Mycorrhizas and regenerative agriculture in horticultural crops.

The AgriRegenCat Project

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Regenerative agriculture

Regenerative agriculture focuses on improving the **soil health**, which has been degraded by the use of heavy machinery, fertilizers and pesticides in intensive farming. *(World Economic Forum)*

Management practices in RA:

- Restoring soil diversity
- Rotation of cultures
- Avoiding external inputs (fertilizers, pesticides)
- Improving soil structure (no tilling)
- Soil covers

Minimize
Soil disturbance

Maximize
Crop diversity

Keep
the soil covered

Maintain
living root year-round

Integrate
livestock

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Soil Health

Soil health is the capacity of a living soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and promote plant and animal health.

(Doran and Zeiss, 2000).

Mycorrhizas and soil health
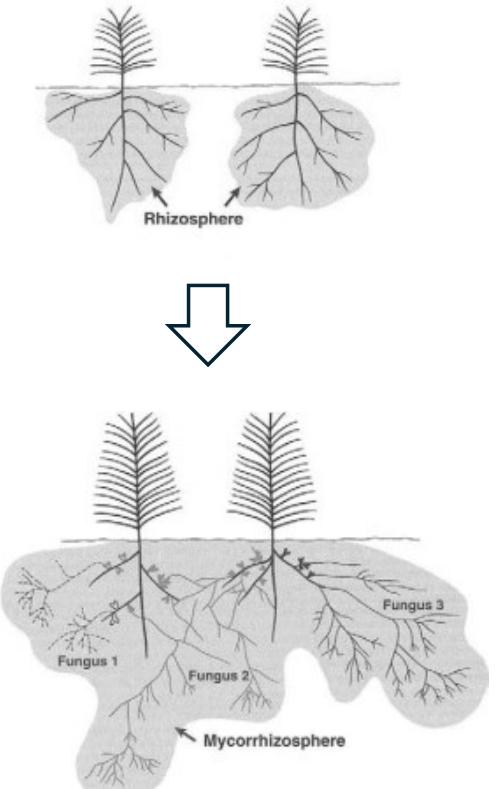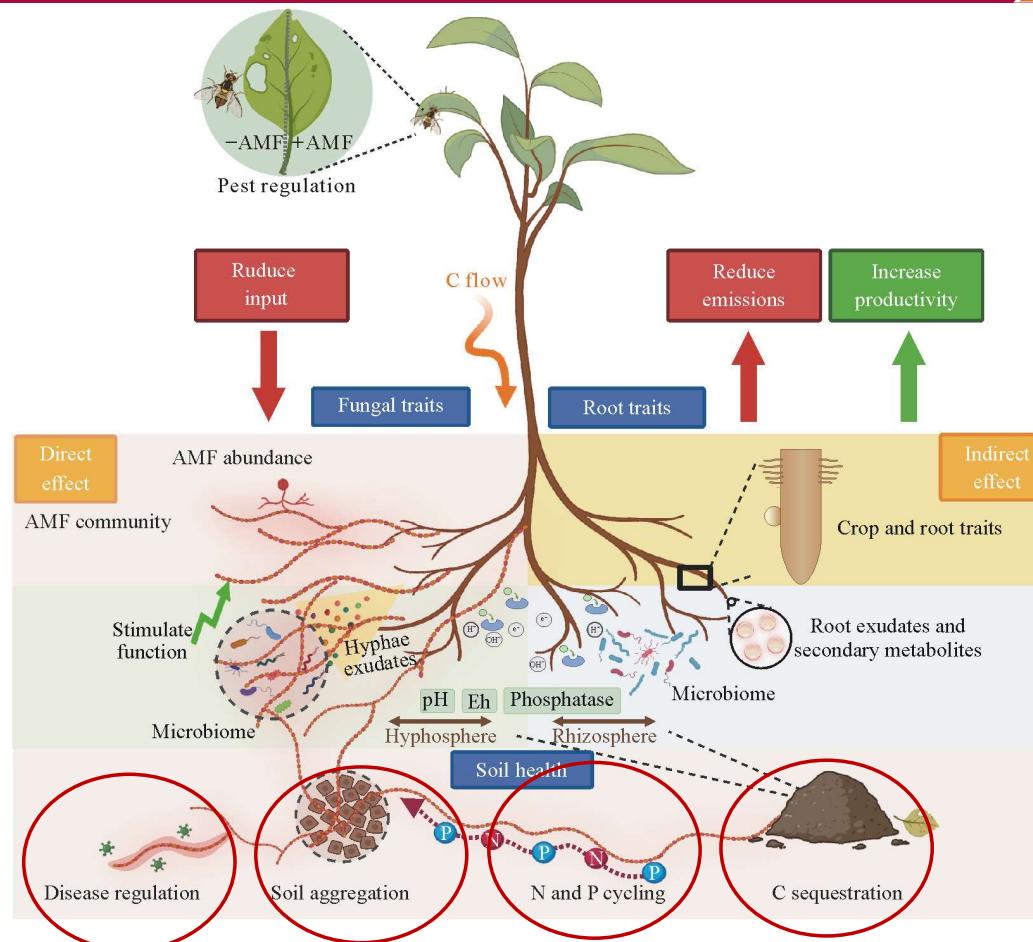
Figure from Zhang et al. 2024. Pedosphere 34: 279-288

