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ENEA
Italian National Agency for New Technologies, Energy and Sustainable Economic Development

GLOBAL SYMPOSIUM ON SOIL BIODIVERSITY | 2-5 February 2021

“Restoring the soil while preserving functions: a winning approach by exploiting microbial biodiversity”

- Soil is considered as a non-renewable resource by the European Union, for a total of about 400 million hectares.
- Soil degradation cost could amount to 38 billion Euros/year
- The remediation of soils contaminated by mining activities is, therefore, a strategic objective for European policies.
- Restoration of natural and semi-natural ecosystems can be achieved by natural attenuation which, however, is extremely slow and often not compatible with the environmental risk.
- Environments contaminated by metals generally suffer from low microbial activity.
- The discovery of new microorganisms may increase the huge potential that microorganisms can contribute to the improvement of phytoextraction/stabilization technologies

The case study concerns the Ingurtosu abandoned mine in Sardinia.

The 7-year-long experience is reviewed

Ingurtosu Mine

Galera (PbS)

Sfalerite (ZnS)

2011
FP7-ENV- “UMBRELLA” N° Project
2012

umbrella

Using MicroBes for the REgulation of heavy metaL mobiLity at ecosystem and landscape scAle

- Chemical characterization
- Mapping native plants and soil bacteria
- Plant Growth Promoting Bacteria (PGPB) Identification
- Physiological profile at community-level
- Testing the combination of plants and microbes
- Field trial

Chemical characterization

Environ Sci Pollut Res (2014) 21:6939–6951
DOI 10.1007/s11356-013-2154-3

USING MICROBES FOR THE REGULATION OF HEAVY METAL MOBILITY AT ECOSYSTEM AND LANDSCAPE SCALE

Assessment of the applicability of a “toolbox” designed for microbially assisted phytoremediation: the case study at Ingurtosu mining site (Italy)

Anna Rosa Sprocati · Chiara Alisi · Valentina Pinto · Maria Rita Montereali · Paola Marconi · Flavia Tasso · Katarzyna Turnau · Giovanni De Giudici · Katarzyna Goralska · Marta Bevilacqua · Federico Marini · Carlo Cremisini

Table 1 Soil chemical properties at the beginning of the experiment

	Total content							pH	HM mobility				
	Cu (mg·kg^{-1})	Pb	Zn	Cd	C total (%)	C organic (%)	N total (%)		DZ (mmol·kg^{-1})	CuDTPA (mg·kg^{-1})	PbDTPA	ZnDTPA	Cd DTPA
Mean value	350	3,350	14,740	86	0.51	0.20	0.02	7.6	22.2	11.5	23	550	4.7
Standard deviation	40	520	1,460	10	0.05	0.03	0.003	0.8	1.1	0.4	2	15	0.4
RSD (%)	11	15	10	12	9	15	16	11	5	4	8	3	8

Results are expressed as mean, standard deviation and relative standard deviation (% RSD). The bioavailable metal content (HM mobility) was evaluated using both a rapid test developed in our laboratory (DZ) and the Italian official method for the measurement of plant available fraction with DTPA extraction

