



Deliverable title	D4.5 Report with the elaborated data overall collected within WP4
Deliverable Lead:	IACKR
Related Work Package:	WP4
Related Task:	Task 4.1 (R). Sustainable production of sea fennel crop in demo field Task 4.2 (R). Sustainable production of sea fennel crop in open field Task 4.3. (D) Analysis of the new sea fennel crops Task 4.4. (D) Statistical analysis
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Dissemination level	PU
Due Submission Date:	MONTH 35 (30.03.2025)
Actual submission:	30.03.2025
Start date of project	30.05.2022
Duration	36 MONTHS
Summary of Deliverable D4.5 – Integrated Report on Sea Fennel Cultivation Trials	The report integrates results from demo and open field trials, covering germination, growth, yield, and by-product generation. Fresh and dry biomass were measured at different growth stages, while laboratory analyses assessed nutritional qualities such as tocopherols, carotenoids, polyphenols, vitamin C, fatty acids, minerals, and volatile compounds. By-products like fibrous stems and older leaves were also quantified, confirming their potential for valorization in foods, nutraceuticals, and animal feed. Results consistently showed that biofertilization and irrigation significantly improved both biomass and quality. Plots treated with biofertilizers exhibited stronger root development, greater shoot biomass, and higher concentrations of bioactive compounds compared to untreated controls. Supplemental irrigation, even when limited to about 100 mm during critical summer months, was crucial to sustaining yields under Mediterranean drought and heat stress, especially in Tunisia and Türkiye. Together, these practices not only boosted productivity but also enhanced the levels of health-promoting compounds such as vitamin C, phenolics, carotenoids, and essential oils—confirming sea fennel's value as a functional food crop. At the same time, local ecotypes and the Atlantic type responded differently depending on environmental conditions. Mediterranean varieties generally showed higher survival and more stable performance under stress, while the Atlantic ecotype thrived in cooler or irrigated sites but was more sensitive to heat and drought. This highlights the importance of matching germplasm choice to local environments, a key guideline for farmers. Beyond plant performance, the trials also examined soil microbiomes. Biofertilized plots revealed richer and more diverse microbial communities, including beneficial bacteria linked to nutrient cycling and plant resilience. These findings suggest that sea fennel not only tolerates harsh conditions but also thrives in synergy with soil microbial life, reinforcing its suitability for organic and low-input farming. A distinctive feature of this deliverable was the use of multispectral drone imagery combined with destructive sampling. By correlating vegetation indices with physical and chemical traits, researchers developed predictive models and vegetation maps. These tools allow farmers to

	monitor crop growth and quality non-invasively, reducing the need for repeated destructive tests and opening the door to precision agriculture practices.
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Versioning and Contribution History

Version	Date	Modified by	Modification reason
v1.0	20/04/2024	Branimir Urlić	First version
v2.0	30/04/2024	Branimir Urlić	Comments after peer reviewing process
v2.0	30/03/2025	Branimir Urlić	Final version

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Report with the elaborated data overall collected within WP4

1 Italian site

1.1 Experimental plan design of cultivation trials

In Italy, two sites for demo field experiments were used during the project. Because in the first year the seeds did not germinate so we used another experimental site already in place with three-year-old plants in Camerano (AN) with mediterranean ecotype to carry out the research. The following year, however, we transplanted the seedlings and started the new experimental site in Agugliano (AN). On both experimental sites, we evaluated the application of THE SAME biostimulant on measured variables such as epigeal biomass, SPAD units in three phenological phases and in Agugliano also evaluated the agronomic performance of two different sea fennel ecotypes (Mediterranean and Atlantic)

1.1.1 Site 1

AGUGLIANO

Soil properties in the 0-30 cm layer in the experimental plots in 2024 (n = 3) are shown below

Soil properties	Values
Sand (g kg ⁻¹)	65 ± 18
Silt (g kg ⁻¹)	504 ± 17
Clay (g kg ⁻¹)	431 ± 20
Volumetric soil water content (%):	
Field Capacity	35.2 ± 1.2
Permanent Wilting Point	18.0 ± 2.4
Total available water	26.0 ± 1.8

values expressed in mean±standard deviation.



Compared ecotypes: *Crithmum maritimum* L ecotype Mediterranean, *Crithmum maritimum* L ecotype Atlantic coast

Seedbed preparation: sowing on February 2023, in greenhouse constantly at 20 °C, in polystyrene alveolate trays with peat, daily irrigation

Biostimulation: In greenhouse, a biostimulant is applied once a week (control: no irrigation), instead in the field, four applications of biostimulant for the growing season (2 L of biostimulant diluted in 10 L of water)

Plant Transplanting: About 800 seedlings of the Atlantic and Mediterranean ecotypes were transplanted in early spring 2024.

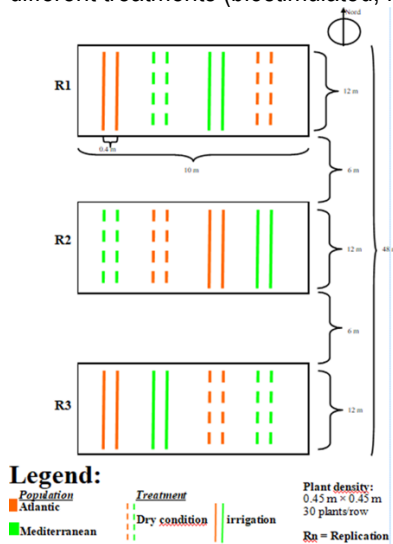


Compared Plant Density 45 x 45 cm

Plot dimension: 9.8 plant m⁻² corresponding 98,000 plants ha⁻¹

Field experiment dimension: 400 m²

Experimental design: Complete randomized blocks with 3 replications (R1, R2, R3), further divided into 3 plots based on different treatments (biostimulated, fertilized, Irrigation, Control) as previously reported by Zenobi et al. (2021, 2022)



The sea fennel plants in the experimental plots were biostimulated using the following mixture of bacterial strains (SAME BIOSTIMULANT as the Camerano site):

- free-nitrogen fixing bacteria: *Azotobacter chroococcum* DSM 2286, and *Azospirillum brasilense* DSMZ 1690 (mixed well together at a 1:1 (v/v) ratio;
- phosphorus-dissolving bacteria: *Priestia megaterium* DSMZ 339;
- potassium-solubilizing bacteria: *Niallia circulans* DSMZ 30598

The three microbial groups were inoculated at a 2:1:1 (v/v/v) ratio in load no lower than 10⁸ cells/mL for each microbial group (details about preparation of the biofertilizer are reported in Section 1.1.1) and fertilized with seaweed-based compound with 5% nitrogen.