Experimental design

Experimental design

Regenerative plot

Fertilization: mineral, compost, wood chips cover+compost (3)
Blocks (3)
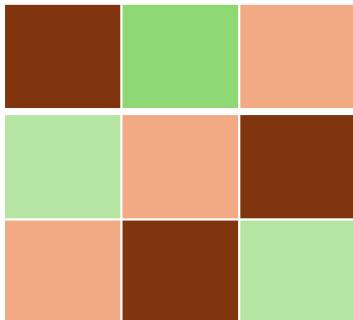
MICO plot

Watering regular and reduced (2) , Inoculation AMF (2)
Blocks (3)

Rotation: cauliflower, lettuce and tomato

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Rotation calendar

Two rotations

YEAR	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
2022												
2023	cauliflower				lettuce		tomato				cauliflower	
2024		lettuce			tomato							

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Measured parameters. Regenerative plot

time (x2 rotations)
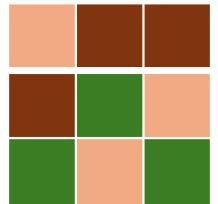
SAMPLINGS (#)

t=0: Initial soil (12)
t=1,4: Soil (9)
t=2,5: Soil (9)
t=3,6: Soil (9)
t=6: Final soil (12)

t=0, t=6
Soil microbiome
(Initial, final)

t=1,4

t=2,5

t=3,6

ANALYSES

- Soil DNA extraction
- Illumina (total fungi/bacteria)
- Soil fungi ΔΔCt qPCR
  (Total / AMF)
- Soil parameters
- Plant production

Soil fungi

Soil fungi

Soil fungi

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Measured parameters. MICO plot
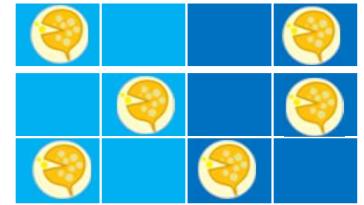
SAMPLINGS (#)

t=0: Initial soil (24)
t=1,4: Soil (12)
t=2,5: Soil (12)
t=3,6: Soil (12)
t=6: Final soil (24)

ANALYSES

- Soil DNA extraction
- Plant DNA extraction
- Illumina (total fungi/bacteria)
- Soil fungi ΔΔCt qPCR
     (Total / AMF)
- Root AM colonisation qPCR/root staining
- Soil parameters
- Plant production

time (x2 rotations)

t=0, t=6
**Soil microbiome**
(Initial, final)

t=1,4

t=2,5

t=3,6

Soil fungi

Soil fungi
AMF colonisation

Soil fungi
AMF colonisation

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Methods to quantify fungi in soil & plant: qPCR

Absolute quantification (standard curve)
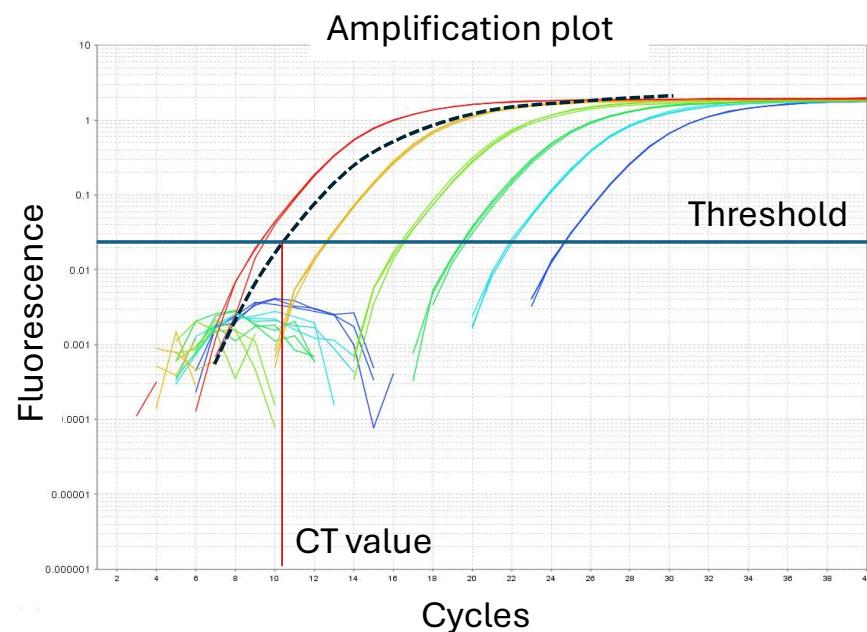
Relative quantification (comparative CT) (requires normalization)

Amplification plot

Fluorescence / Cycles / Threshold / CT value

Amplification curve
real-time PCR

High DNA Copy number / Low DNA Copy number / Relative Fluorescence / Threshold / Low CT value / High CT value / Cycles

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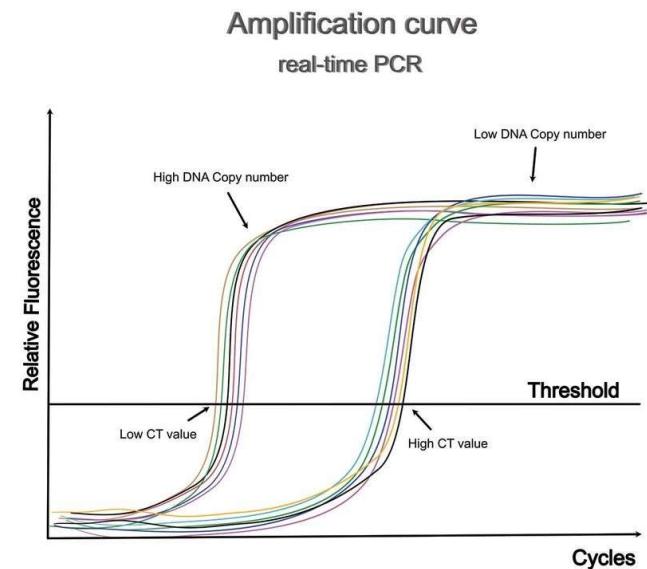
Methods to quantify fungi in soil. Concepts