2011

Mapping native plants

Cistus salvifolius,

Rosmarinus officinalis,

Ranunculus bullatus,

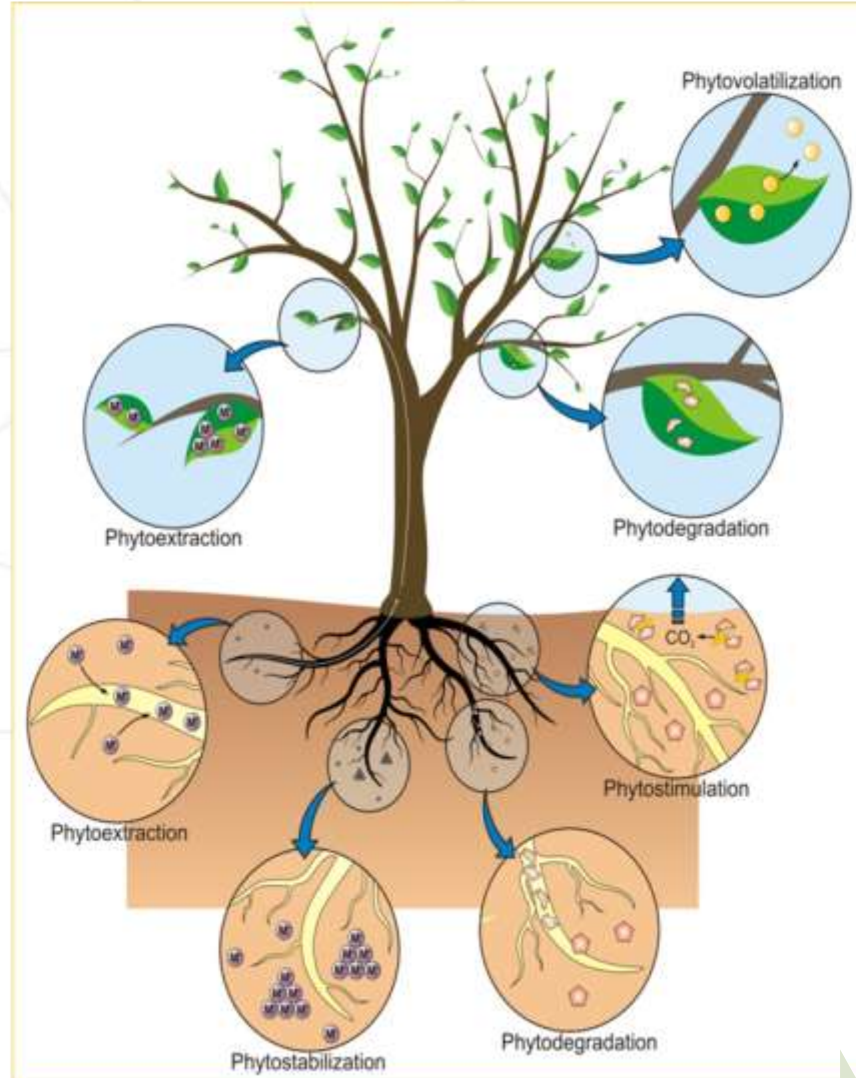
Festuca sp.,

Helichrysum italicum,

Ptilostemon casabonae,

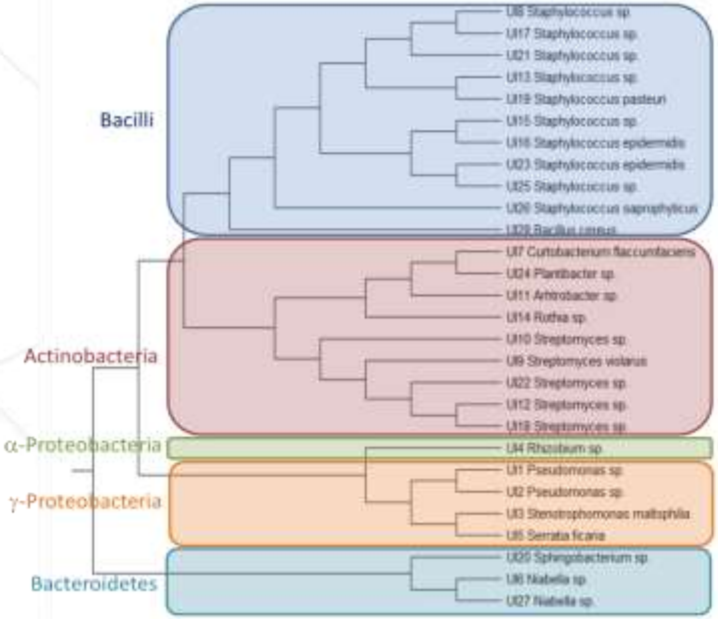
Juncus acutus

Euphorbia pithyusa

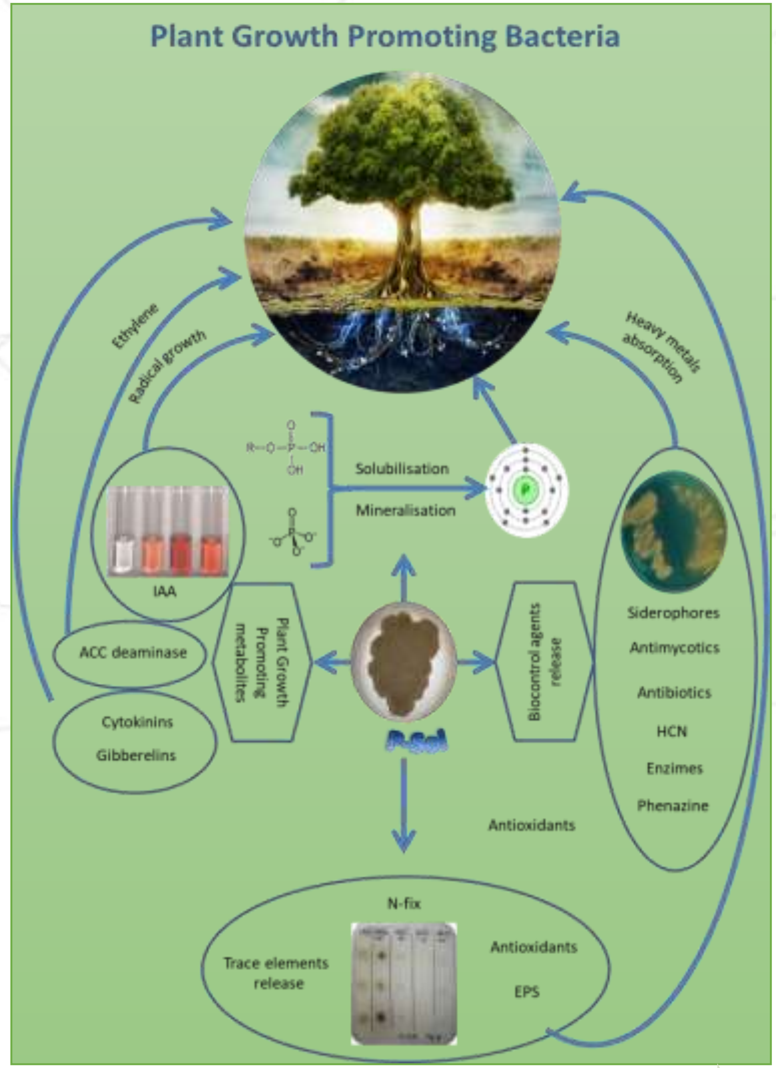


2011

Soil bacteria

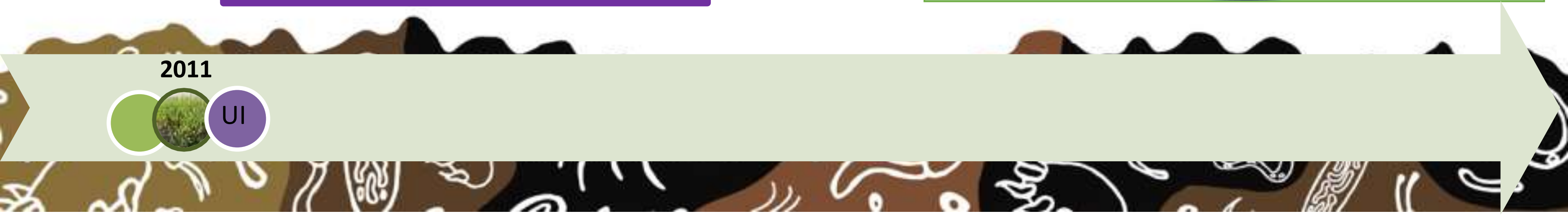


Microbial load CFU/g soil	9*10E6
Colony morphotypes	41
N_2-fix	90%
PO_4 mob	44%
Siderophore producer	63%
Auxine producer	32%
Soil extract (metal resistant)	54%



UI Consortium

UI2 *Pseudomonas sp.*
UI3 *Stenotrophomonas maltophylia*
UI4 *Rhizobiom sp.*
UI6 *Niabella sp.*
UI7 *Curtobacterium flaccumfaciens*
UI9 Streptomyces ambofaciens
UI24 *Plantibacter flavus*
UI27 *Niabella sp.*
UI28 Bacillus cereus



Testing the combination of plants and microbes

Environ Sci Pollut Res
DOI 10.1007/s11356-013-1928-y

USING MICROBES FOR THE REGULATION OF HEAVY METAL MOBILITY AT ECOSYSTEM AND LANDSCAPE SCALE

Plant growth promotion by inoculation with selected bacterial strains versus mineral soil supplements

S. Wernitznig · W. Adlassnig · A. R. Sprocati ·
K. Turnau · A. Neagoe · C. Alisi · S. Sassmann ·
A. Nicoara · V. Pinto · C. Cremisini · I. Lichtscheidl