1.1.2 Site 2

CAMERANO

Soil characterisation was performed before the *C. maritimum* L. growing season. According to the USDA classification, the soil detected is clayey loam, therefore with good water retention but subject to water stagnation.

Soil properties in the 0-30 cm layer in the experimental plots in 2023 (n = 3) are shown below:

Soil properties	Values
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Sand (g kg ⁻¹)	125 ± 19
Silt (g kg ⁻¹)	445 ± 12
Clay (g kg ⁻¹)	430 ± 15
Volumetric soil water content (%):	
Field Capacity	44.3 ± 1.1
Permanent Wilting Point	18.4 ± 2.2
Total available water	26.2 ± 1.7

values expressed in mean±standard deviation.

Farm “Paccasassi del Conero” located in Camerano – Ancona – Italy. (43_530 N, 13_550 E).



Ecotypes: *Crithmum maritimum* L ecotype Mediterranean

Seedbed preparation: sowing on 26th January 2023, in greenhouse constantly at 20 °C, in polystyrene alveolate trays with peat, daily irrigation

Biostimulation: In greenhouse, a biostimulant applied once a week (control: no irrigation), instead in the field, four applications of biostimulant for the growing season (2 L of biostimulant diluted in 10 L of water)

Plant Transplanting: In March 2023, the transplantation failed due to low seed germination; hence a demonstration plot consisting of 3-year-old sea fennel plants (Mediterranean ecotype) was used for bio-fertilization experiments in 2022-2023. These plants had previously been planted as part of the regional funded project "Bio-Veg-Conserve" (<http://www.biovegconserve.it/>).

Compared Plant Density 45 x 45 cm

Plot dimension: 9.8 plant m⁻² corresponding 98,000 plants ha⁻¹

Field experiment dimension: 400 m²

Experimental design: Complete randomized blocks with 3 replications (R1, R2, R3), further divided in 3 plots based on different treatments (biostimulated, Irrigation, Control) as previously reported by Zenobi et al. (2021, 2022)

Experimental plot with sampling points in selected coastal demonstration plot – Farm “Paccasassi del Conero” (Ancona) (Zenobi et al., 2021)

The three year-old sea fennel plants in the experimental plots were biostimulated using the following mixture of bacterial strains:

- free-nitrogen fixing bacteria: *Azotobacter chroococcum* DSM 2286, and *Azospirillum brasilense* DSMZ 1690 (mixed well together at a 1:1 (v/v) ratio;
- phosphorus-dissolving bacteria: *Priestia megaterium* DSMZ 339;
- potassium-solubilizing bacteria: *Niallia circulans* DSMZ 30598

The three microbial groups were inoculated at a 2:1:1 (v/v/v) ratio in load no lower than 10⁸ cells/mL for each microbial group (details about preparation of the biofertilizer are reported in Section 1.1.1)

1.1.3 Germination tests in growth chamber

Objective

The objective of this activity was the evaluation of seed germination capacity under controlled growth conditions, including the application of a biostimulant.

Materials and methods

Three tests were conducted in the growth chamber, the first on 27th January 2023, the second on 16th February 2023, and the third on 16th March 2023, following the method described by Meot-Duros and Magné (2008). Each treatment was replicated three times, using 30 seeds per replication for the following treatments:

- Biostimulated Atlantic ecotype
- Control Atlantic ecotype
- Biostimulated Marche ecotype
- Control Marche ecotype

Briefly, seeds were placed in sterile Petri dishes and pre-treated with 10 mL of ethanol (96% solution, v/v) at room temperature to enhance germination, as suggested by Meot-Duros and Magné (2008). After 24 hours, the seeds of each ecotype were rinsed with sterile deionized water and divided into two groups:

1. Bio-fertilized group: seeds were treated with a bio-fertilizer mixture:
BS1

- *Azospirillum brasilense* (strain DSMZ 1690), for atmospheric nitrogen fixation
- *Priestia megaterium* (strain DSMZ 339), as a phosphorus-solubilizing bacterium
- *Niallia circulans* (strain DSMZ 30598), as a potassium-solubilizing bacterium

These strains were mixed in a 2:1:1 (v/v/v) ratio.

BS2

- *Azospirillum brasilense* (strain DSMZ 1690), for atmospheric nitrogen fixation
 - *Priestia megaterium* (strain DSMZ 339), as a phosphorus-solubilizing bacterium
 - *Niallia circulans* (strain DSMZ 30598), as a potassium-solubilizing bacterium
 - *Azotobacter chroococcum* (strain DSM 2286), for atmospheric nitrogen fixation
- These strains were mixed in a 1:1:1:1 (v/v/v/v) ratio.

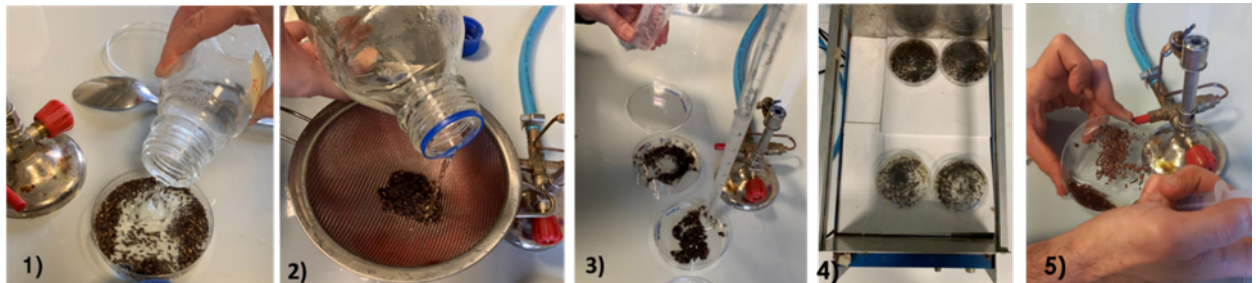
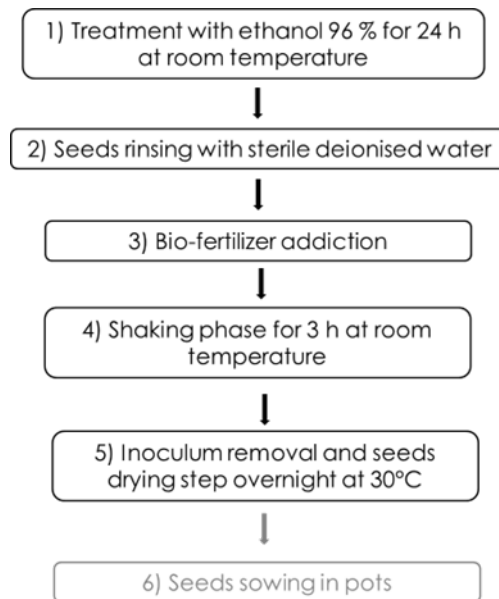
2. Control group: Seeds were treated with sterile deionized water only (Abdallah et al., 2021).