- **Normalization by volume in soil DNA extractions.**

  Initial quantity = Efficiency^{-Ct}

Efficiency (%) = $(10^{-1/slope} -1) \times 100$

-3,32 = 100% efficiency (=2 ratio)

slope = -3.49
E = 96%
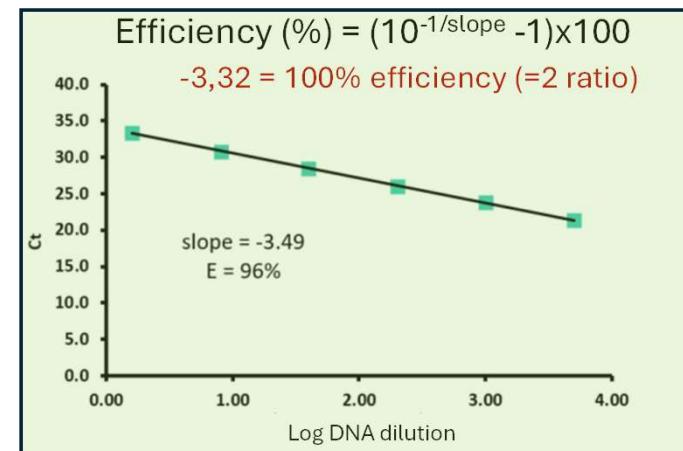
- **Normalization with housekeeping gene (AMF vs. total fungi in soil vs. root tissue in plant)**

  Relative quantity = $2^{-\triangle\triangle Ct}$

  ΔΔCt = ΔCt (sample) – ΔCt (control or reference sample)
  ΔCt = Ct (target gene) – Ct (housekeeping gene)

Housekeeping (constitutive) genes: **GAPDH** for plant, and **rDNA (ITS)** for fungi

Indicators:

AMF normalized to plant tissue

AMF normalized to total soil fungi

Methods to quantify fungi in plant. Concepts

Percentage of root colonization (Intersection grid method, Brundrett 2008)

Stained root
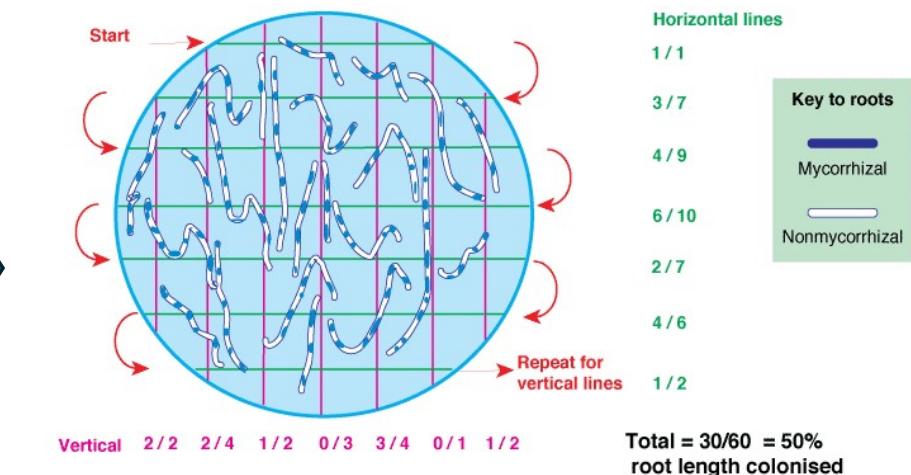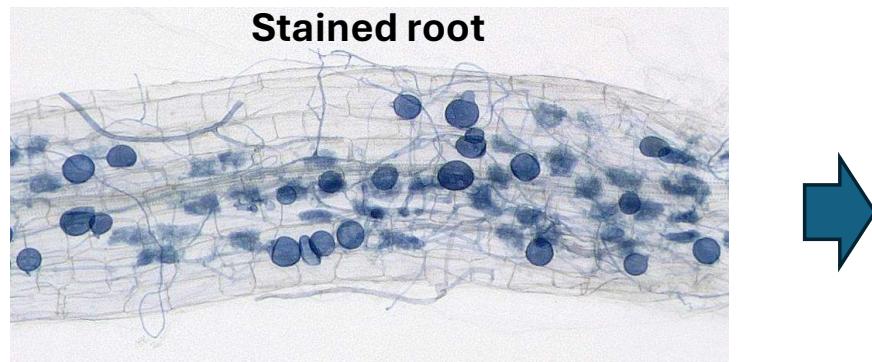
With normalization (housekeeping gene) plant

$$\text{Relative quantity} = 2^{-\triangle\triangle Ct}$$

Validation of the relationship morphological vs. molecular AMF count
Lettuce

Most Probable number (MPN)

