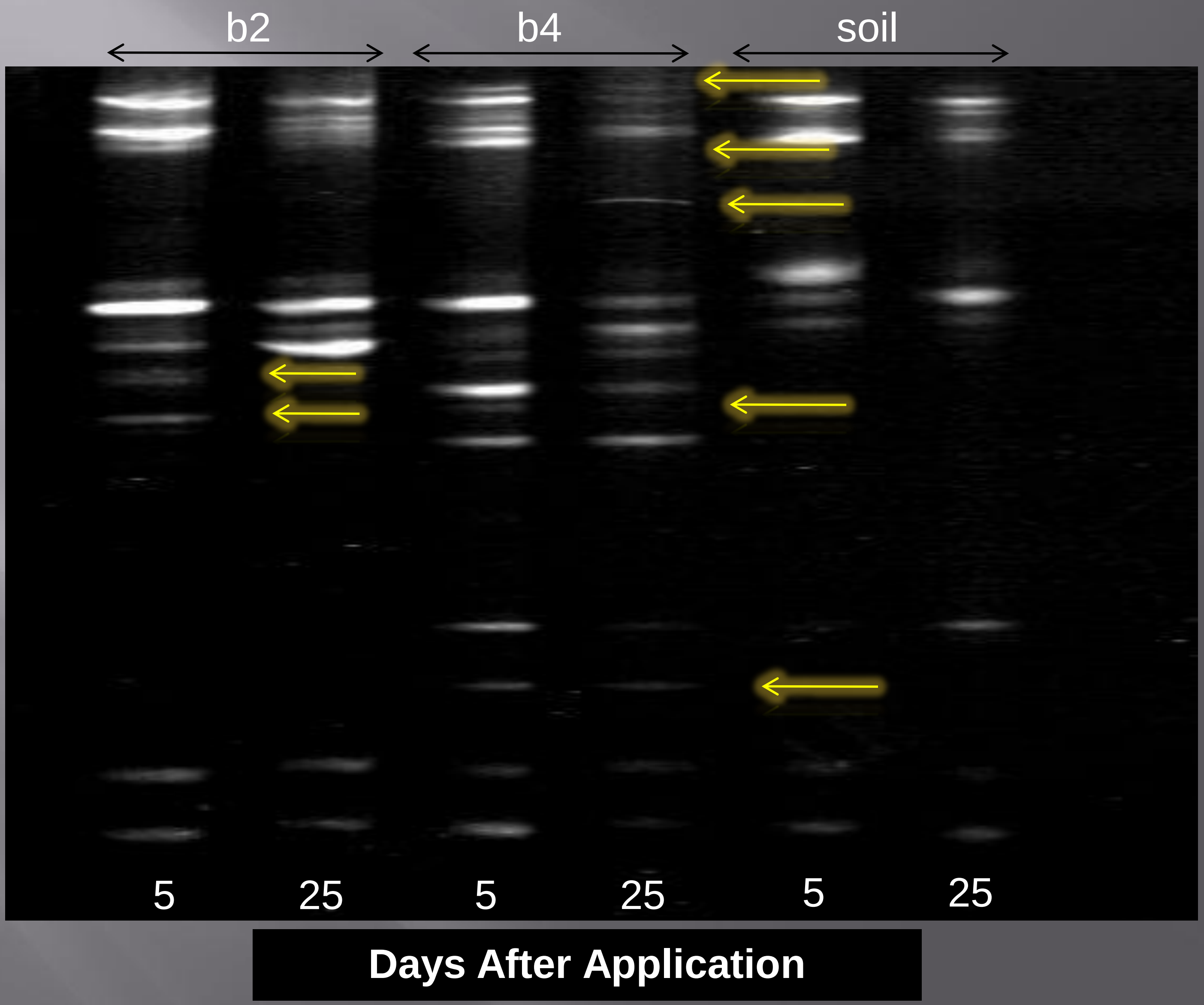
- Microbial community structure
  - *PCR – DGGE for Pseudomonas (common degraders)*
  - *Biomixtures - Samples examined:*
    - *b2, b4 and soil*
    - *5 and 25 days samples (fast degradation phase)*