The bacterial strains were obtained from the International Culture Collection DSMZ (<https://www.dsmz.de/>). Each strain was cultivated independently in Nutrient Broth (NB) for 48 hours at 30°C in a rotary shaking incubator (SKI 8R, ArgoLab, Carpi, Italy) at 150 rpm. The biomass was harvested by centrifugation (Rotofix 32 A, Hettich, Tuttlingen, Germany) at 4000 rpm for 5 minutes. After discarding the supernatant, the cell pellet was resuspended in 10 mL of sterile deionized water. The bacterial cell concentration was determined spectrophotometrically at 600 nm using a Shimadzu UV-1800 spectrophotometer (Shimadzu Corporation, Kyoto, Japan), and cell viability was verified using the spread plate method on Nutrient Agar.

The bacterial suspensions, each containing no fewer than 10^8 cells/mL, were mixed before being applied to the seeds. For bacterization, 10 mL of the bacterial mixture was added to the seeds in sterile Petri dishes and left at room temperature under shaking conditions (100 rpm) for 3 hours. The excess inoculum was removed using a sterile syringe, and the seeds were left to dry overnight at 30°C before being sown in pots.

Additionally, 2 liters of bacterial suspension prepared as described above was applied weekly as a bio-fertilizer to the seeds after sowing.

The following figures illustrate the sea fennel bacterization procedure and Petri dishes containing control and treated seeds.



Procedure for bacterization of sea fennel seeds.



Petri dishes with treated and untreated sea fennel seeds in germination chamber.

Germination % and root germination parameters were evaluated onto 10 plants for each replicate of each treatment (30 total seedlings per treatment). Sea fennel roots were first scanned, and the resulting captured images were analyzed using the winRhizo software. This latter is an image analysis system specifically designed for measurement of different morphological root parameters (length, area, volume...), topology, architecture, and color analysis.



Control and biostimulated roots of germinated sea fennel seeds

Results

The data collected onto sea fennel roots are reported below.

Ecotype	Treatment	BBCH	UDB (mg)	LNG (cm)	DMT (mm)
AT	CT	06	1.01 (± 0.10) ¹ d ²	3.92(± 0.53) d	0.45(± 0.04) c
ME	CT	06	0.96(± 0.05) d	3.76(± 0.37) d	0.46(0.07) c
AT	BS1	06	1.59(± 0.09) c	7.59(± 0.40) c	0.56(± 0.06) ab
ME	BS1	06	1.53(± 0.15) c	7.48(± 1.21) c	0.54(± 0.06) b
AT	BS2	06	2.06(± 0.23) b	10.38(± 0.89) a	0.60(± 0.05) a
ME	BS2	06	2.34(± 0.36) a	9.73(± 1.10) b	0.60(± 0.06) a
Mean			1.58(± 0.55)	7.14(± 2.80)	0.54(± 0.06)

Estimated root parameters differences calculated with Shapiro-Wilk normality test in three treatments in both ecotypes.

¹Mean $\pm$ standard deviation. ² Means within each column among the same BBCH followed by different letters are significantly different (ANOVA, $p < 0.05$) respectively among the ecotype and treatment combination

BBCH= Phenological Phase of radicle 06 = Elongation of radicle, formation of root hairs and/or lateral roots

ME = Marche region ecotype

AT = Atlantic ecotype

CT = control treatment

BS1 = Biostimulant composition: Azospirillum brasilense, Priestia megaterium var. phosphaticum, Bacillus circulans,

BS2 = Biostimulant composition: Azotobacter chroococcum plus, Azospirillum brasilense, Priestia megaterium var. phosphaticum, Bacillus circulans,

UDB = unit dry biomass root

LNG = length root

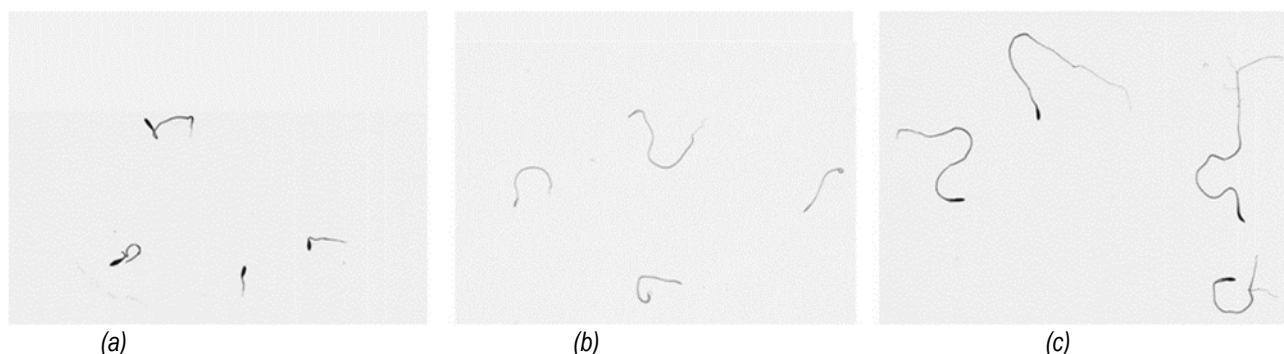
DMT = diameter root.

The overall data collected showed that the biostimulant significantly promoted germination in both the Marche region ecotype (57% higher than the control) and the Atlantic ecotype (40% higher than the control).

Regarding the evaluation of root growth, the following evidence emerged:

- Length (cm): The data indicate that biostimulated roots are nearly twice as long as the control roots, with a 97% increase observed in the Marche region ecotype and a 90% increase in the Atlantic ecotype.
- Diameter (mm): The data indicate that biostimulated roots are thicker than those in the control group for both ecotypes, showing a 23% increase in diameter for the Atlantic ecotype and a 21% increase for the Marche region ecotype.
- Tips (n°): In laboratory tests, no significant differences were observed in the number of tips, as the number was consistently close to 1 per root.

- Dry weight (g): The dry weight of biostimulated roots was approximately 15% higher than the control for both ecotypes, specifically 15.3% for the Atlantic ecotype and 14.6% for the Marche region ecotype.



Root system: without biostimulant (CT) somministration (a) and with BS1 (b) and BS2 (c) somministration

1.1.4 Germination tests in greenhouse

Objective

Evaluate the efficacy of sea fennel seed bacterization in germination % and root growth (length, diameter, number of tips and dry weight, under greenhouse conditions).