Calculation of AMF viable propagules in a soil sample

- Approx.1 L soil (pooled sample, 0-20 cm)
- Keep at 4ºC until processing
- Serial dilutions in sterile sand(1:10)
- Trap plant: *Allium porrum*
- Check colonisation (2 months)
- Calculate MPN
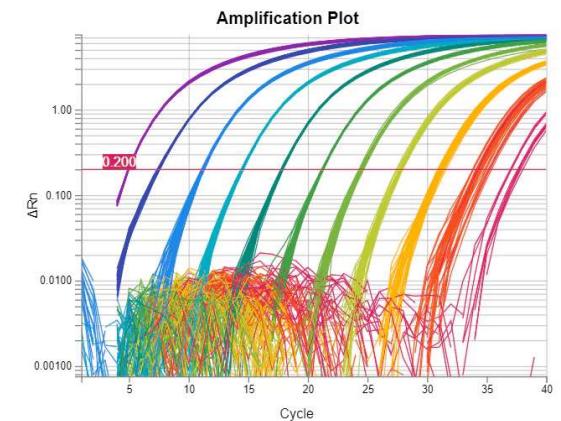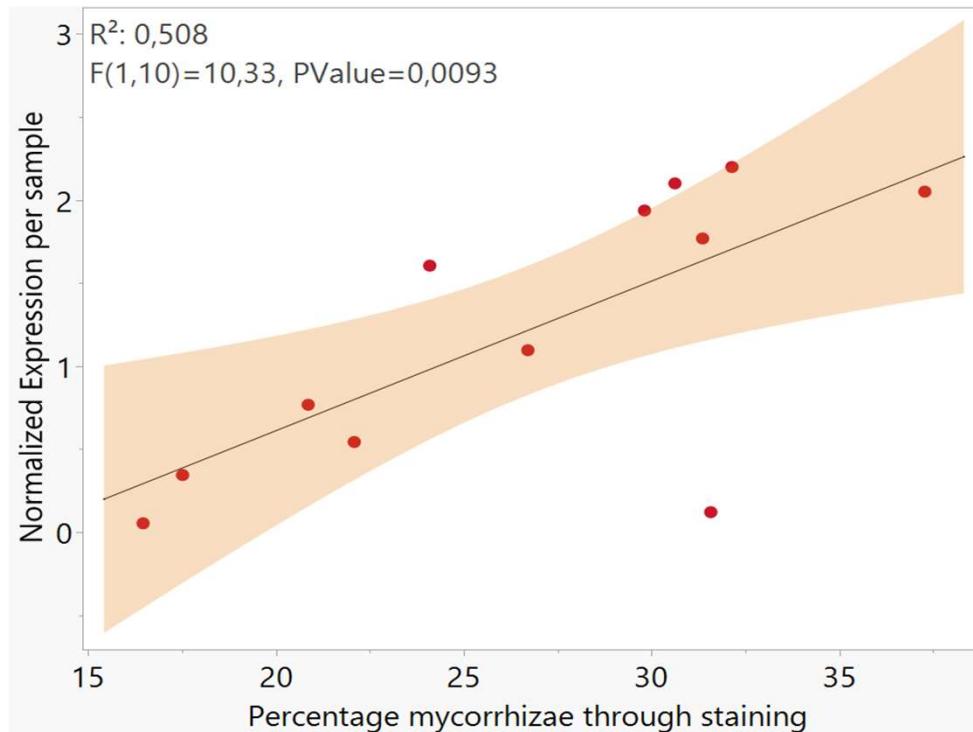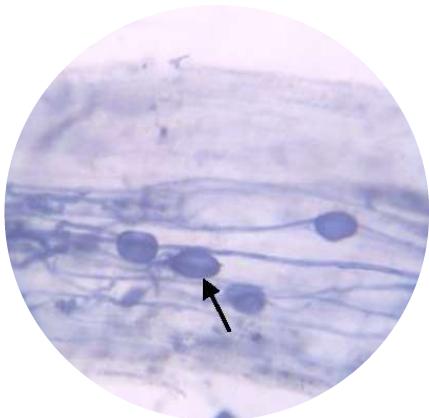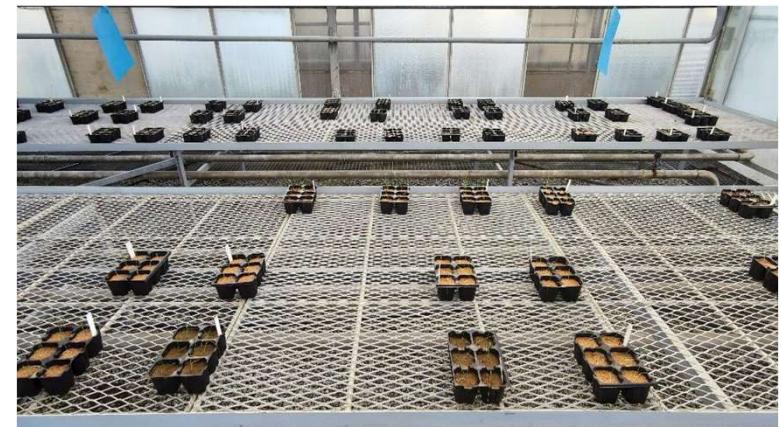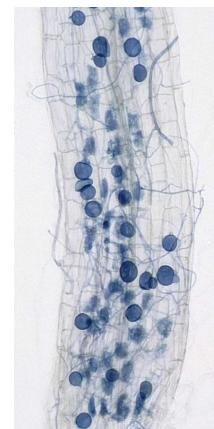
Methods to quantify fungi in soil. Concepts

Most Probable number (MPN)
Statistical calculation

https://mpncalc.galaxytrakr.org/
https://mostprobablenumbercalculator.epa.gov/mpnForm

