# Recovery of microbiological status with organic amendments on soils affected by mining activity in a decadal temporal scale.


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# LABORATORIO DE MICROBIOLOGÍA DE SUELOS

Centro de Investigación en Agrosistemas Intensivos Mediterráneos y Biotecnología Agroalimentaria (CIAMBITAL)
Universidad de Almería (UAL)

ANÁLISIS DE PROPIEDADES MICROBIOLÓGICAS (METAGENÓMICA)

ANÁLISIS DE PROPIEDADES FÍSICAS Y QUÍMICAS

MICROSCOPÍA ÓPTICA Y ELECTRÓNICA

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## MIEMBROS DEL LABORATORIO

RNM-934: Agronomía y Medio Ambiente (AGROMA)

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# Introduction

*Almería, SE Spain*

*Source: Albert Solé*

*Source: Isabel Miralles*

Opencast mining for aggregate extraction

+

Semiarid climate with extreme weather conditions

+

high solar radiation

+

loss plant cover

↓

**Completely degraded soil with high erosion rates**

**Limitations on physical, chemical and biological properties**

*Luna et al., 2016*

# Introduction

*Almería, SE Spain*

Opencast mining for aggregate extraction

+

Semiarid climate with extreme weather conditions

+

high solar radiation

+

**Loss of productivity and soil fertility**

(decreasing organic matter, N, structure, etc)

**Loss of soil quality**

**Loss of ecosystem functionality**

**DESERTIFICATION!!**

*2016*

# Introduction

Why use organic amendments?

Organic amendments → Restoration → Accelerates recovery → Improve soil properties

**Soil microorganims could be excellent indicators to evaluate quality recover in restoring soils**

**Soil bacteria communities**

**Improve soil structural formation**

*(Bender et al., 2016)*

**Contribute to plant establishment**

*(Zink and Allen, 1998)*

**Organic matter transformation**

*(Zhao et al., 2019)*

**Improve biogeochemicals cycles (N, C, P)**

*(Bastida et al., 2015)*

# Introduction

# Introduction

**Experimental plots 2008**

Experimental soil restoration in limestone quarry in semiarid climate (RMN-5887)

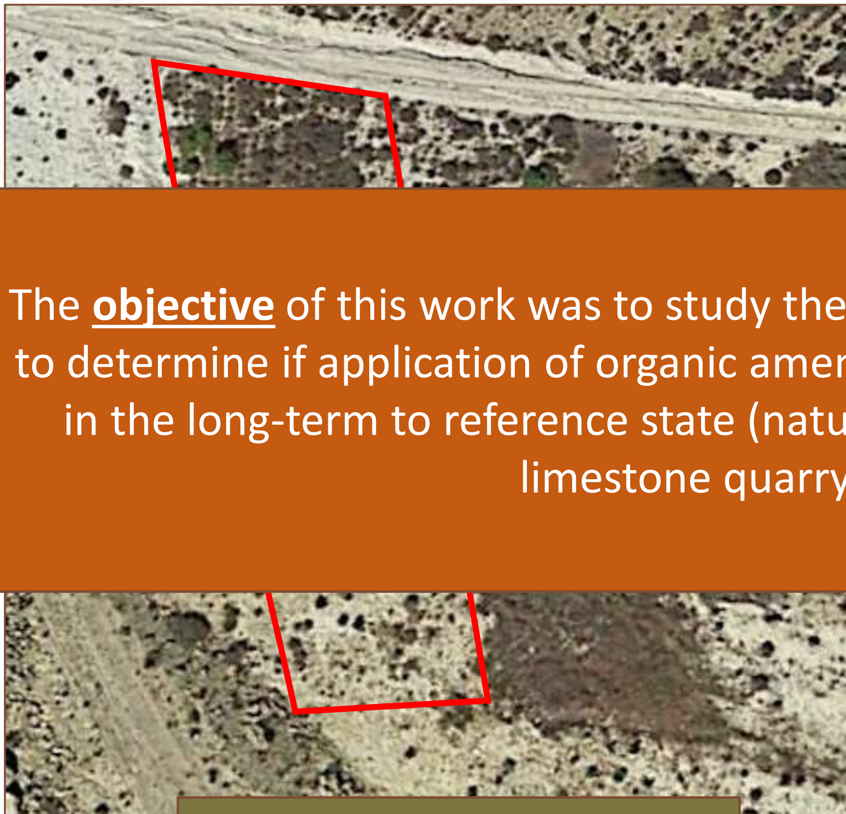
**Experimental plots 2018**

New approacehes to soil restoration in semi-arid environments $CO_2$ Flows and molecular indicators (CGL2017-88734-R; BIORESOC)