Materials and methods

Sowing for the four treatments (Control Marche region ecotype, Biostimulated Marche region ecotype, Control Atlantic ecotype, Biostimulated Atlantic ecotype) in a heated greenhouse took place on 26th January 2023. Seeds were sown in alveolated trays (alveolus volume $\sim 5 \text{ cm}^3$) containing a peat-based sowing substrate. A total of 550 seeds were planted for each treatment.

Irrigation was performed almost daily, maintaining a constant greenhouse temperature of 20°C . After sowing, the biostimulant mixture was applied via nebulization once a week.



Sowing

Germination % and root germination parameters were evaluated on 10 seedlings per treatment, as previously described in section 1.1.1.

Results

Data overall collected is reported in the following table.

Ecotype	Treatment	BBC H	UDB (mg)	LNG (cm)	DMT (mm)	Tips (n°)
ME	CT	13	1.98(±0.21)	11.73(±2.85)	0.26(0.05)	19.9(±1.21)
AT	CT	13	2.00(±0.47)	8.43(±0.67)	0.26(±0.015)	14.60(±2.07)
Mean			1.99(±0.02) ¹ c ²	10.09(±2.33) c	0.26(±0.0000 7) c	17.25(±3.75) c
ME	BS1	13	2.56(±0.14)	20.18(±3.72)	0.33(±0.008)	44.7(±3.79)
AT	BS1	13	2.55(±0.15)	22.61(±1.05)	0.32(±0.02)	56(±4.14)
Mean			2.56(±0.01) b	21.40(±1.71) b	0.32(±0.005) b	50.35(±7.99) b
ME	BS2	13	2.95(±0.13)	29.91(±6.4)	0.35(±0.004)	82.8(±3.84)
AT	BS2	13	2.80(±0.10)	34.93(±3.15)	0.34(±0.008)	88.6 (±1.40)
Mean			2.88(±0.11) a	32.42(±3.55) a	0.35(±0.009) a	85.70(±4.10) a
Mean	13		2.48(±0.45)	21.30(±11.17)	0.31(±0.046)	51.1(±34.23)
ME	CT	15	3.22(±0.59)	62.52(±3.81)	0.29(±0.004)	173.3(±17.30)
AT	CT	15	3.16(±0.36)	60.88(±2.00)	0.29(±0.007)	172.86(±4.75)
Mean			3.20(±0.05) c	61.70(±1.17) c	0.29(±0.004) c	173.08(±0.31) c
ME	BS1	15	4.55(±0.15)	82.86(±3.07)	0.35(±0.02)	213.33(±8.80)
AT	BS1	15	4.64(±0.10)	75.16(±1.81)	0.35(±0.014)	219.83(±23.60)
Mean			4.60(±0.06) b	79.01(±5.44) b	0.35(±0.0003) b	216.58(±4.60) b
ME	BS2	15	7.36(±0.52)	121.60(±3.82)	0.37(±0.003)	259.12(±12.48)
AT	BS2	15	7.24(±0.97)	117.47(±13.6 5)	0.36(±0.009)	229.6(±11.88)
Mean			7.31(±4.08) a	119.53(±2.92) a	0.37(±0.01) a	244.34(±35.92) a
Mean	15		5.03(±2.09)	86.75 (±29.68)	0.34(±0.04)	211.34(±35.92)

Estimated root parameters differences calculated with Shapiro-Wilk normality test in three treatments in both ecotypes. ¹Mean ± standard deviation. ²Means within each column followed by different uppercase and lowercase letters are significantly different (ANOVA, p < 0.05) respectively among the ecotype in the same treatment and between the three treatments (by averaging in each the values of the two ecotypes).

BBCH = Phenological Phase 13 = three true leaves, pairs of leaves or unfolded whorls (BBCH scale); 15 = five true leaves, pairs of leaves or unfolded whorls (BBCH scale)

ME = Marche region ecotype

AT = Atlantic ecotype

CT = control treatment

BS1 = Biostimulant composition: Azospirillum brasilense, Priestia megatherium var. phosphaticum, Bacillus circulans, BS2 = Biostimulant composition: Azotobacter chroococcum, Azospirillum brasilense, Priestia megatherium var. phosphaticum, Bacillus circulans,

UDB = root unit dry biomass

LNG = root length

DMT = root diameter



Germination tests at green house conditions with a detail of young sea fennel plan

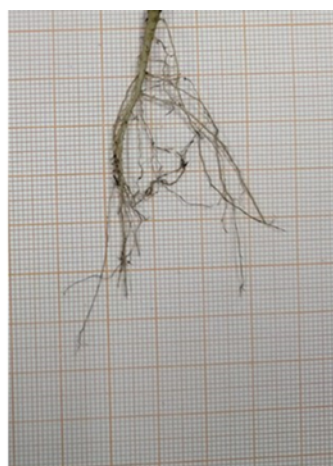
The evaluation of root growth reveals significant improvements in various parameters due to biostimulation compared to control roots across both ecotypes. Here's a breakdown of the findings:

1. Length (cm):
Biostimulated roots exhibited more than double the length of control roots, indicating a significant increase in root elongation for both ecotypes.
2. Diameter (mm):
Biostimulated roots showed a larger diameter compared to control roots:
-Atlantic ecotype: 21% increase.
-Marche region ecotype: 51% increase.
3. Tips (number):
The number of root tips was substantially higher in biostimulated roots:
-Atlantic ecotype: 286% increase.
-Marche region ecotype: 128% increase.
4. Dry weight (g):
Biostimulated roots demonstrated a higher dry weight:
-Atlantic ecotype: 28% increase.
-Marche region ecotype: 25% increase.

These findings highlight the positive impact of biostimulation on root growth, with marked improvements in length, diameter, root tip number, and dry weight in both ecotypes.



(a)



(b)



(c)

Root system at the stage when the plants had developed five true leaves: (a) without biostimulant treatment (CT), (b) with BS1, and (c) with BS2.

1.2 Fresh biomass (edible aerial parts) from the new organic sea fennel crops

Materials and methods

The epigeal biomass was cut with scissors at 5 cm from the ground level and after plant sampling, the leaves and branches were separated and carefully cleaned from soil residues, limiting the loss of leaves as much as possible. Each fresh plant was separately weighed using a laboratory balance to assess the fresh weight. After that, the biomass was placed in an oven at 105° C for two days, after which its dry weight was determined. Samples were taken in a randomised manner.

The biomass of the plant was collected at three distinct phenological stages: Side Shot formation, Vegetative parts development, Flower buds visible; for each treatment studied: biostimulated, irrigated and control.