Cauliflower production

Production increase in inflorescence, leaves and roots

RESULTS
Regenerative plot 2024

Soil fungi
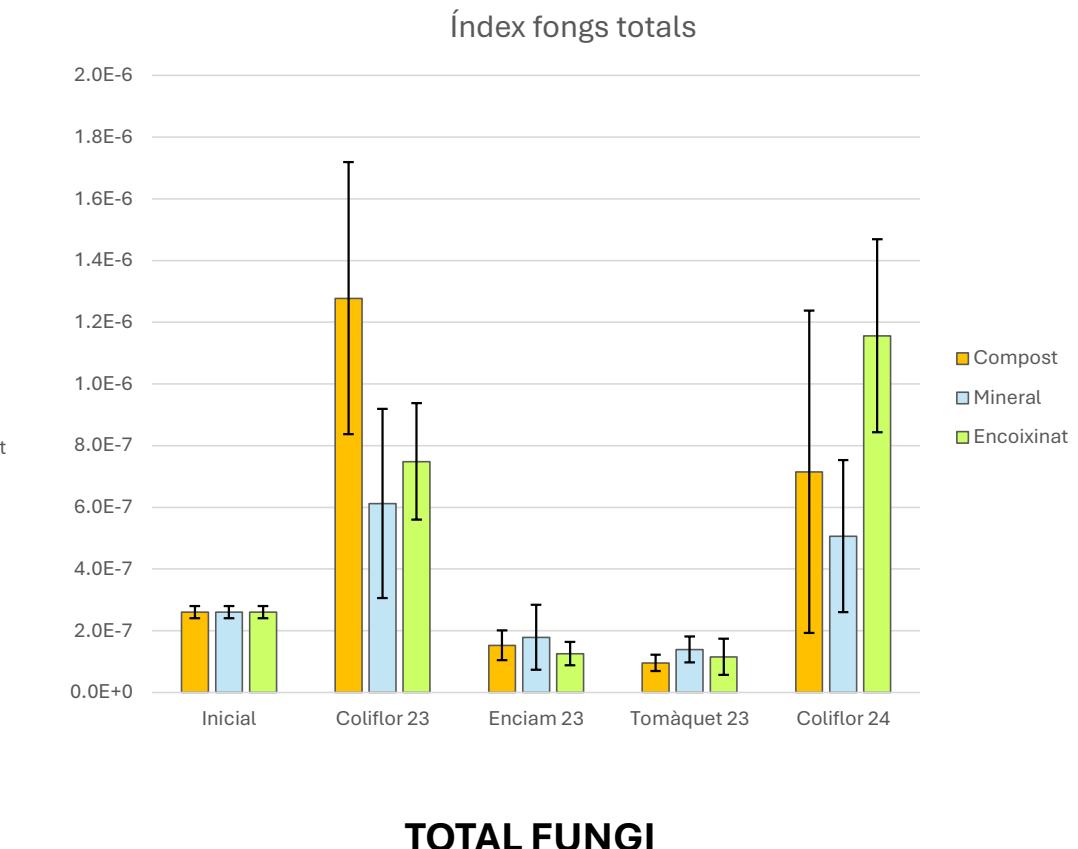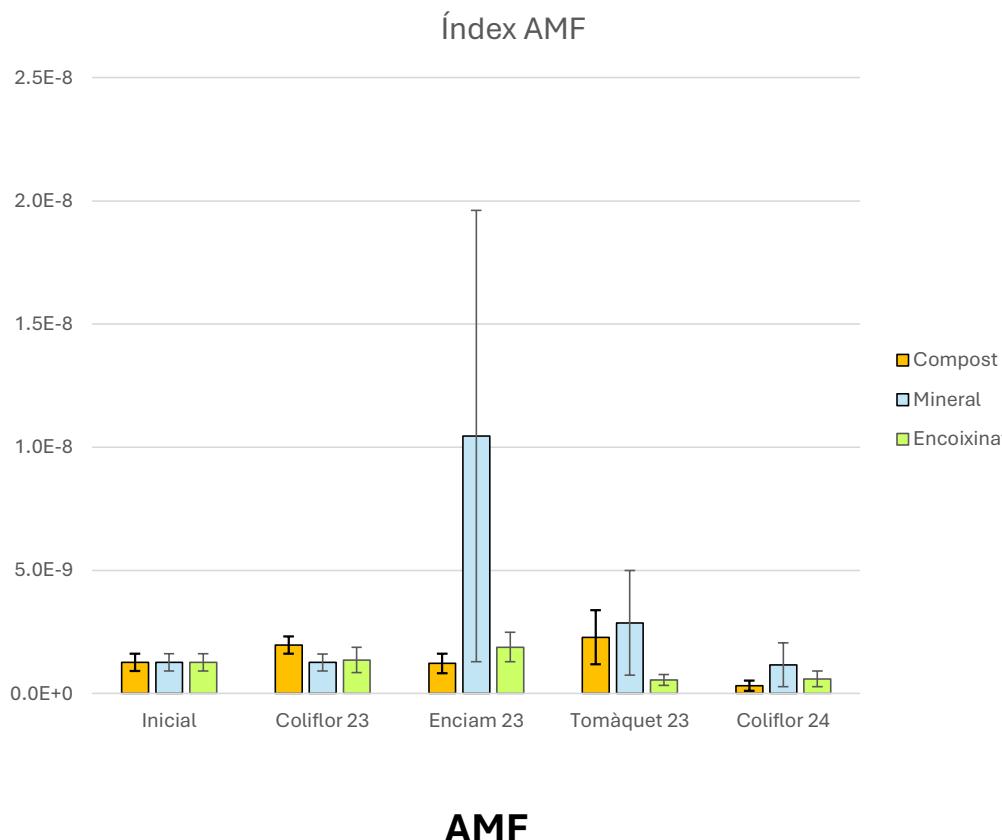
Cauliflower. Inflorescence weight