# Introduction

**Long term study**

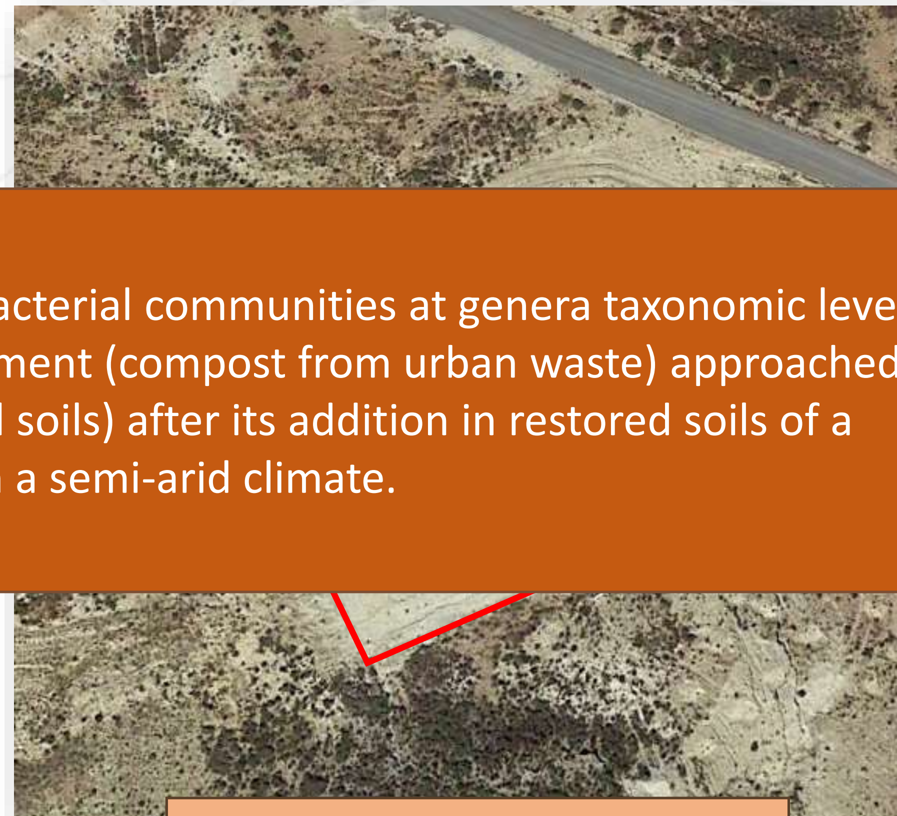
**Experimental plots 2008**



**Experimental soil restoration in limestone quarry in semiarid climate (RMN-5887)**

**Short term study**

**Experimental plots 2018**



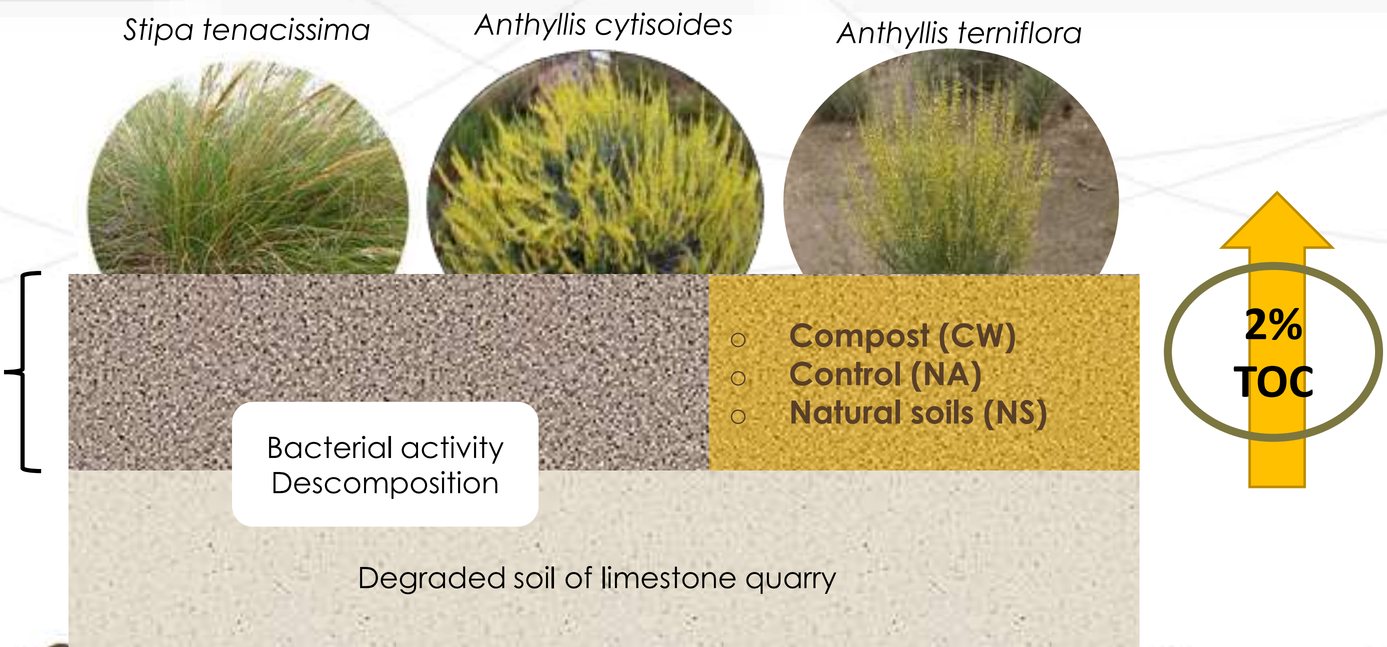
**New approacehes to soil restoration in semi-arid environments $CO_2$ Flows and molecular indicators (CGL2017-88734-R; BIORESOC)**

The **objective** of this work was to study the bacterial communities at genera taxonomic level to determine if application of organic amendment (compost from urban waste) approached in the long-term to reference state (natural soils) after its addition in restored soils of a limestone quarry in a semi-arid climate.