Weighing of fresh biomass

Results

Biomass data refer only for Mediterranean population. Results collected at the Italian demo field in Agugliano (AN) in 2023 are reported below.

Treatment	BBCH	UFW (g plant ⁻¹)	TFW (t ha ⁻¹)	UDW (g plant ⁻¹)	TDW (t ha ⁻¹)
CT	20-29	95.3(±24.8) b	9.3(±2.4) a	14.0(±2.5) b	1.4 (±0.4) b
BIO	20-29	151.1(±41.4) a	14.8(±4.1) a	27.4(±2.6) a	2.7(±0.3) a
IRR	20-29	141.6(±24.1) a	13.9 (±2.4) a	24.1(±4.0) a	2.4(±0.4) a
Mean 20-29		129.3(±38.9)	12.7(±3.8)	21.9(±6.5)	2.1 (±0.6)
CT	40-49	380.7(±32.1) c	37.3(±3.2) c	59.4(±8.6) b	5.8(±0.8) b
BIO	40-49	548.6 (±39.8) b	53.8(±3.9) b	86.6(±7.9) a	8.5(±0.8) a
IRR	40-49	596.8(±37.9) a	58.4(±3.7) a	88.0(±12.0) a	8.6 (±1.2) a
Mean 40-49		508.7(±100.8)	49.9(±9.9)	78.0(±16.3)	7.6 (±1.6)
CT	51	523.7(±28.2) c	51.3(±2.8) c	88.1(±7.5) c	8.6(±0.7) c
BIO	51	936.1(±70.6) a	91.7(±6.9) a	153.7(±19.0) a	15.1(±1.9) a
IRR	51	820.4(±35.0) b	80.4(±3.4) b	122.9(±9.3) b	12.0(±0.9) b
Mean 51		760.1(±183.0)	74.5(±17.9)	121.6(±30.0)	11.9 (±2.9)

Means (+/- standard deviation) within each column followed by different letters are significantly different (ANOVA, p < 0.05).

BIO: Biofertilized

IRR: Irrigated

CT: Control (no treatment)

BBCH = phenological phase 20-29 Side shoots formation; 40-49 Vegetative parts development; 51 Flower buds visible

UFW Unit Fresh Weight

TFW Total Fresh Weight

UDW Unit Dry Weight
TDW Total Dry Weight

Results collected in Agugliano (AN) in 2024 are reported below, the data refers for Mediterranean and Atlantic population.

AT = Atlantic population

ME = Mediterranean population,

BBCH	Treatment	Ecotype	UFB g pt ⁻¹	TFB t ha ⁻¹	UDB g pt ⁻¹	TDB t ha ⁻¹
20-29 (side shoots formation)	Biostimulated		1.76(±0.44) a	0.172(±0.043) a	0.26(±0.07) a	0.025(±0.007) a
		AT	1.88(±0.51)	0.184(±0.050)	0.28(±0.06)	0.028 (±0.006)
		ME	1.64(±0.32)	0.161(±0.031)	0.24(±0.07)	0.023 (±0.007)
	Irrigated		1.77(±0.50) a	0.173(±0.049) a	0.28(±0.10) a	0.029 (±0.010) a
		AT	1.82(±0.53)	0.179(±0.052)	0.28(±0.10)	0.029 (±0.010)
		ME	1.71(±0.48)	0.168(±0.047)	0.29(±0.11)	0.029 (±0.011)
	Control		1.67(±0.40) a	0.163(±0.039) a	0.29(±0.09) a	0.028 (±0.008) a
		AT	1.79(±0.45)	0.176(±0.044)	0.29(±0.10)	0.028 (±0.009)
		ME	1.54(±0.33)	0.151(±0.032)	0.30(±0.08)	0.028 (±0.008)
Mean 20-29			1.73(±0.44)	0.170(±0.043)	0.28(±0.09)	0.027(±0.009)
40-49 (vegetative parts development)	Biostimulated		23.80(±3.62) a	2.332(±0.355) a	3.43(±0.64) a	0.336(±0.063) a
		AT	23.64(±3.36)	2.317 (±0.329)	3.19(±0.40)	0.313(±0.039)
		ME	23.96(±4.01)	2.348(±0.393)	3.66(±0.77)	0.359(±0.075)
	Irrigated		23.01(±4.31) a	2.255(±0.423) a	3.27(±0.68) a	0.320(±0.066) a
		AT	23.00(±5.07)	2.254(±0.497)	3.31(±0.83)	0.324(±0.082)
		ME	23.01(±3.63)	2.255(±0.355)	3.23(±0.51)	0.317(±0.050)
	Control		14.28(±4.94) b	1.399(±0.484) b	2.43(±0.97) a	0.238(±0.095) a
		AT	14.03(±4.39)	1.375(±0.430)	2.32(±0.71)	0.228(±0.070)
		ME	14.52(±5.62)	1.423(±0.551)	2.54(±1.19)	0.249(±0.117)
Mean 40-49			20.36(±6.09)	1.995(±0.597)	3.04(±0.88)	0.298(±0.086)
51 (visible flower buds)	Biostimulated		107.05(±35.03) a	10.491(±3.433) a	16.35(±5.46) a	1.602(±0.535) a
		AT	110.25(±32.03)	10.804(±3.139)	15.10(±4.42)	1.479(±0.434)
		ME	103.86(±40.62)	10.178(±3.980)	17.61(±6.49)	1.725(±0.636)
	Irrigated		89.49(±21.40) a	8.770(±2.097) a	13.29(±3.28) ab	1.303(±0.322) ab
		AT	89.87 (±17.79)	8.807(±1.744)	12.78(±2.39)	1.253(±0.234)
		ME	89.11(±25.31)	8.732(±2.481)	13.80(±4.03)	1.352(±0.395)
	Control		42.89(±13.36) b	4.203(±1.309) b	7.64 (±3.16) b	0.749(±0.310) b
		AT	40.30(±13.27)	3.949(±1.300)	7.18(±3.06)	0.704(±0.300)
		ME	45.48(±13.51)	4.457 (±1.324)	8.10(±3.33)	0.794(±0.326)
Mean 51			74.36(±34.40)	7.287(±3.372)	11.64(±5.08)	1.141(±0.498)

UFB = Unit Fresh Biomass,

TFB = Total Fresh Biomass,

UDB = Unit dry biomass,

TDB = Total Dry Biomass,

Signif. codes: 0 '****' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Values are shown as mean ± standard deviation

1.2.1 Field experiments: demo field

Objective

Sustainable production of sea fennel crops in demo field (Task 4.1)