Boxplot for log_Total.inflorescence.weight

Lettuce. Total leaf weight.
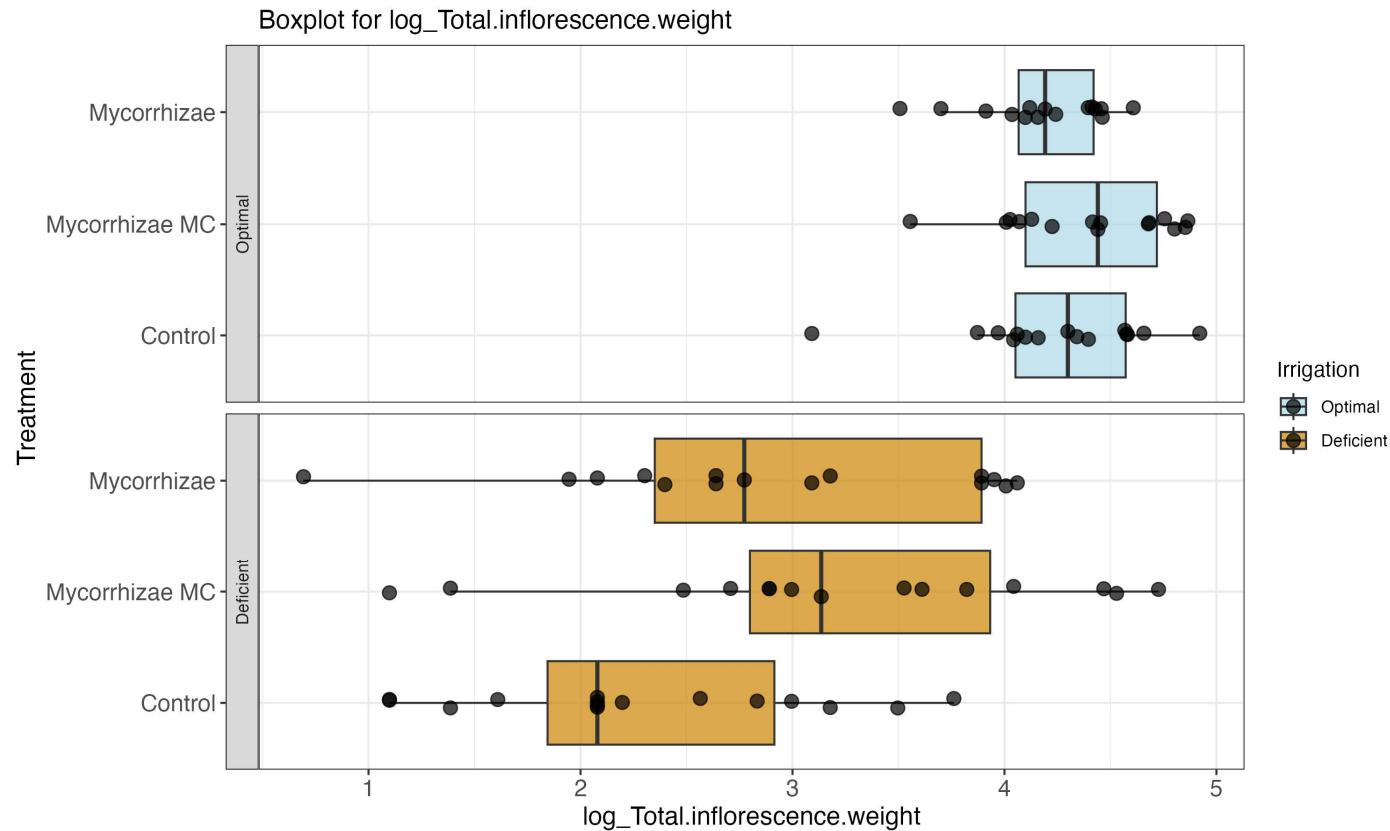
Boxplot for lettuce Total.leaf.weight