# Methodology experimental plots 2008 (Luna et al., 2016)

75 m$^2$ surfaces (15 m × 5 m)

20 cm depth
Organic
amendments
addition

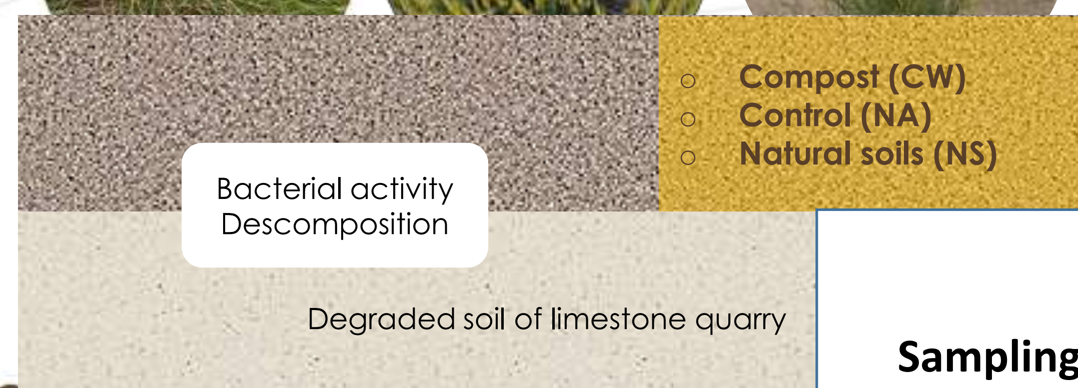



# Methodology experimental plots 2008 (Luna et al., 2016)

75 m² surfaces (15 m × 5 m)

*Stipa tenacissima* *Anthyllis cytisoides* *Anthyllis terniflora*

20 cm depth Organic amendments addition




2%
TOC

**Sampling 10 years later**
**10 cm depth, 3 replies**

# Methodology experimental plots 2008 (Luna et al., 2016)

75 m$^2$ surfaces (15 m × 5 m)

*Stipa tenacissima*

*Anthyllis cytisoides*

*Anthyllis terniflora*

20 cm depth
Organic amendments addition


**2% TOC**

**OBJETIVES**

- **Chemical properties**
- **Bacterial taxa**
- **Relatioship between bacteria and chemical properties**

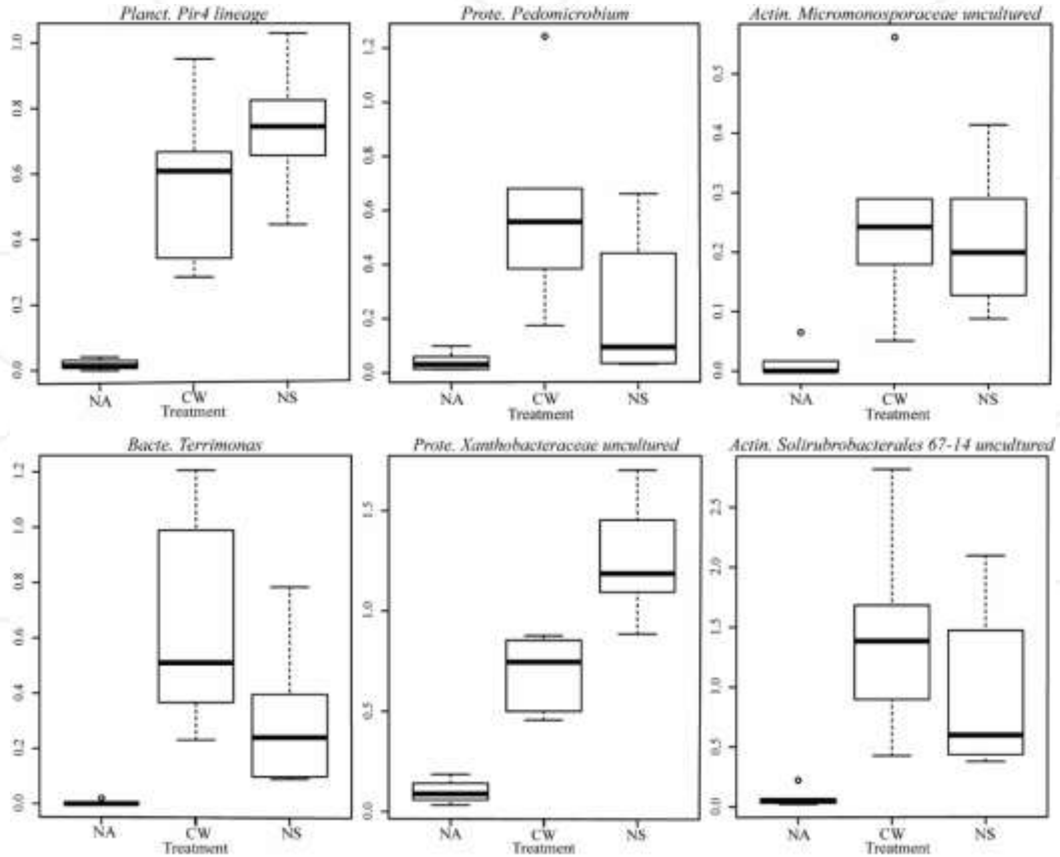
# Methodology experimental plots 2008 (Luna et al., 2016)