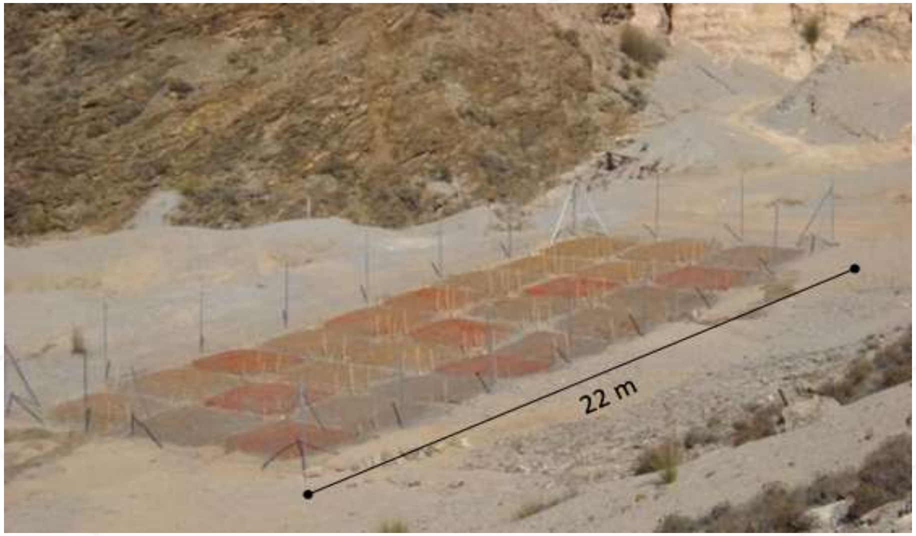
The plants were tested in :

- soil with only autochthonous microflora
- soil with the addition of the native bacterial consortium
- soil with the addition of mycorrhiza

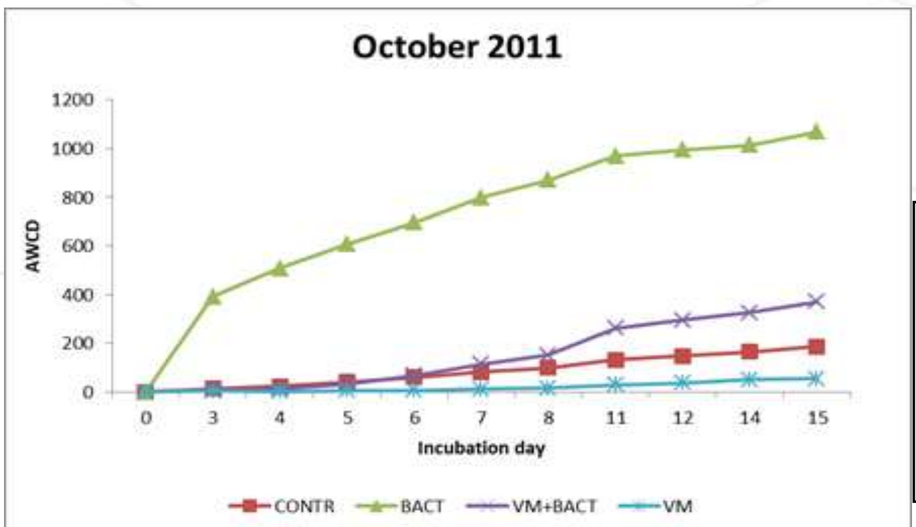
2011

UI

Field trial

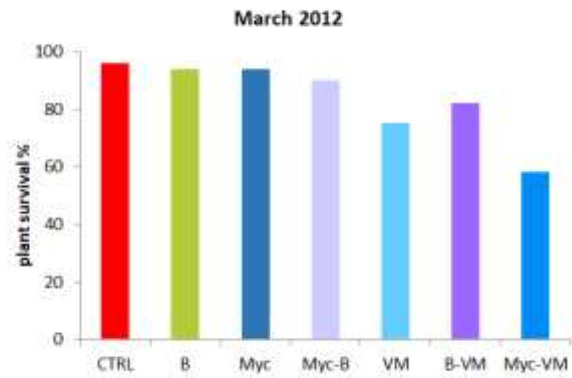


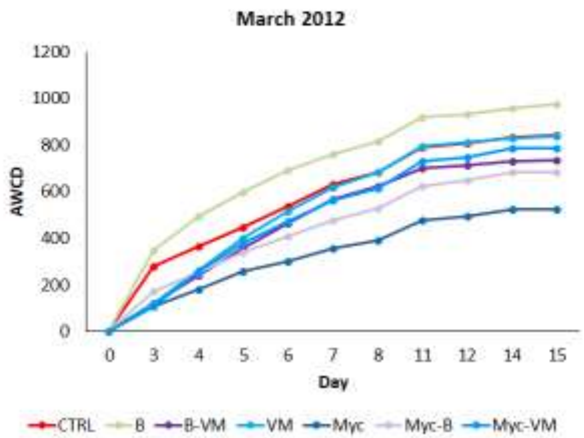
	A	B	C
1	Myc-B	Myc	CTRL
2	B	Myc-B	B-VM
3	Myc	Myc-VM	Myc-B
4	B-VM	CTRL	Myc
5	Myc-VM	B-VM	B
6	CTRL	B	Myc-VM
7	VM	B-S	S
8	S	VM	Myc-B-S
9	B-S	Myc-B-S	VM



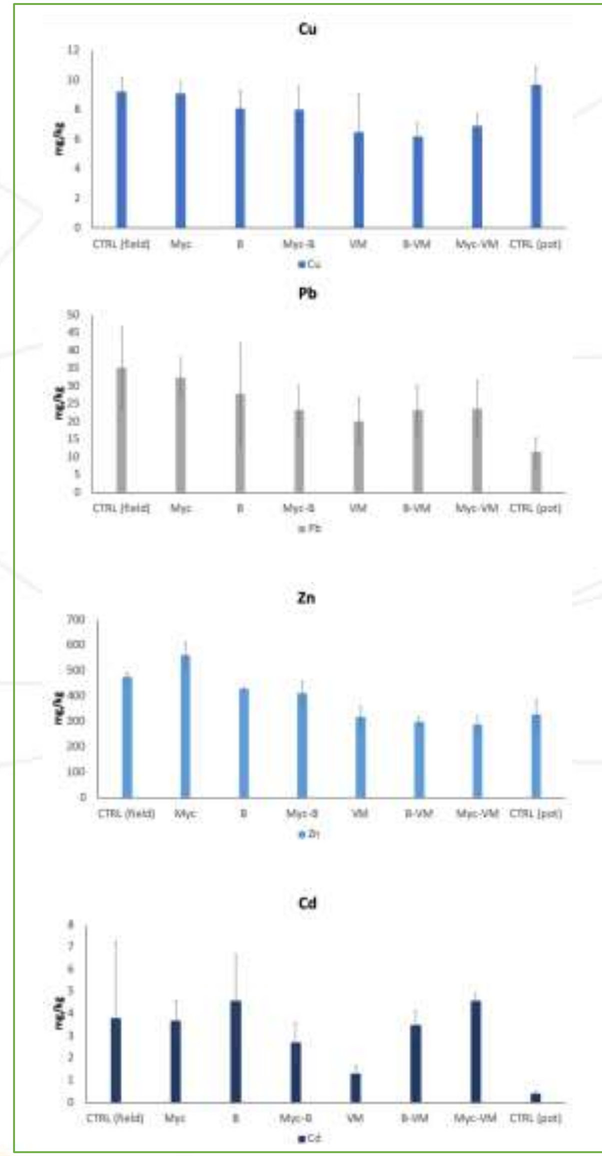
Myc-B	Mycorrhyze + Bacteria	Myc-VM	Mycorrhyze + Viromine™
Myc	Mycorrhyze	VM	Viromine™
CTRL	Control with E. pithyusa	B-S	Bacteria + Bare soil
B	Bacteria	S	Bare soil
B-VM	Bacteria + Viromine™	Myc-B-S	Mychorryze + Bacteria + Bare soil