Tomato. Total weight (kg/m2).
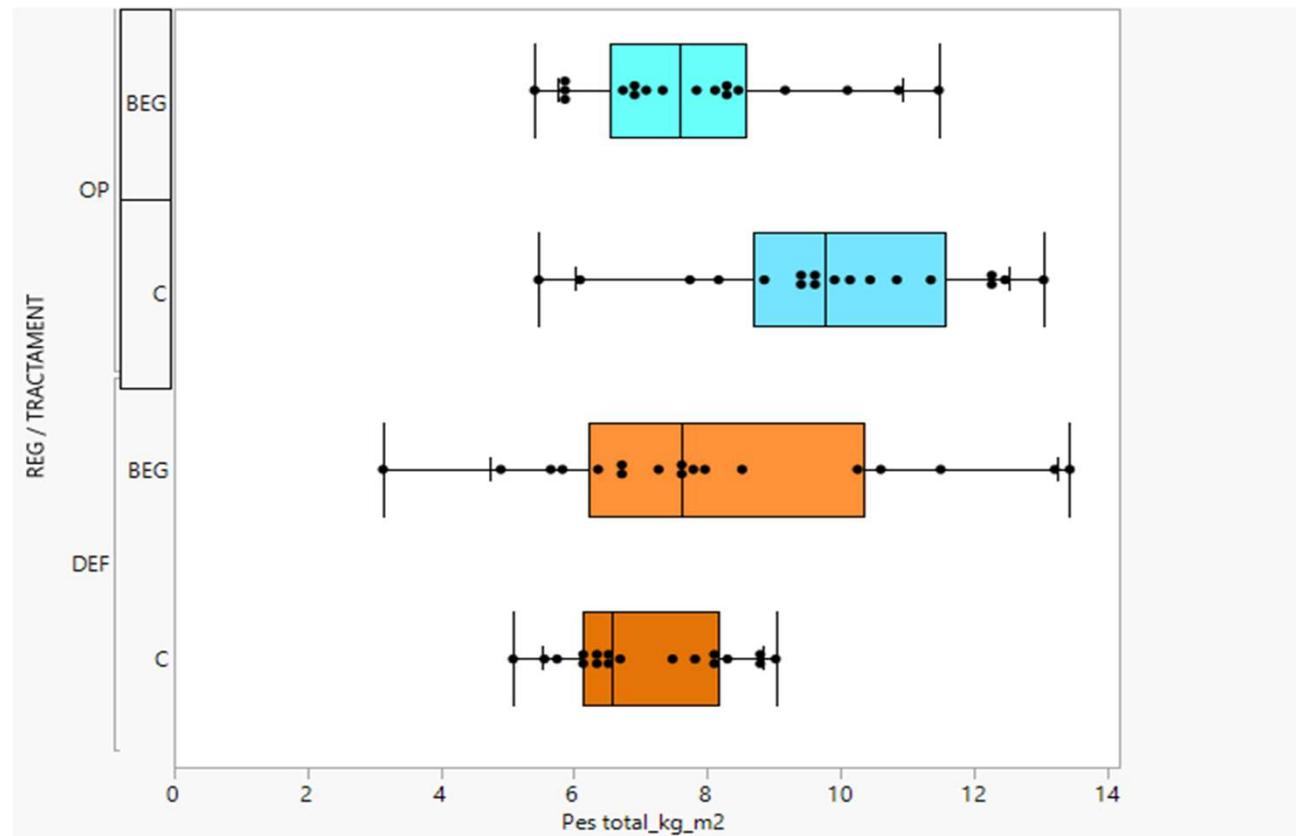
RESULTS
MICO plot 2023

AMF **root** colonisation normalized to plant
Lettuce
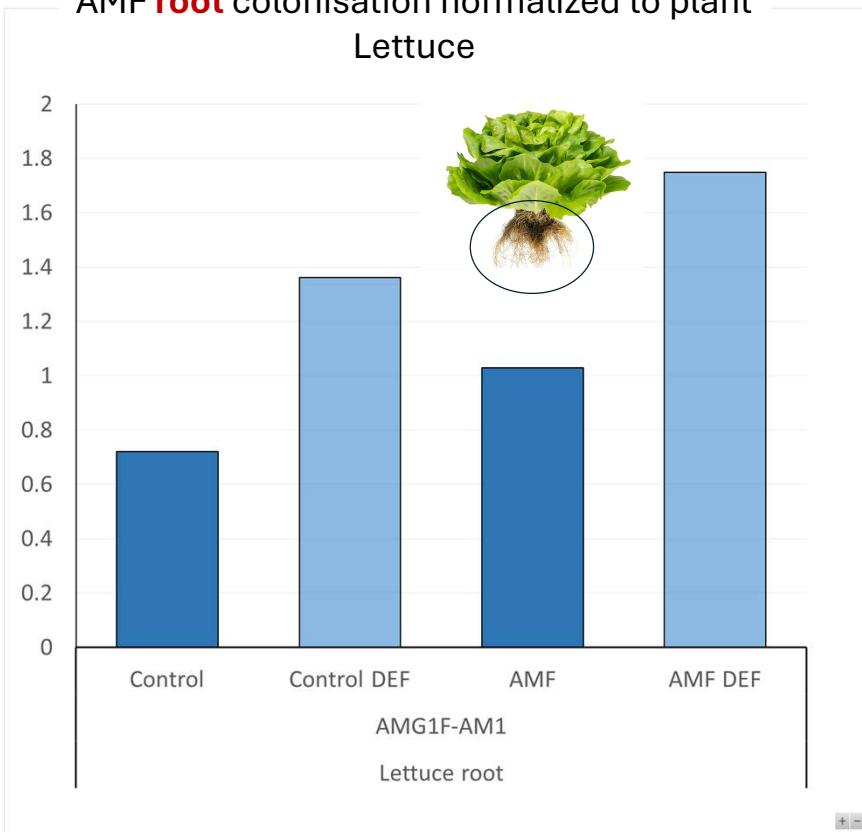
More AMF under inoculation & reduced irrigation