Materials and methods

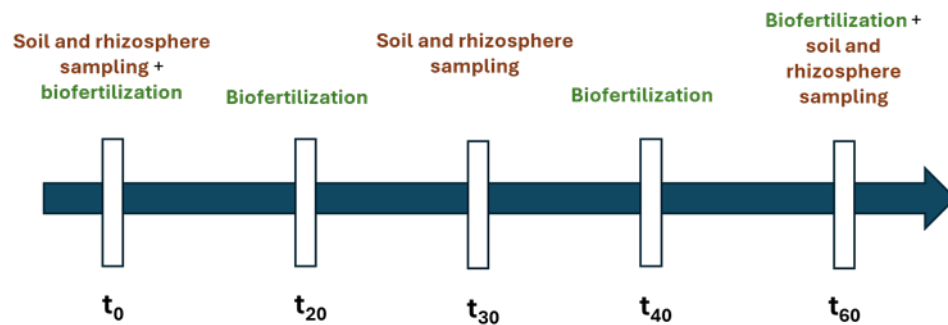
Due to the very low germination rate of seeds collected in autumn 2022, no transplanting was performed in March 2023. Consequently, new germination tests were conducted using seeds collected in autumn 2023; transplantation of the seedlings was done at the demo field of Agugliano (see D4.1) in Spring 2024.

Immediately after transplantation, sea fennel plants were bio-fertilized using the following mixture of bacterial strains (BS2):

- o free nitrogen-fixing bacteria: *Azotobacter chroococcum* DSM 2286, and *Azospirillum brasilense* DSMZ 1690 (mixed well together at a 1:1 (v/v) ratio);
- o phosphorus-dissolving bacteria: *Priestia megaterium* DSMZ 339;
- o potassium-solubilizing bacteria: *Niallia circulans* DSMZ 30598

The microbial groups were inoculated at a 2:1:1 (v/v/v) ratio, each with a load no lower than 10^8 cells/mL (details about the biofertilizer preparation are reported in Section 1.1.1).

The bio-fertilization was performed periodically according to time schedule reported below.



Sampling and field bio-fertilization timeline – Agugliano site

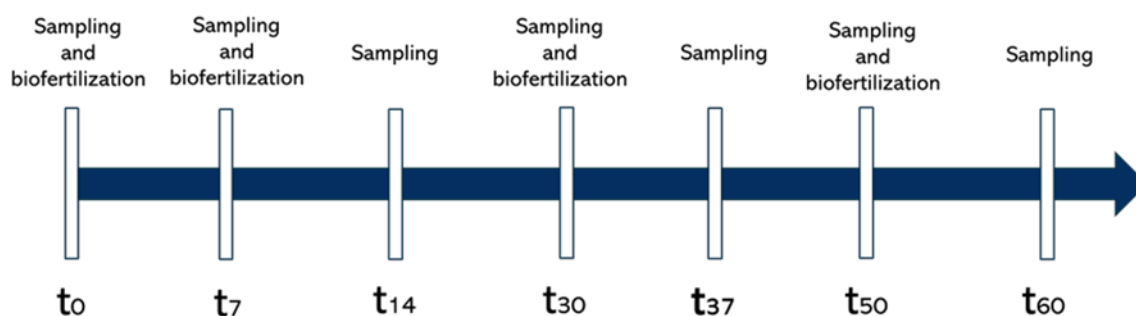
The influence of bio-fertilization on the microbiome of the transplanted sea fennel rhizosphere and root-free soil samples was evaluated in comparison with two control plots: one irrigated with water (I), and the other with no irrigation (C) by applying culture-dependent techniques (viable counting) and a culture-independent approach (next generation sequencing, NGS).

For root-free soil, three soil samples (collected at 0–30 cm depth) were taken across each experimental plot according to time schedule reported above and pooled together before the analysis. The samples were collected using a corer.

For sea fennel rhizosphere soil, three samples were taken across each experimental plot according to time schedule reported above and pooled together before the analysis.

In parallel, an additional experimental plot established with three-year-old sea fennel plants (Marche ecotype, planted in March 2020 as part of the regionally funded project “Nuove conserve vegetali biologiche, Bio-veg-conserve,” <http://www.biovegconserve.it/>) was used to evaluate the effect of periodic biostimulant supplementation on sea fennel crop yield and/or root growth. This experimental plot was organized using a completely randomized block design with three replications (R1, R2, R3), each further divided into three subplots based on different treatments (Bio-fertilization, Irrigation, Control), as previously described by Zenobi et al. (2021, 2022) (refer to D4.1 for details).

The three-year-old sea fennel plants in the selected experimental plots were again bio-fertilized using the bacterial mixture BS2. The three microbial groups were inoculated at a 2:1:1 (v/v/v) ratio, each with a load no lower than 10^8 cells/mL (details about the biofertilizer preparation is reported in Section 1.1.1). The bio-fertilization was performed periodically according to time schedule reported below.



Sampling and field bio-fertilization timeline - Camerano site

The influence of bio-fertilization on the three-year-old sea fennel root microbiome was evaluated in comparison with two control plots: one irrigated with water (I), and the other with no irrigation (C) by applying culture-dependent techniques (viable counting) and a culture-independent approach (next generation sequencing, NGS).

For root-free soil, three soil samples (collected at 0–30 cm depth) were taken across each experimental plot according to time schedule reported above and pooled together before the analysis. The samples were collected using a corer.

For sea fennel rhizosphere soil, three samples were taken across each experimental plot according to time schedule reported above and pooled together before the analysis.



Soil sampling at the demo field

Viable counting the sea fennel rhizosphere soil and root-free soil.

For the viable counts, aliquots (10 g) of each pooled soil sample were homogenized with 90 mL of peptone water (peptone 1 g/L) at 260 rpm for 3 min. The serial ten-fold dilutions of each sample were prepared for the enumeration of:

- v) total mesophilic aerobes on Plate Count Agar (PCA) incubated at 30°C, ii) spore forming bacteria after pre-treatment of soil dilutions at 80°C for 10 min and at 100°C for 30 min for a heat-resistant (HR) spore count, followed by plating on Tryptic Soy Agar (TSA) incubated at 30°C for 72 h for recovery of mesophilic bacteria and at 55°C for thermophilic bacteria (Eijlander et al., 2020),
- v) yeasts and moulds on Rose Bengal Agar (RBA) incubated at 25°C,
- v) actinomycetes on Actinomycetes Agar (AA) added with 40 µg/mL of cycloheximide to inhibit yeasts and incubated at 30°C,
- v) free nitrogen-fixing bacteria on Norris Glucose Nitrogen Free Agar incubated at 30°C
- v) lactic acid bacteria on De Man Rogosa and Sharp Agar MRS agar (Oxoid) incubated at 30°C.