**Test carried out**

- **Chemical properties analysis**
  - Total Organic Carbon (TOC)
  - pH
  - Total Nitrogen (TN)
- **Metagenomic analysis**
  - DNA Extraction by comecial kit
  - Illumina sequencing
- **Bioinformatic and biostatistics analysis**
  - Qiime software
  - General Lineal Models
  - Pearson’s correlation

# Results

- 162 soil bacteria taxa with abundance upper 0.1%.
- GLMs showed compost from urban waste (CW) significantly influenced ($p<0.05$) the 59% of bacterial taxa and 14% by natural soils (NS).
- Boxplots revealed some bacteria taxa most abundant in both compost (CW) and natural soils (NS).



# Results

- **Chemical soil properties**

| | pH | TOC | TN |
|---|---|---|---|
| **Compost (CW)** | 8.0 ± 0.1 a | 3.4 ± 1.5 a | 0.5 ± 0.2 a |
| **Reference Soil (NS)** | 8.1 ± 0.1 a | 3.1 ± 0.5 a | 0.3 ± 0.1 c |
| **No Amendment (NA)** | 8.7 ± 0.1 b | 0.2 ± 0.0 b | 0.02 ± 0.01 b |

Values represent average ± standard deviation ($p$<0.05).
Letters show statistical differences between treatments.

# Results

Pearson's correlation analysis of soil bacteria taxa that were common between CW-treated soils and natural soils and chemical soil properties showed significant ($p < 0.05$) and positive correlations with TOC and TN and significant negative correlation with soil pH.

| | pH | TOC | TN |
|---|---|---|---|
| *Actin. Blastococcus* | *-0,336* | 0,305 | 0,416 |
| *Actin. Geodermatophilus* | *-0,498* | 0,561 | 0,612 |
| *Actin. Micromonosporaceae uncultured* | *-0,629* | 0,784 | 0,717 |
| *Actin. Solirubrobacterales 67-14 uncultured* | *-0,651* | 0,680 | 0,747 |
| *Bacte. Chryseolinea* | *-0,414* | 0,452 | 0,115 |
| *Bacte. Terrimonas* | *-0,731* | 0,725 | 0,770 |
| *Planc. Pir4 lineage* | *-0,761* | 0,852 | 0,736 |
| *Planc. Singulisphaera* | *-0,552* | 0,551 | 0,351 |
| *Prote. Pedomicrobium* | *-0,615* | 0,802 | 0,805 |
| *Prote. Xanthobacteraceae uncultured* | *-0,636* | 0,709 | 0,378 |

# Discussion

- Compost treatment (CW) promoted in the long-term soil restoration, showing TOC and TN values similar to natural soils (NS).
- Organic amendments have contributed in different studies to increase and improve the microbial proliferation of the soil (Bastida et al., 2008; Tejada et al., 2006).
- Type of organic matter and plant residues influence the composition of soil microbial communities (Kramer and Gleixner, 2008).
- The soil treated with compost was the most influential in the bacterial taxa filling us to believe that this organic amendment favoured the bacterial proliferation due to the improvement of the chemical properties of the soil which in turn favoured increased plant growth by Luna et al. (2016) five years after restoration.
- Interestingly, these bacterial taxa were present almost exclusively in soils amended with compost and natural soils as showed boxplots.
- May have been the organic amendment of compost was the one that came closest to the reference soils, confirming again that it is possibly due to the vegetal stabilization that favours rhizospheric niches and also to the improvement of the chemical properties of the soil (Bastida et al., 2016).

# Discussion

- Several common bacterial taxa among CW-treated and NS soils also showed significant positive correlations with soil TOC and TN (Table 1). Besides, bacterial taxa as *Pedomicrobium* was observed in soils rich in organic matter (Lima et al., 2015) or as *Terrimonas* that was found in developed soils near our study area (Sánchez-Marañón et al., 2017), corroborating that compost-treated soils improved their quality in ten years.
- Similarly, these bacterial taxa also strong negative correlations with soil pH (Table 1). Lauber et al. (2009) established that soil pH plays a crucial role on soil microbial composition which drive changes in soil bacterial taxa.