SOIL





PLANT



2011

UI

2012

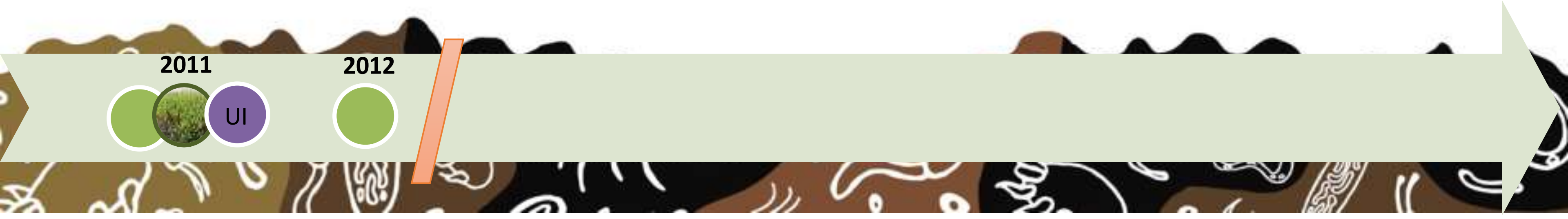
Results at the end of UMBRELLA

Assessment of the toolbox after 5 months:

- Euphorbia pithyusa proved to be a well-performing metallophyte species, which is
- able to absorb, in the aerial part of the plant, Cd, Pb and Zn.
- Despite the cold season 90% of the plants survived and the positive effect of bacteria on metabolic activity in soil was detectable as well
- Multivariate analysis supports the conclusion that the proposed toolbox, composed of endemic *E. pithyusa* and the native UI consortium, can be established in the soil of the mining area of Ingurtosu.

Significant effect of Viromine ™ and its combinations with bacteria and mycorrhizae :

- Soil: higher pH, C org, C tot; reduction of HM mobility , microbial activity improved
- Plants: reduction of Cd and Zn uptake , growth promotion
- Positive correlation between HM mobility test and plant uptake



2013 S.ME.RI Cluster Project 2013-2015

- Enlargement of plant association by introducing *Juncus maritimus*
- Updating the bioaugmentation following the spontaneous changes of microbial community

USMI Consortium

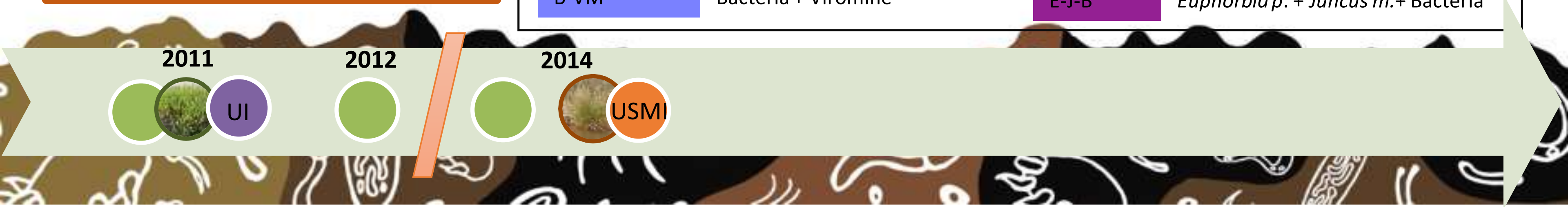
UI2 *Pseudomonas sp.*
UI3 *Stenotrophomonas maltophylia*
UI4 *Rhizobiom sp.*
UI6 *Niabella sp.*
UI7 *Curtobacterium flaccumfaciens*
UI9 Streptomyces ambofaciens
UI24 *Plantibacter flavus*
UI27 *Niabella sp.*
UI28 Bacillus cereus

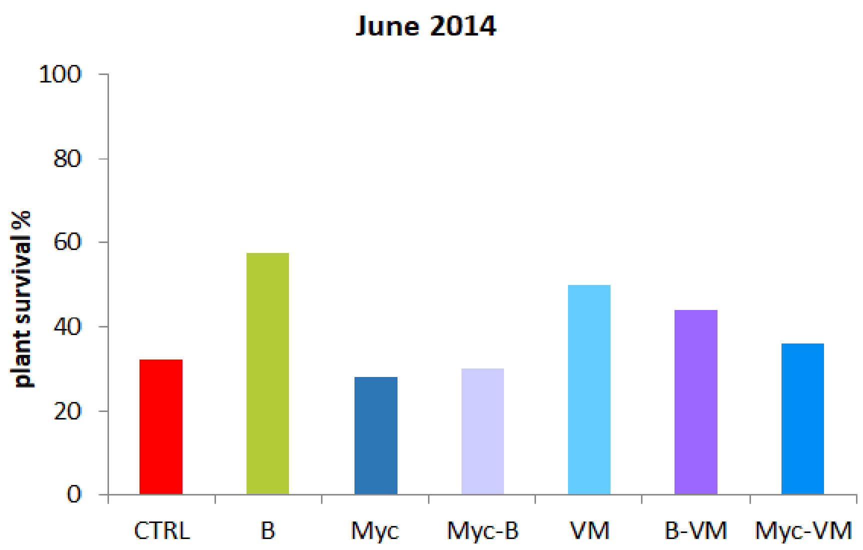
A1.8 *Bacillus ayabhattai*
A8.1 *Micrococcus sp.*
A9 *Streptomyces sp.*
B3.1 *Streptomyces thermocarboxydus*
B5.3 *Bacillus safensis*
B6.1 *Kocuria rhizophila*
B9.2 *Streptomyces bobili*
C3.1 *Streptomyces resistomycificus*