The statistical analysis of viable count texture data was performed to determine differences among samples using the JMP v11.0.0 software (SAS Institute Inc., Cary, NC). To this end, the Tukey-Kramer's Honest Significant Difference (HSD) test (level of significance 0.05) was used by one-way analysis of variance (ANOVA). To evaluate the relationship between the

number of microorganisms and different treatments, a Principal Component Analysis (PCA) was performed using JMP v11.0.0 software (SAS Institute Inc., Cary, NC).

Regarding NGS, aliquots (1.5 mL) of the obtained homogenates (10^{-1} dilution) were centrifuged at 14'000 g for 10 min to collect the pellet formed by microbial cells; hence each pellet was then used for the extraction of the total microbial DNA using the E.Z.N.A. soil DNA kit (Omega Bio-tek, GA, USA) following the manufacturer's instructions. DNA was quantified using the QUBIT dsHS kit (Thermo Fisher, Milan, Italy), and purity was checked by measuring absorbance with a Nanodrop 2000 spectrophotometer (Thermo Fisher). The bacterial community was analyzed by amplifying the V3–V4 hypervariable region of the 16S rRNA gene using primers and procedure described by Klindworth et al. (2013). The fungal population was studied by the amplification of the D1 domain of the 26S rRNA gene according to Mota-Gutierrez et al. (2019). PCR amplicons were purified following the Illumina metagenomic pipeline (Illumina Inc., San Diego, CA, United States). Pair-end sequencing (2X250bp) was performed with a MiSeq platform (Illumina) using V2 chemistry according to the manufacturer's instructions.

Raw reads were analyzed by using QIIME2 software (Bolyen et al., 2019); primers and adapters were first trimmed by using Cutadapter and then quality filtered using the DADA2 algorithm (Callahan et al., 2016). Amplicon sequence variants (ASVs) generated through DADA2 were used for taxonomic assignment against the SILVA database for bacteria, and a manually built database for fungi (Mota-Gutierrez et al., 2019). When the taxonomy assignment was not able to reach species level, the genus or family name was displayed.

ASV tables and taxonomic classifications were analyzed using MicrobiomeAnalyst (Chong et al., 2020) to assess alpha diversity (Shannon index) and beta diversity (Bray-Curtis index). Statistical differences in alpha diversity among samples were evaluated using the Kruskal-Wallis test based on the Shannon index. Beta diversity was visualized through Principal Coordinate Analysis (PCoA) based on the Bray-Curtis index, and significant differences between groups were determined using PERMANOVA ($p < 0.05$).

Chemical analysis of soil

Soil samples underwent also the following chemical analyses (at the beginning of the cultivation trials) to evaluate the influence of soil composition and salinity on crop yield, using standard optimized protocols:

- pH
- organic matter
- total N
- total organic C
- total inorganic C
- C/N
- Exchangeable K
- Humic and fulvic acids

The Soil chemical analyses were performed by the A.M.A.P. laboratories in Jesi (Ancona, Marche). The analyses were performed according to the following methods:

- Total Inorganic Carbon (TIC900): Internal method by oxidative combustion
- Humic and Fulvic Acid Carbon: D.M. 13/09/99 GU SO no. 248 of 21/10/1999 - VIII.1
- pH in H₂O: D.M. 13/09/99 GU SO no. 248 of 21/10/1999 - III.1
- Organic Matter D.M. 13/09/99 GU SO no. 248 of 21/10/1999 - VII.3, VII.3.6 low
- Total Nitrogen D.M. 13/09/99 GU SO no. 248 of 21/10/1999 - XIV.1
- Exchangeable Potassium: D.M. 13/09/99 GU SO no. 248 of 21/10/1999 - XIII.2, XIII.2.6 high
- Organic Carbon: Internal method by oxidative combustion

Analysis of crop yield/quality

The new sea fennel crops produced through the demo field cultivation trials, were analysed once a year (harvesting period: may – august) using destructive analyses (biomass sampling) for the determination of:

- phenological development;
- fresh and dry biomass yield;

- multispectral imagery captured with unmanned aerial vehicles (UAVs) (drones) equipped with multispectral cameras.