# Conclusions

These results suggest that long-term restored soils with compost treatment have established microbial communities similar to surrounding reference natural soils. These results show that compost management of urban waste is a suitable method to recover microbial communities of degraded soils in a temporal scale.

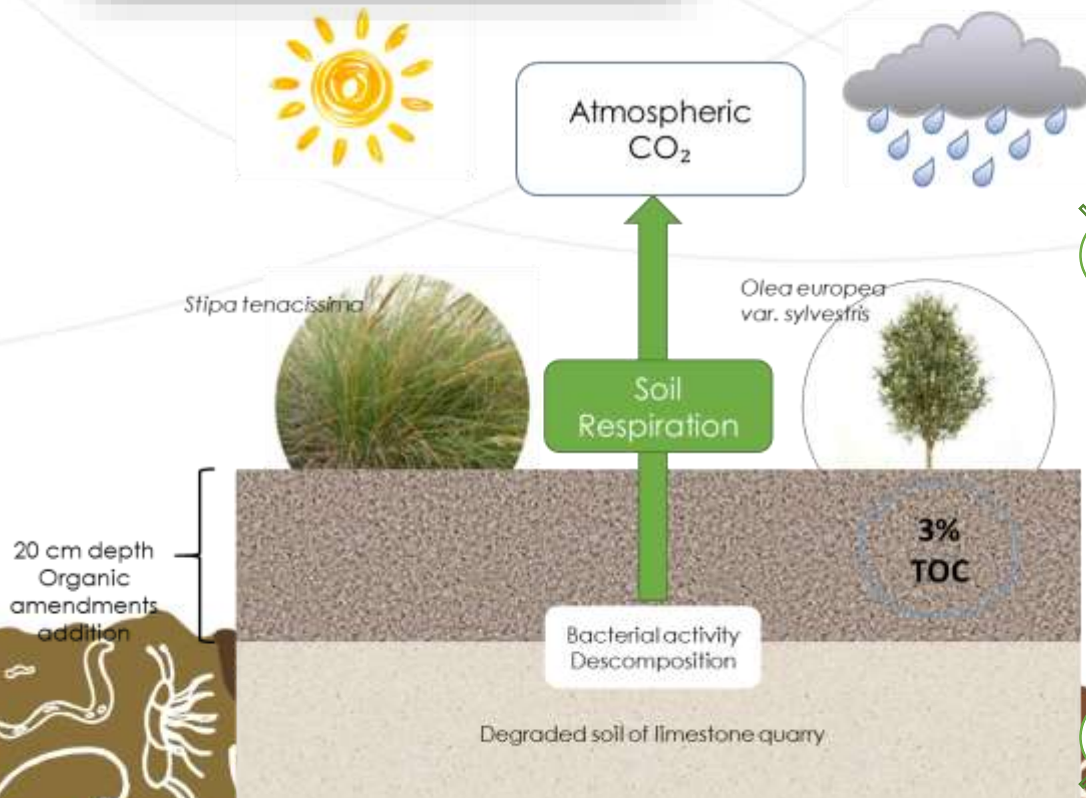
# At present, we are working on plots installed in 2018, in the same limestone quarry, studying the effect of the amendments from the moment of their application over time.