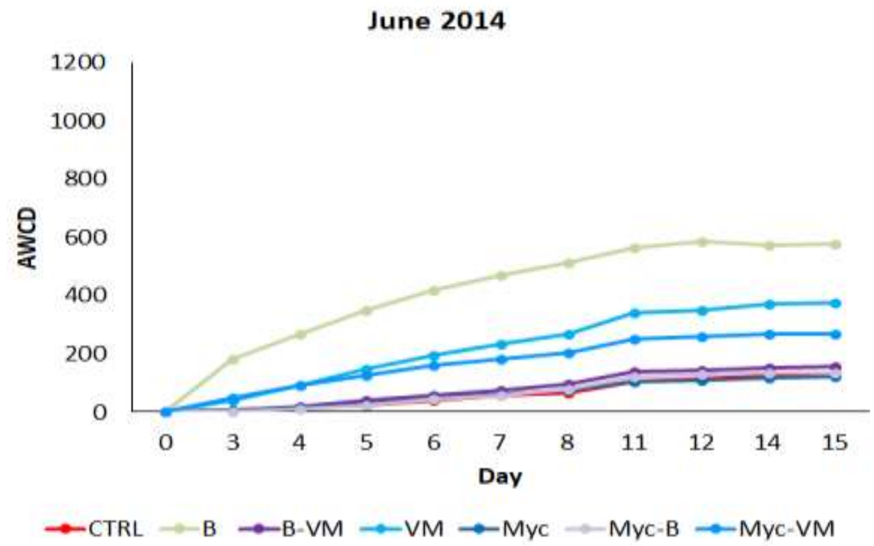
Colony morphotypes	94
N_2-fix	82%
PO_4 mob	35%
Siderophore producer	93%
Auxine producer	21%

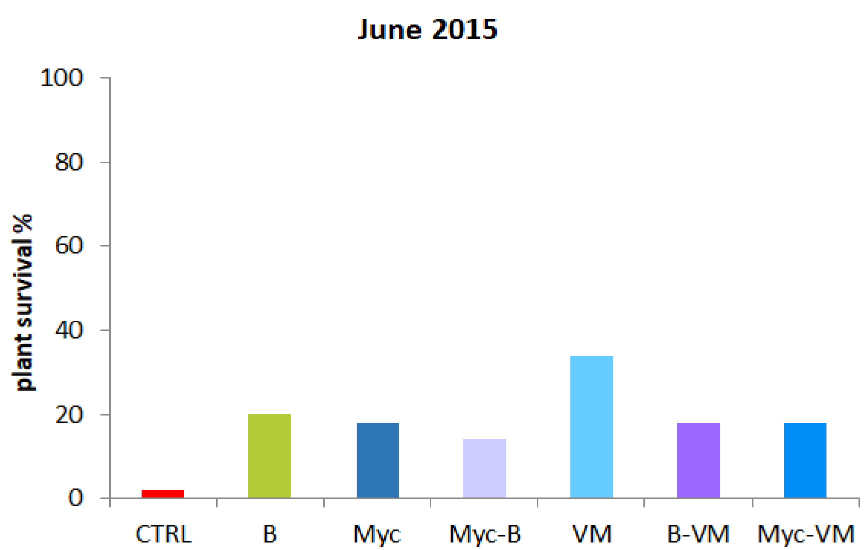
	A	B	C
1	Myc-B-J	Myc-J	CTRL
2	B	Myc-B-J	B-VM
3	Myc-J	Myc-VM-J	Myc-B-J
4	B-VM	CTRL	Myc-J
5	Myc-VM-J	B-VM	B
6	CTRL	B	Myc-VM-J
7	VM	E-J-B	E-J
8	E-J	VM	E-J-B
9	E-J-B	E-J	VM

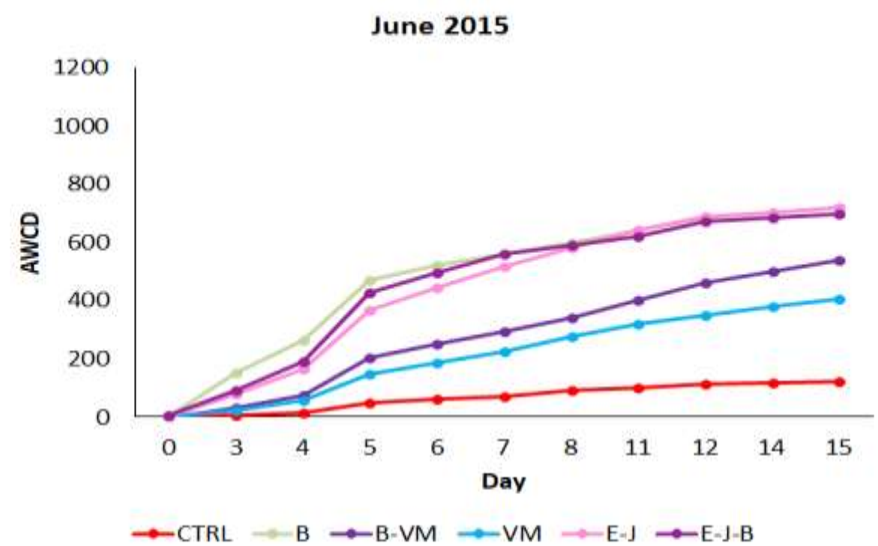
Myc-B-J	Mycorrhyze + Bacteria + *Juncus m.*
Myc-J	Mycorrhyze + *Juncus m.*
CTRL	Control with E. pithyusa
B	Bacteria
B-VM	Bacteria + Viromine™
Myc-VM-J	Mycorrhyze + Viromine™ + *Juncus m.*
VM	Viromine™
B-S	Bacteria + Bare soil
E-J	*Euphorbia p.* + *Juncus m.*
E-J-B	*Euphorbia p.* + *Juncus m.*+ Bacteria




June 2014
plant survival %
100
80
60
40
20
0
CTRL
B
Myc
Myc-B
VM
B-VM
Myc-VM


June 2014
AWCD
1200
1000
800
600
400
200
0
0
3
4
5
6
7
8
11
12
14
15
Day
CTRL
B
B-VM
VM
Myc
Myc-B
Myc-VM