Microbial respiration

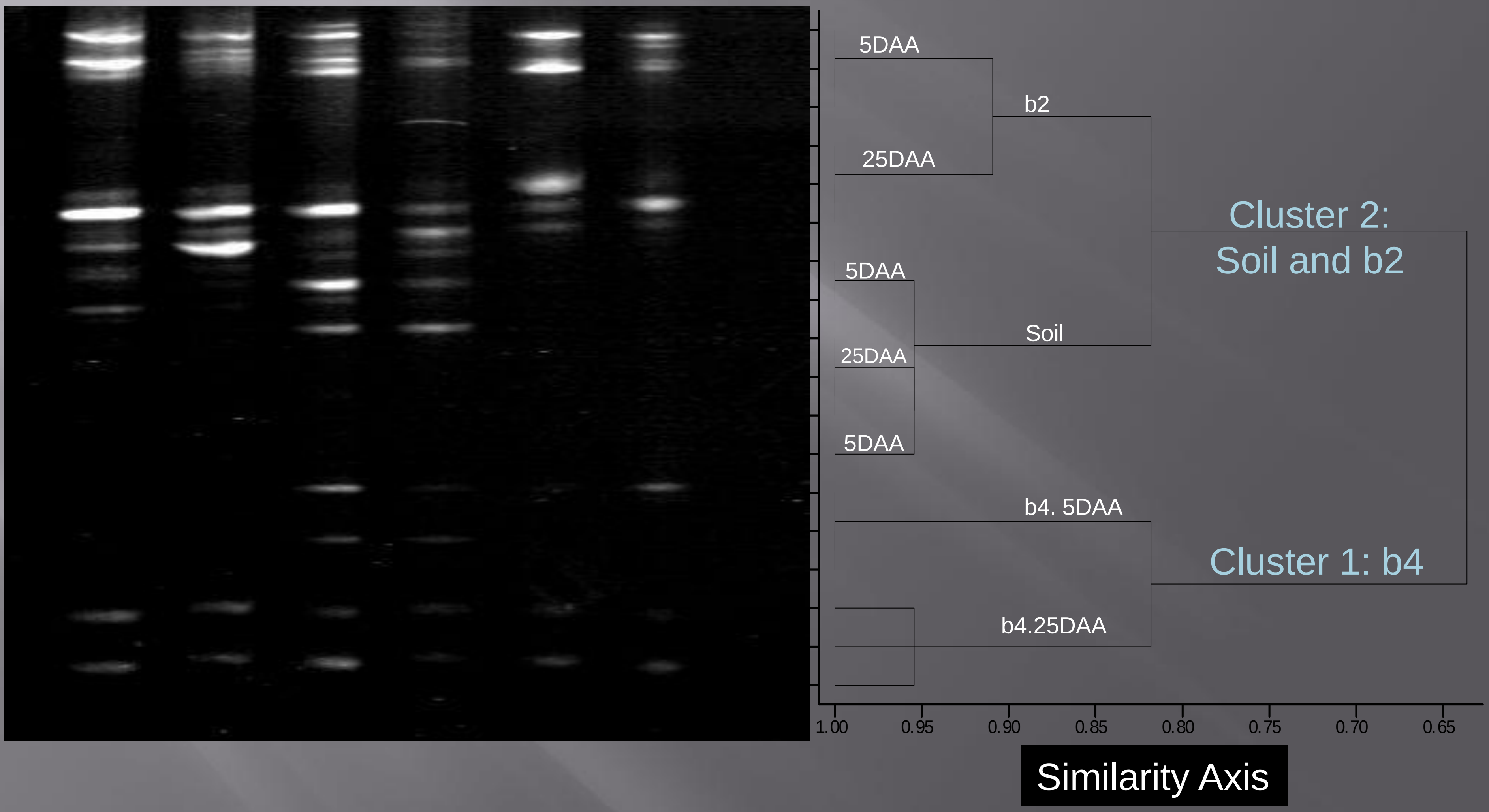
- **Biomixtures b4** and **b5** showed highest microbial respiration
- Lowest microbial respiration in soil