Natural reference ecosystem

vegetable compost garden waste

vegetable compost from horticulture greenhouse crop residues

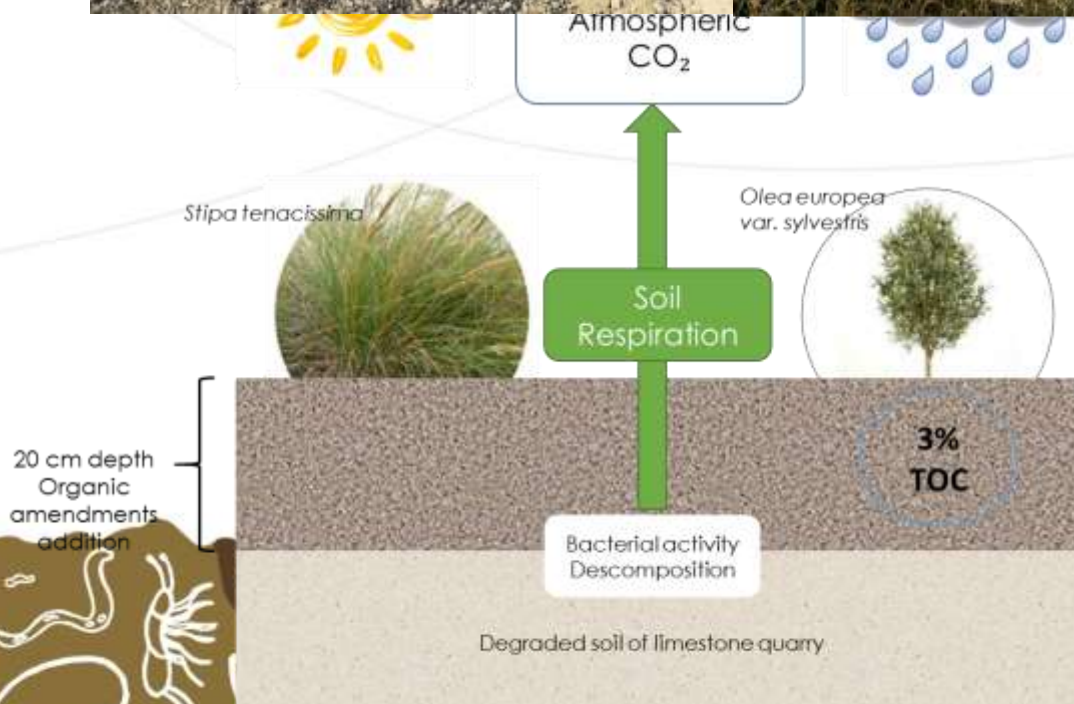
stabilized sewage sludge




- vegetable compost garden waste
- vegetable compost from horticulture crop residues
- stabilized sewage sludge
- mixtures amendments
- mixtures amendments
- Control (no amendment)

# At present, we are working on plots installed in 2018, in the same limestone quarry, studying the effect of the amendments from the moment of their application over time.



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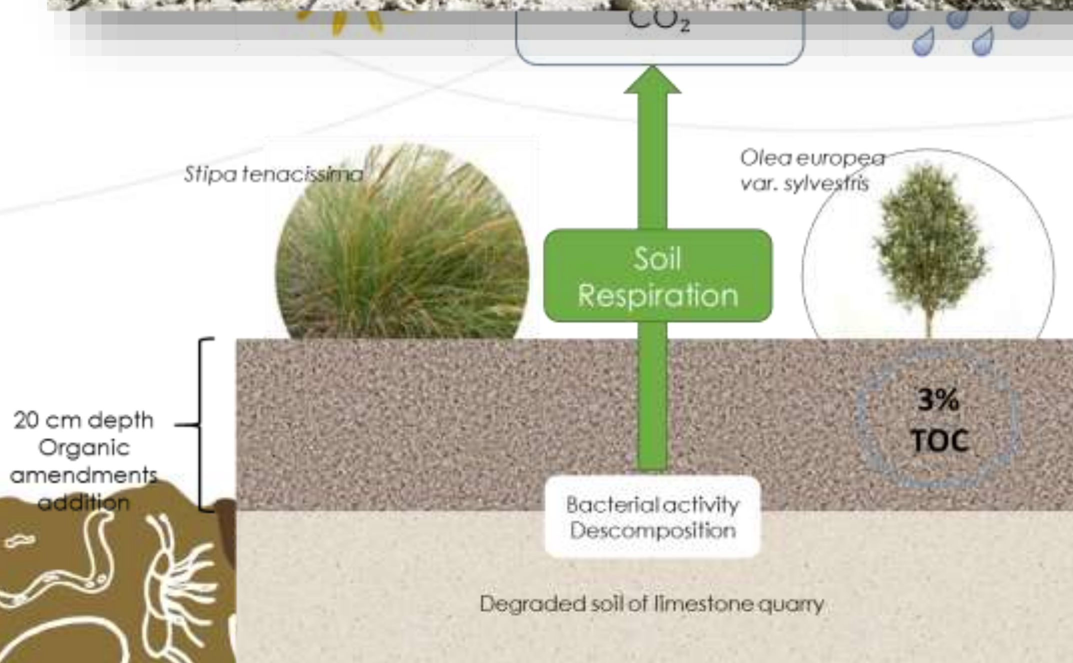


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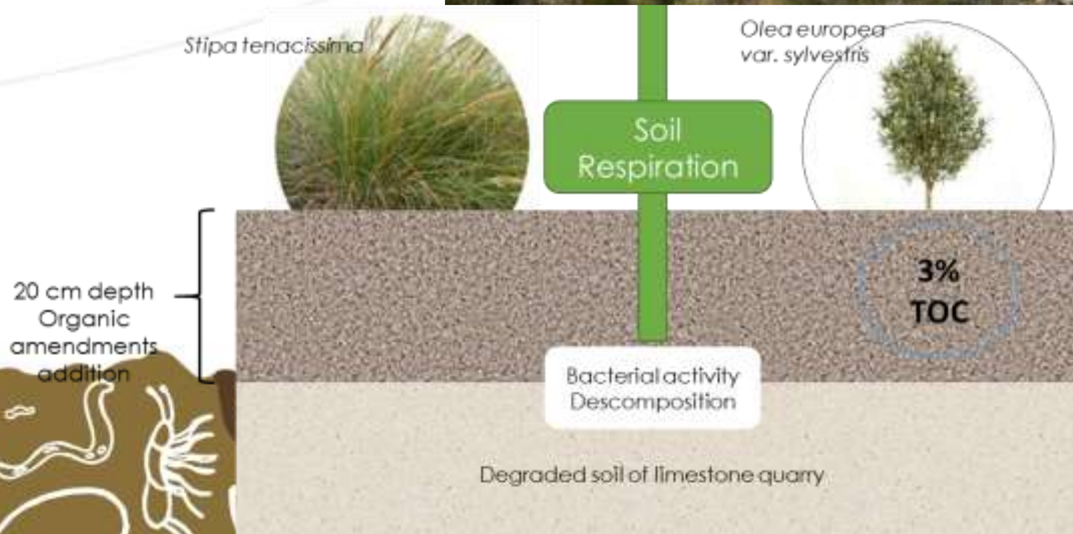