June 2015
plant survival %
100
80
60
40
20
0
CTRL
B
Myc
Myc-B
VM
B-VM
Myc-VM


June 2015
AWCD
1200
1000
800
600
400
200
0
0
3
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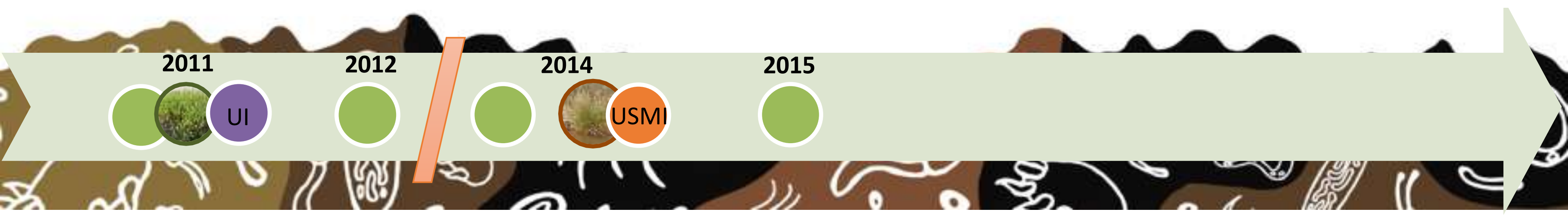
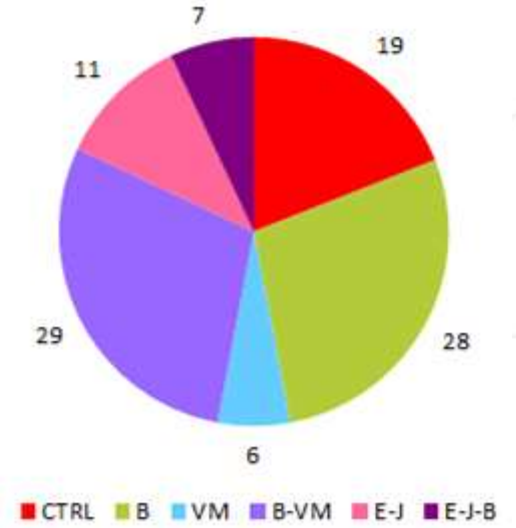
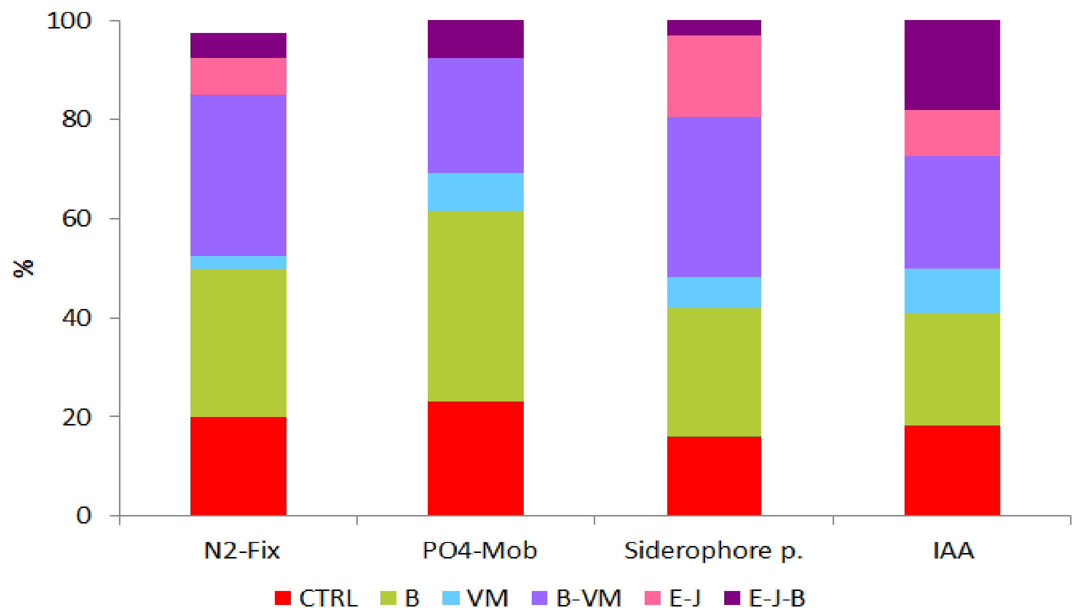
5
6
7
8
11
12
14
15
Day
CTRL
B
B-VM
VM
E-J
E-J-B


2011
UI
2012
2014
USMI
2015

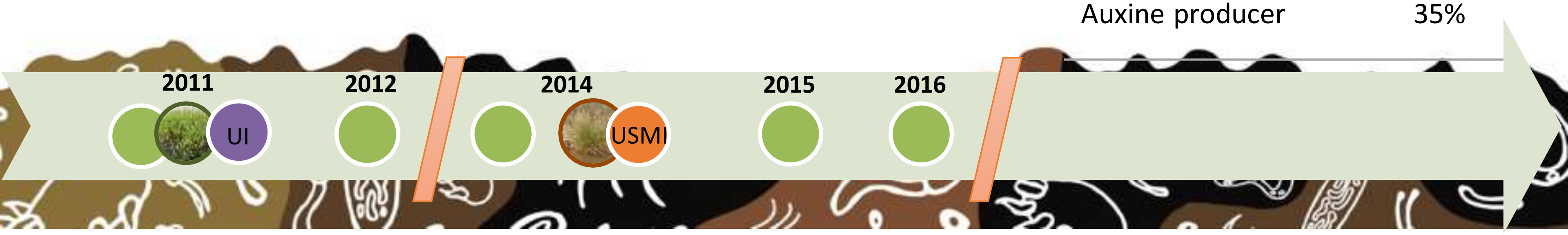
2016

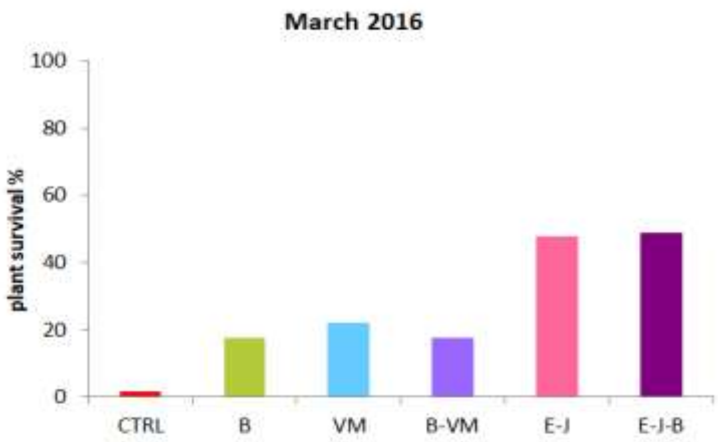
	CTRL	B	VM	B-VM	E-J	E-J-B
CFU*10^6/g soil	7,3	5,4	2,7	17,4	1,2	3,5
PGPB %	19	28	6	29	11	7