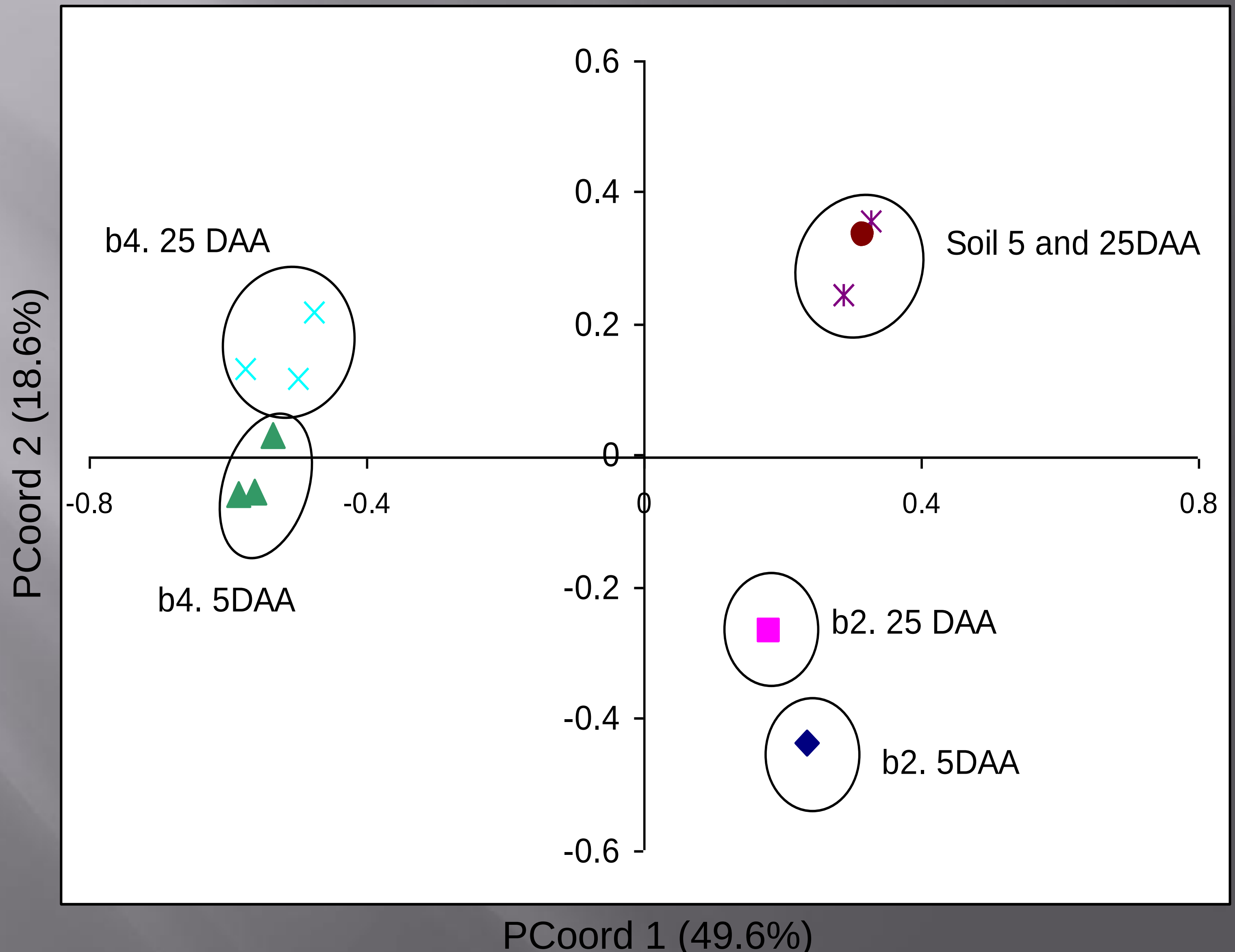
DGGE - Pseudomonas community 5 and 25 days after pesticide application



Hierarchical Cluster analysis



Principle Coordinate Analysis



Conclusions

- Compost-containing biomixtures showed higher degrading efficiency compared to soil
- **Biomixtures containing winery by-products (b4, b5)** were the most effective in the degradation of most pesticides tested
- Respiration activity correlated well with the high degradation capacity of b4 and b5
- *Pseudomonas* community was altered after the addition of the insecticide in biomixtures b2 and b4

Work that is running in ARI

- Identification of *Pseudomonas* potentially involved in pesticide degradation (clone libraries)
- PCR-DGGE of basidiomycetes (just started)

Microbiology of biobeds

- Leaching tubes experiments (running)
  - Different H_2O flows
  - 2 different biomixtures
- Pilot "biobed" plant in the Experimental Research Station of ARI at Zygi (under construction now)

Application of biobeds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Thank you for your attention