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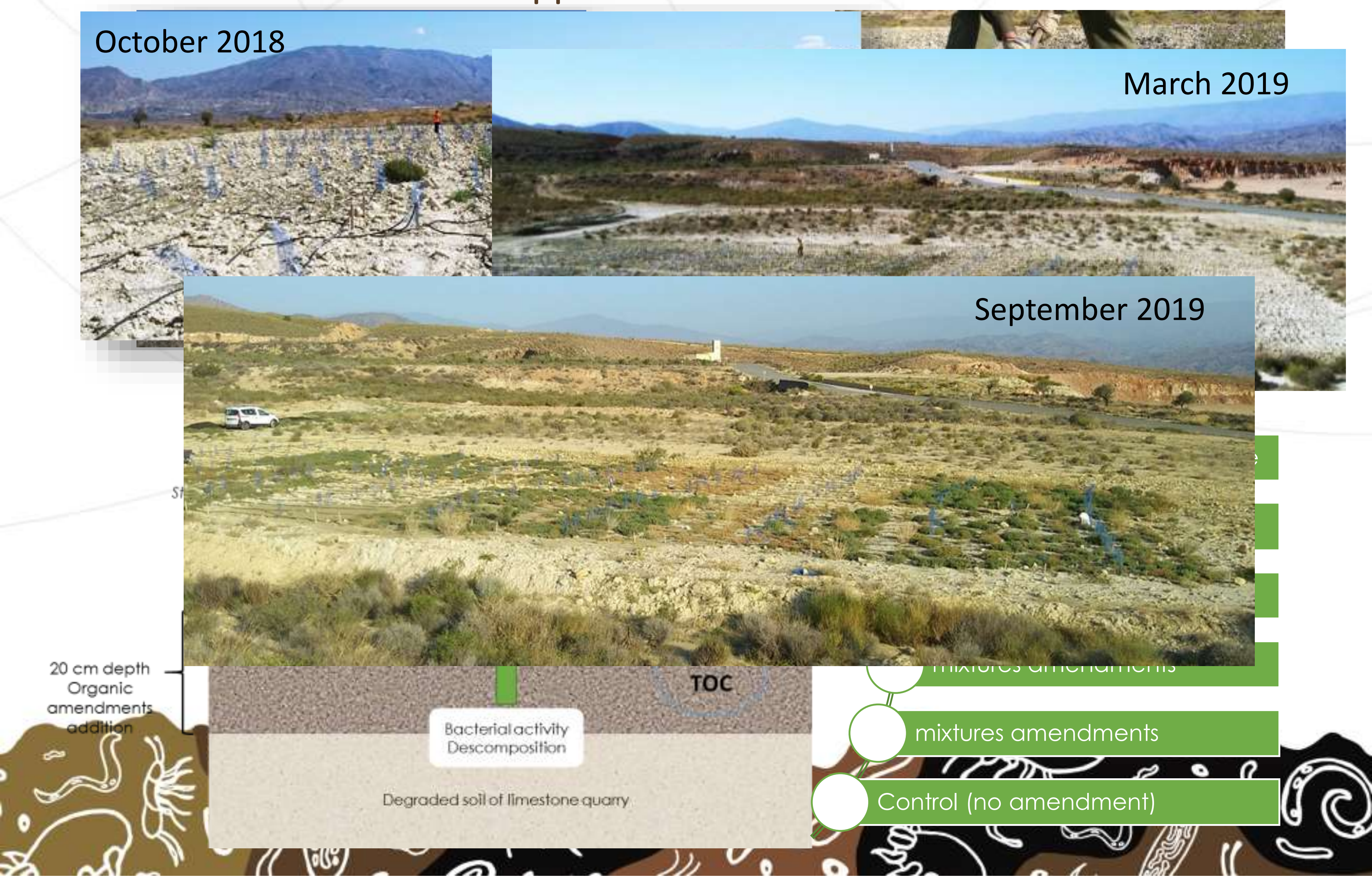

At present, we are working on plots installed in 2018, in the same limestone quarry, studying the effect of the amendments from the moment of their application over time.