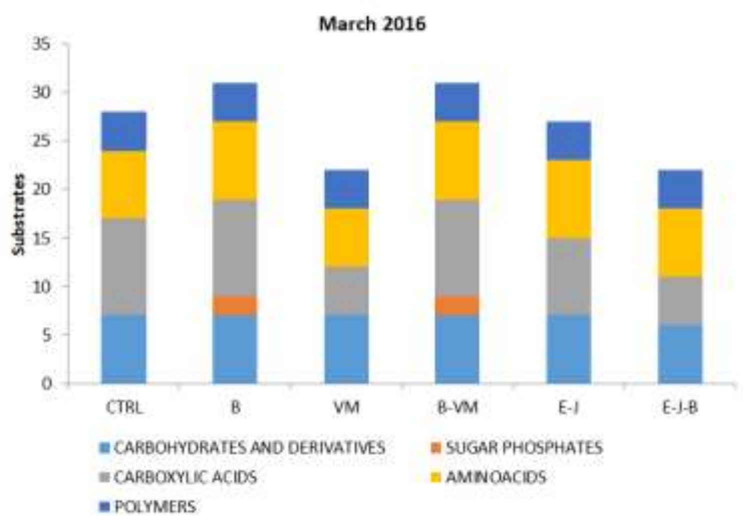


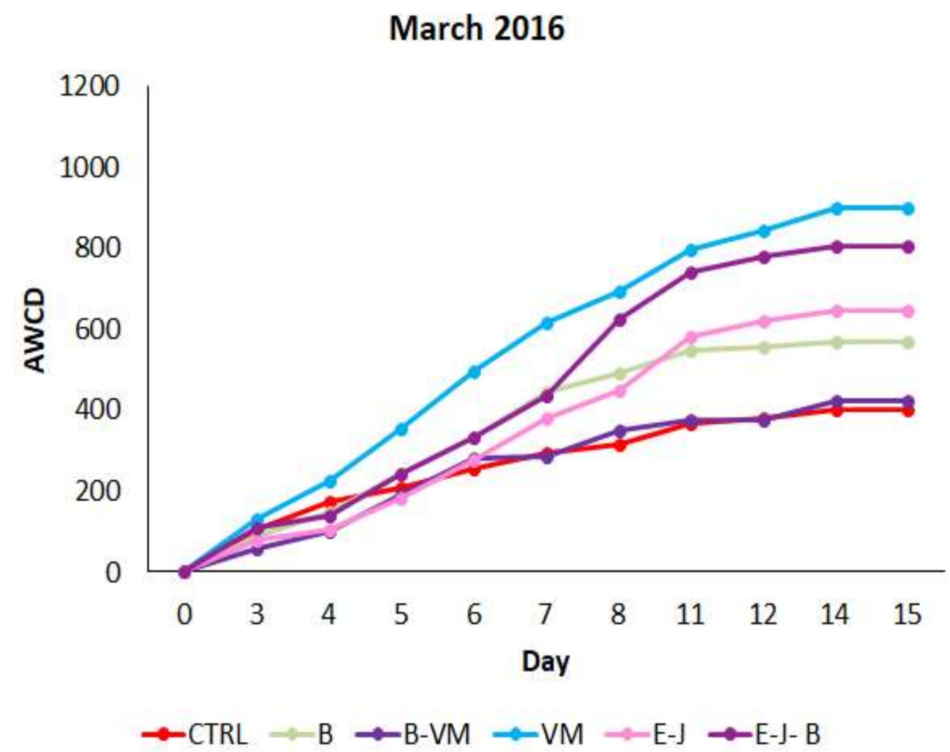


Colony morphotypes	63
N_2-fix	63%
PO_4 mob	21%
Siderophore producer	49%
Auxine producer	35%




March 2016
plant survival %
100
80
60
40
20
0
CTRL
B
VM
B-VM
E-J
E-J-B


March 2016
Substrates
35
30
25
20
15
10
5
0
CTRL
B
VM
B-VM
E-J
E-J-B
CARBOHYDRATES AND DERIVATIVES
SUGAR PHOSPHATES
CARBOXYLIC ACIDS
AMINOACIDS
POLYMERS


March 2016
AWCD
1200
1000
800
600
400
200
0
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3
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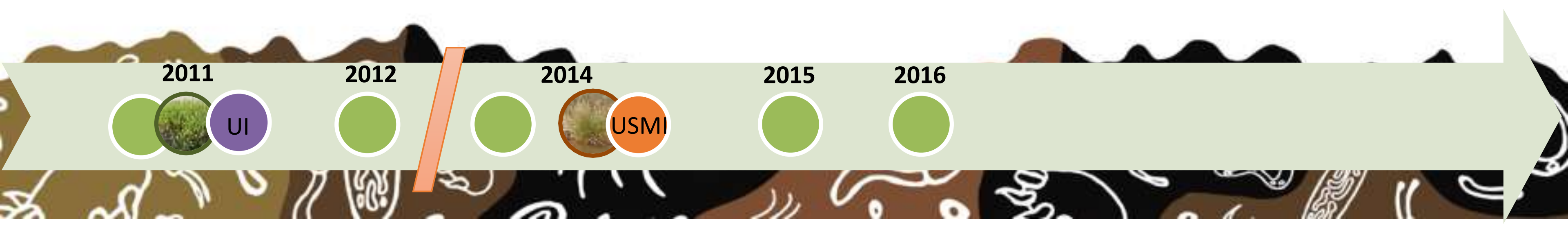
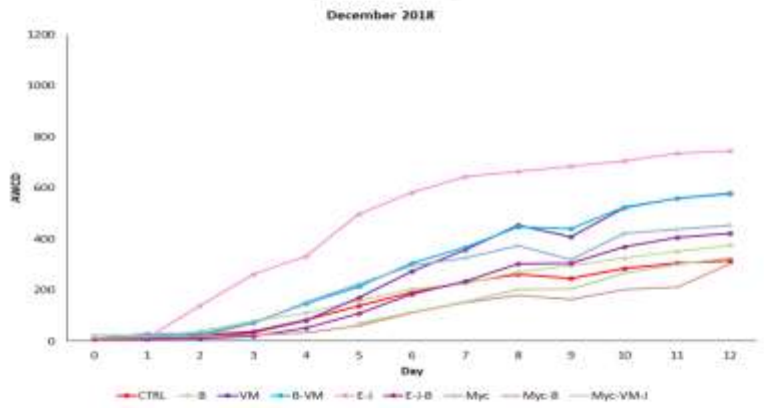
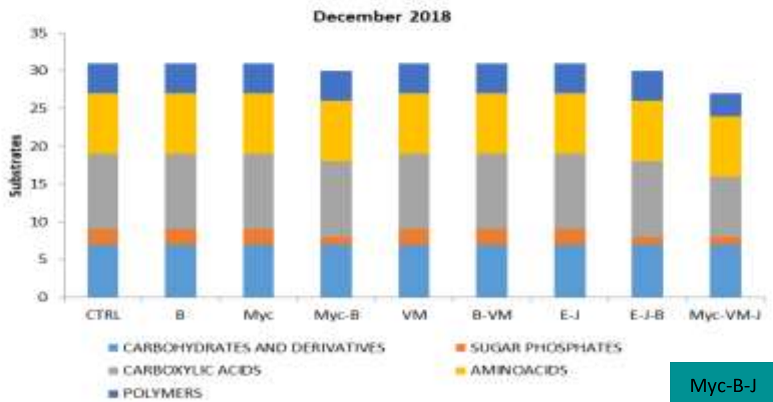
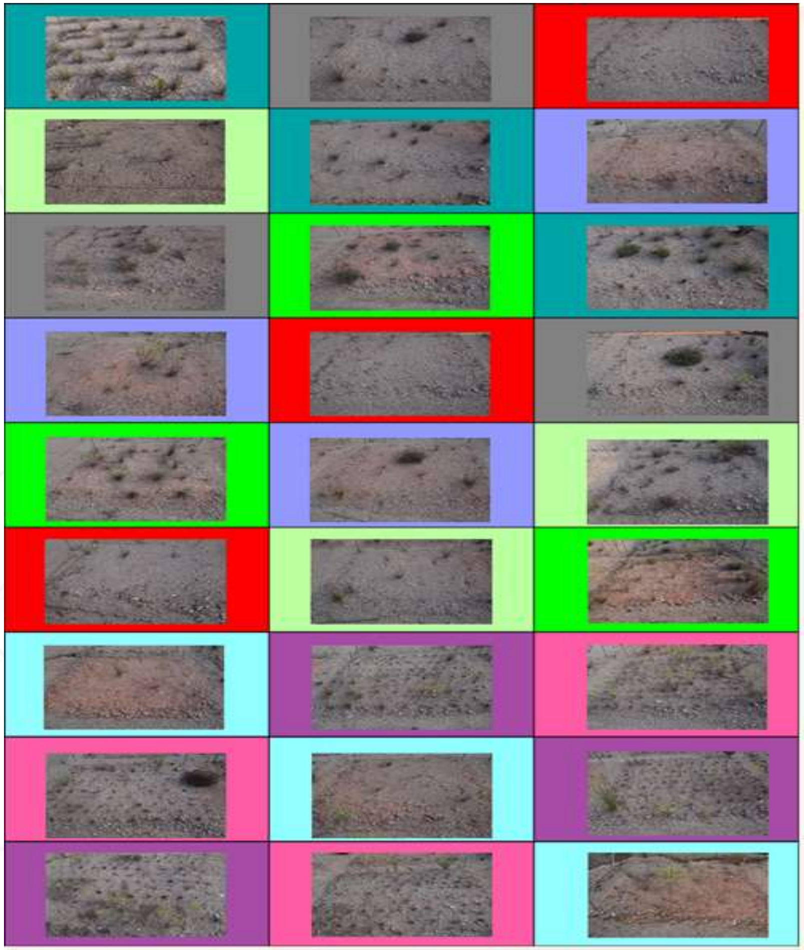
14
15
Day
CTRL
B
B-VM
VM
E-J
E-J- B