AMF in **soil** normalized to total fungi
Lettuce
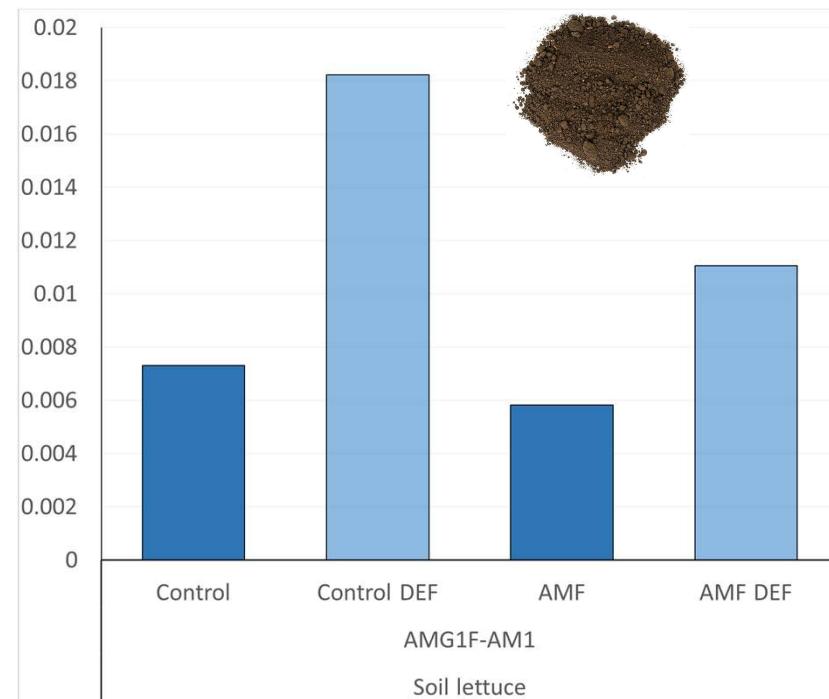
More AMF under reduced irrigation

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Preliminary results/conclusions

❑ No tilling increased cauliflower production in regenerative plot

❑ Total soil fungi tended to increase after the cauliflower crop.

❑ Molecular quantification of AMF in roots is highly correlated with morphological count.

❑ Water regime influences production in cauliflower and lettuce but not in tomato in MICO plot

❑ AMF inoculation had no effect in production in MICO plot

❑ AMF root and soil colonisation in lettuce increased under reduced irrigation in MICO plot

❑ AMF inoculation enhanced root colonization in lettuce even in the presence of native fungal symbionts.

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The AgriRegenCat Team
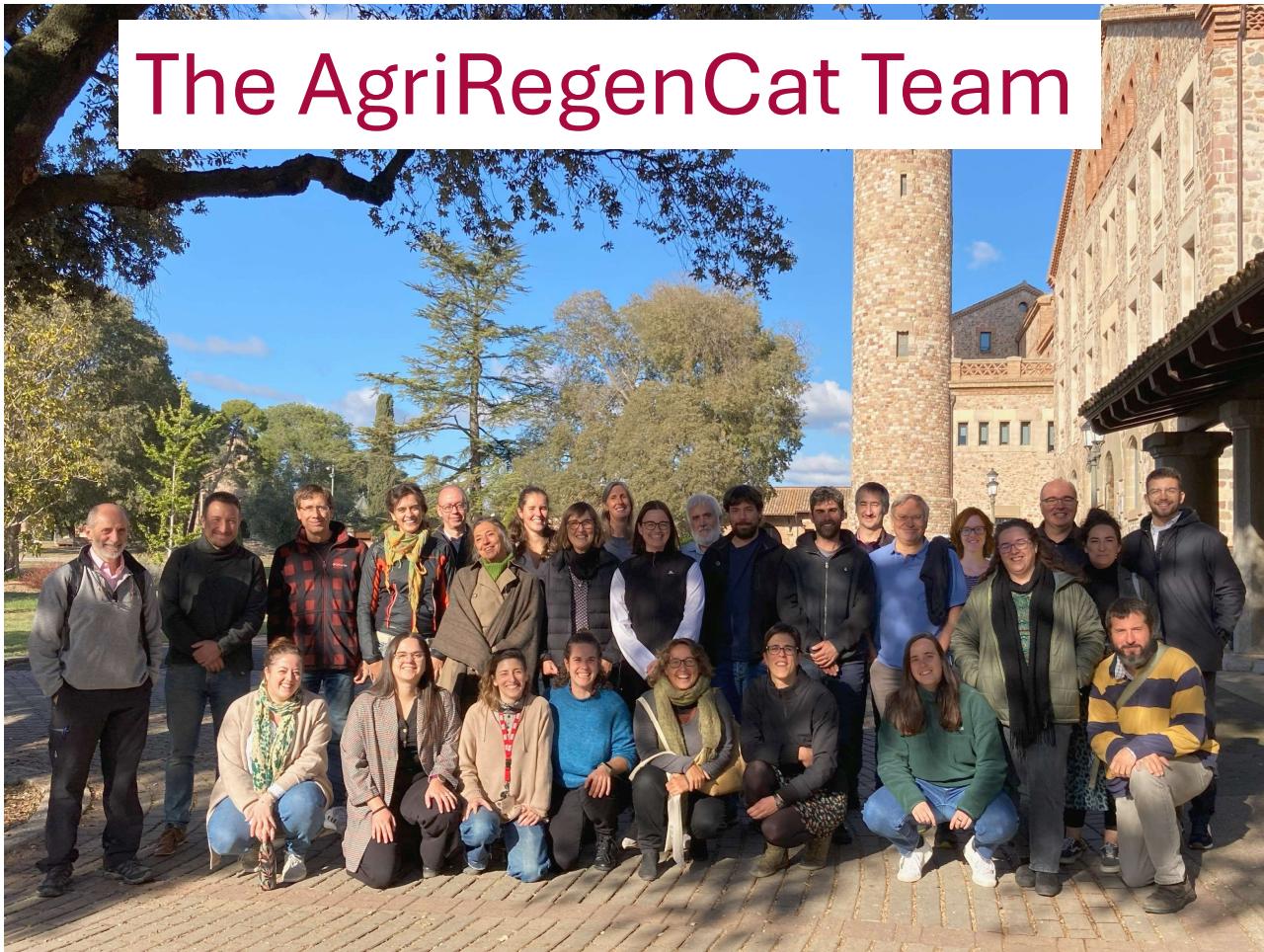
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Thanks for your attention

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