# Experimental plots installed in 2018 with different types of organic amendments. Date 06-02-2020

**No amendment plots**

# Experimental plots installed in 2018 with different types of organic amendments. Date 06-02-2020

**Vegetable compost from garden waste**

# Experimental plots installed in 2018 with different types of organic amendments. Date 06-02-2020

**Vegetable compost from horticulture greenhouse crop residues**

## Experimental plots installed in 2018 with different types of organic amendments. Date 06-02-2020

**Stabilized sewage sludge**

# Experimental plots installed in 2018 with different types of organic amendments. Date 06-02-2020

**Mixtures amendments**

Stabilized sewage sludge

+

Vegetable compost garden waste

# Experimental plots installed in 2018 with different types of organic amendments. Date 06-02-2020

**Mixtures amendments**
Stabilized sewage sludge
+
Vegetable compost from horticulture greenhouse crop residues

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The preliminary results indicate that there are short-term changes in bacterial communities and their relationships with carbon emissions and sequestration, so we think this is an interesting line of thought for restoring fragile arid ecosystems to promote their functionality and improve their global change disease.

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# Thank you for your attention

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# References


- **Adak, T. & Sachan, R.S.** 2009. Effect of co-inoculation of Sinorhizobium meliloti and Bacillus megaterium on yield and nutrient uptake of fenugreek (Trigonella foenum-graecum L.) in Mollisol soil. *Journal of Medicinal and Aromatic Plant Sciences*, 31(2): 124–130.
- **Bastida, F., Kandeler, E., Moreno, J.L., Ros, M., García, C. & Hernández, T.** 2008. Application of fresh and composted organic wastes modifies structure, size and activity of soil microbial community under semiarid climate. *Applied Soil Ecology*, 40(2): 318–329.
- **Bastida, F., Selevsek, N., Torres, I. et al**. Soil restoration with organic amendments: linking cellular functionality and ecosystem processes. Sci Rep 5, 15550 (2015).
- **Bastida, F., Torres, I.F., Moreno, J.L., Baldrian, P., Ondoño, S., Ruiz-Navarro, A., Hernández, T., Richnow, H.H., Starke, R., García, C. & Jehmlich, N.** 2016. The active microbial diversity drives ecosystem multifunctionality and is physiologically related to carbon availability in Mediterranean semi-arid soils. *Molecular Ecology*, 25: 4660–4673.
- **Bender, S.F., Wagg, C. & van der Heijden, M.G.A.** 2016. An Underground Revolution: Biodiversity and Soil Ecological Engineering for Agricultural Sustainability. *Trends in Ecology & Evolution*, 31(6): 440–452.
- **Deng, J., Zhang, Y., Yin, Y., Zhu, X., Zhu, W. & Zhou, Y.** 2019. Comparison of soil bacterial community and functional characteristics following afforestation in the semi-arid areas. *PeerJ*, 7: e7141.
- **Juwarkar, A.A. & Jambhulkar, H.P.** 2008. Phytoremediation of coal mine spoil dump through integrated biotechnological approach. *Bioresource Technology*, 99(11): 4732–4741.
- **Kramer, C. & Gleixner, G.** 2008. Soil organic matter in soil depth profiles: Distinct carbon preferences of microbial groups during carbon transformation. *Soil Biology and Biochemistry*, 40(2): 425–433.
- **Lauber, C.L., Hamady, M., Knight, R. & Fierer, N.** 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Applied and Environmental Microbiology*, 75(15): 5111–5120.
- **Lima, A.B., Cannavan, F.D.S., Germano, M.G., Dini-andreote, F., Paula, A.M. De, Franchini, J.C., Teixeira, W.G. & Tsai, S.M.** 2015. Effects of vegetation and seasonality on bacterial communities in Amazonian dark earth and adjacent soils. *African Journal of Microbiology Research*, 9(40): 2119–2134.
- **Luna, L., Pastorelli, R., Bastida, F., Hernández, T., García, C., Miralles, I. & Solé-Benet, A.** 2016. The combination of quarry restoration strategies in semiarid climate induces different responses in biochemical and microbiological soil properties. *Applied Soil Ecology*, 107: 33–47.
- **Rodríguez-Berbel, N., Soria, R., Ortega, R. & Miralles, I.** 2019. Biochemistry and Metagenomic Techniques in Restored Soils with Organic Amendments. *Agricultural Research and Technology*, 20(5): 1–4.
- **Sánchez-Marañón, M., Miralles, I., Aguirre-Garrido, J.F., Anguita-Maeso, M., Millán, V., Ortega, R., García-Salcedo, J.A., Martínez-Abarca, F. & Soriano, M.** 2017. Changes in the soil bacterial community along a pedogenic gradient. *Scientific Reports*, 7: 1–11.
- **Tejada, M., Hernandez, M.T. & Garcia, C.** 2006. Application of two organic amendments on soil restoration: Effects on the soil biological properties. *Journal of Environmental Quality*, 35(4): 1010–1017.
- **Zhao, X., Wei, Y., Zhang, F. et al.** How do fungal communities and their interaction with bacterial communities influence dissolved organic matter on the stability and safety of sludge compost?. Environ Sci Pollut Res 26, 4141–4146 (2019).
- **Zink, T.A. & Allen, M.F.** 1998. The Effects of Organic Amendments on the Restoration of a Disturbed Coastal Sage Scrub Habitat. *Restoration Ecology*, 6: 52–58.