2011
UI
2012
2014
USMI
2015
2016

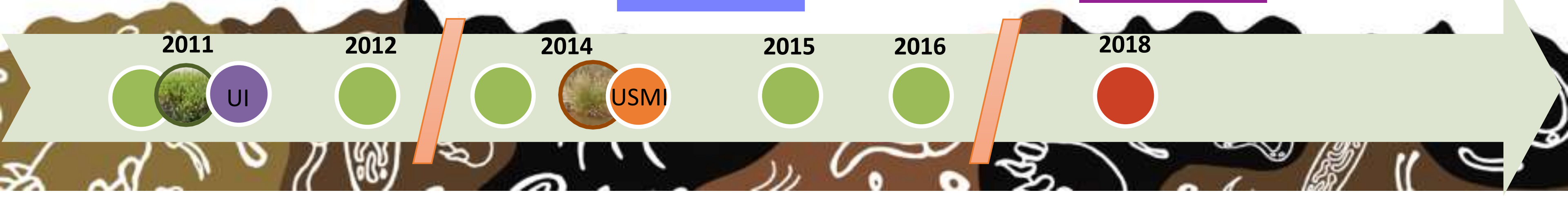
2018



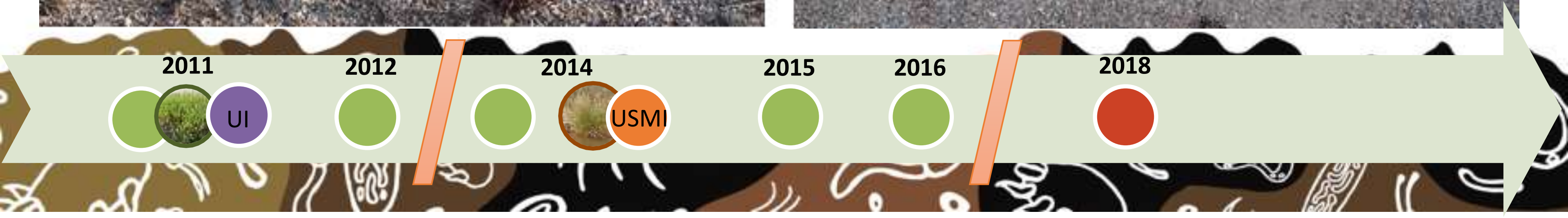




Myc-B-J	Mycorrhyze + Bacteria + *Juncus m.*	Myc-VM-J	Mycorrhyze + Viromine™ + *Juncus m.*
Myc-J	Mycorrhyze + *Juncus m.*	VM	Viromine™
CTRL	Control with E. pithyusa	B-S	Bacteria + Bare soil
B	Bacteria	E-J	*Euphorbia p.* + *Juncus m.*
B-VM	Bacteria + Viromine™	E-J-B	*Euphorbia p.* + *Juncus m.*+ Bacteria




CTRL
B
Myc-B-J
E-J-B


2011
UI
2012
2014
USMI
2015
2016
2018

In conclusion...

- Bioaugmentation with a consortium of selected endemic bacteria enhances PGP functions in the soil and allows their maintenance over time
- The inoculum directs and shapes community development as demonstrated by the increased metabolic activity and functional diversity
- The positive effect of bioaugmentation on plant survival gradually decreases over 5 years, in the absence of field management.
- It is necessary to define guidelines and protocols that include repeated interventions over time to maintain high levels of microbial activity and functional diversity in the soil.
- The combination of bioaugmentation with ViroMineTM treatment gave the best results in terms of soil metabolic activity, long-term plant survival and maintenance and stabilization of effects over time.
- A rational selection of the microbial inoculum, that takes into account the ecological context can help to capture and exploit the intrinsic bioremediation potential of contaminated environmental systems. Such processes take time and energy to achieve equilibrium before showing real benefits, but they represent a sustainable, low-impact, low-cost solution.


Thank you
for your
attention