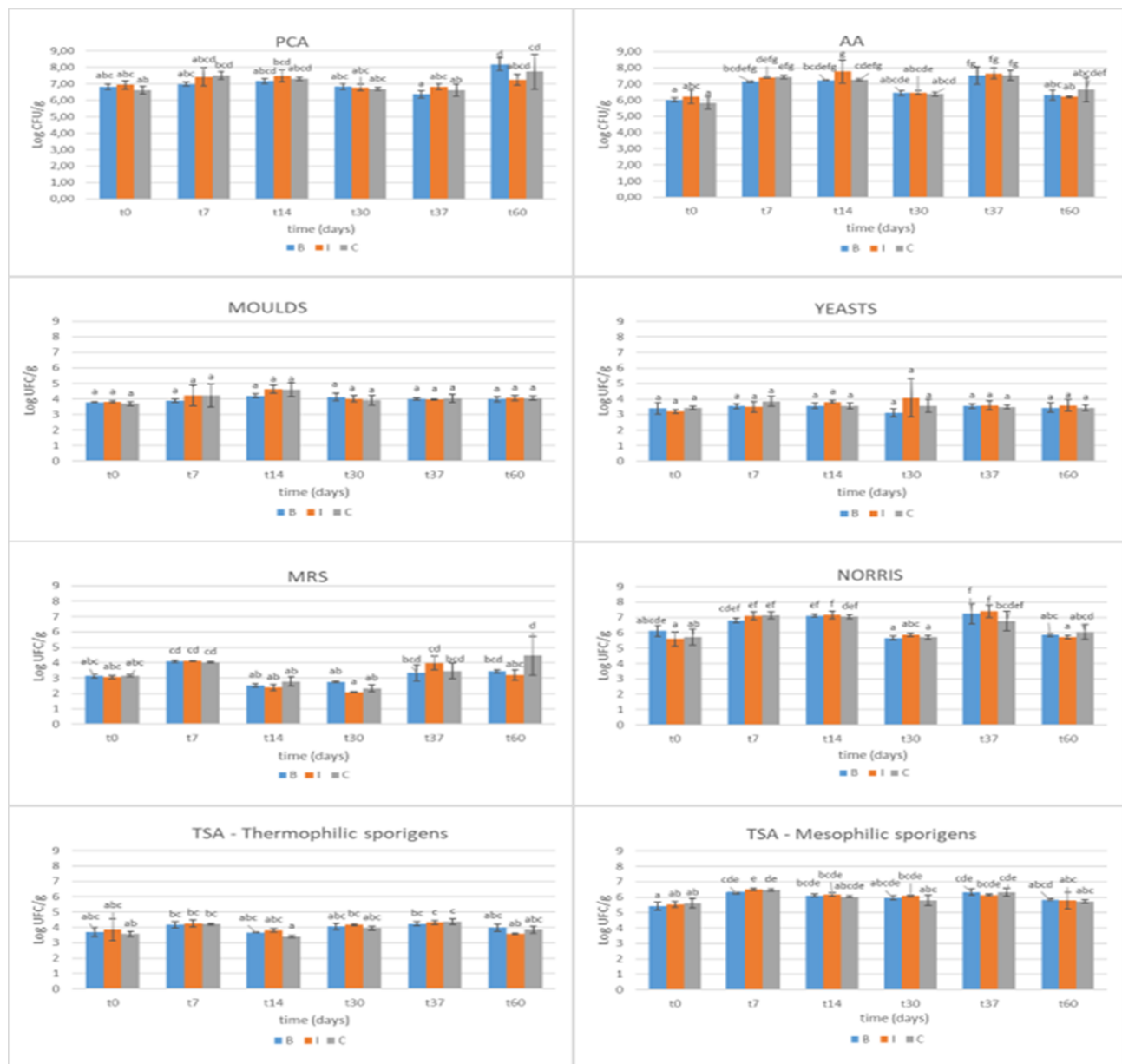
Results

Data collected at the two Italian sites for demo field experiments are reported below, grouped per site.

Site CAMERANO - demo field with three-year-old sea fennel crop

Viable counting of root-free soil

Data of the viable counts of root-free soils are reported below.



Means $\pm$ standard deviations are shown. For each microbial group and sample, overall means with different small letters are significantly different ($p < 0.05$).

B: Biostimulated

I: Irrigated

C: Control (no treatment)

PCA: Plate count agar (total mesophilic aerobes)

AA: Actinomyces agar (actinomycetes)

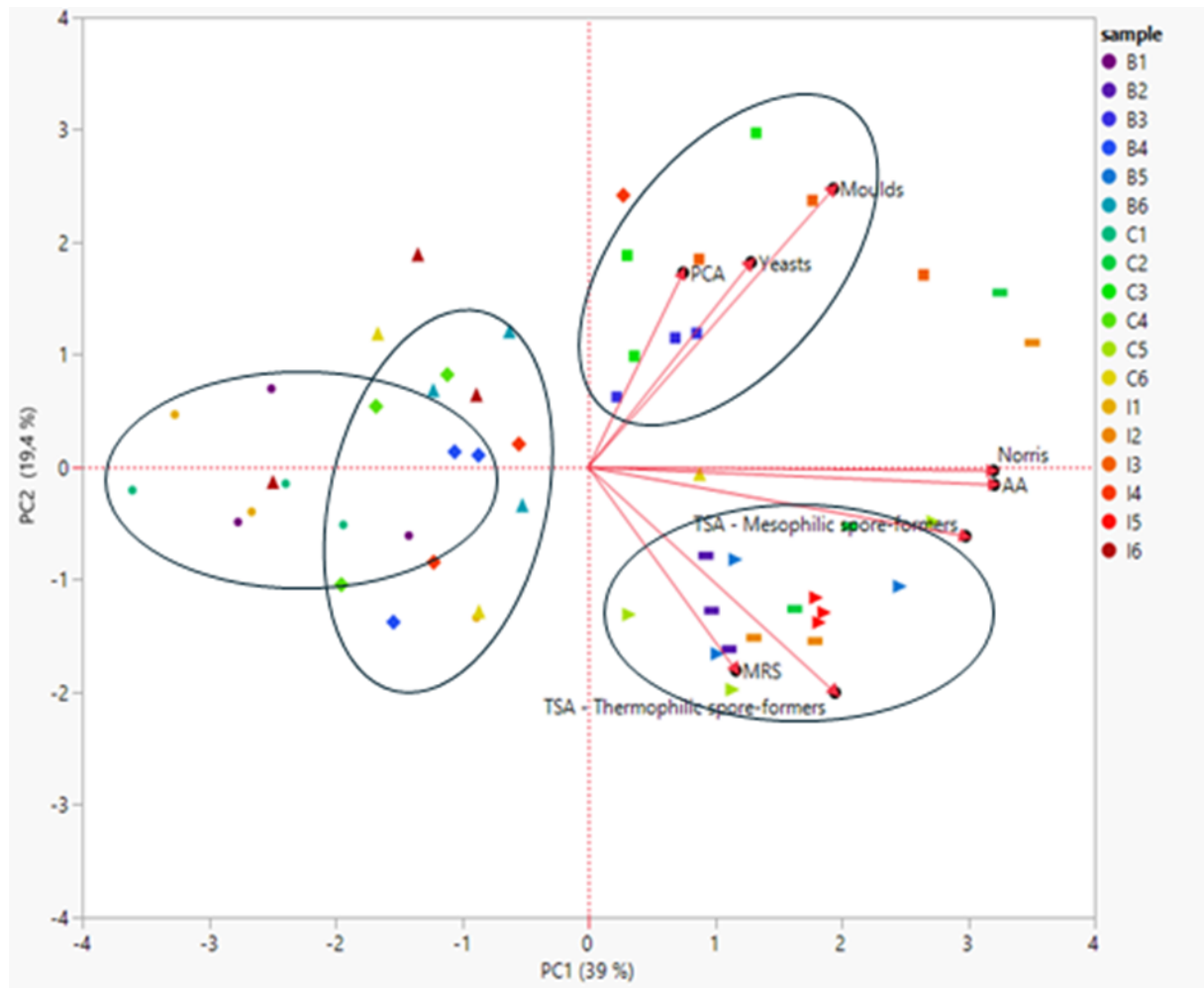
NORRIS: Norris Glucose Nitrogen Free Medium (free nitrogen-fixing bacteria)

MRS: De Man–Rogosa–Sharpe Agar (lactic acid bacteria)

TSA: Tryptic Soy Agar

The results overall collected showed only sporadic statistically significant differences between the treatments and within them, between the six points (t0, t7, t14, t30, t37 and t60).

Bi-plot of PCA carried out onto viable counts of root-free soil samples are shown below.



- 1: 0 days •
- 2: 7 days ■
- 3: 14 days ▣
- 4: 30 days ◆
- 5: 37 days ►
- 6: 60 days ▲

B: Biostimulated

I: Irrigated

C: Control (no treatment)

PCA: Plate count agar (total mesophilic aerobes)

AA: Actinomyces agar (actinomycetes)

NORRIS: Norris Glucose Nitrogen Free Medium (free nitrogen-fixing bacteria)

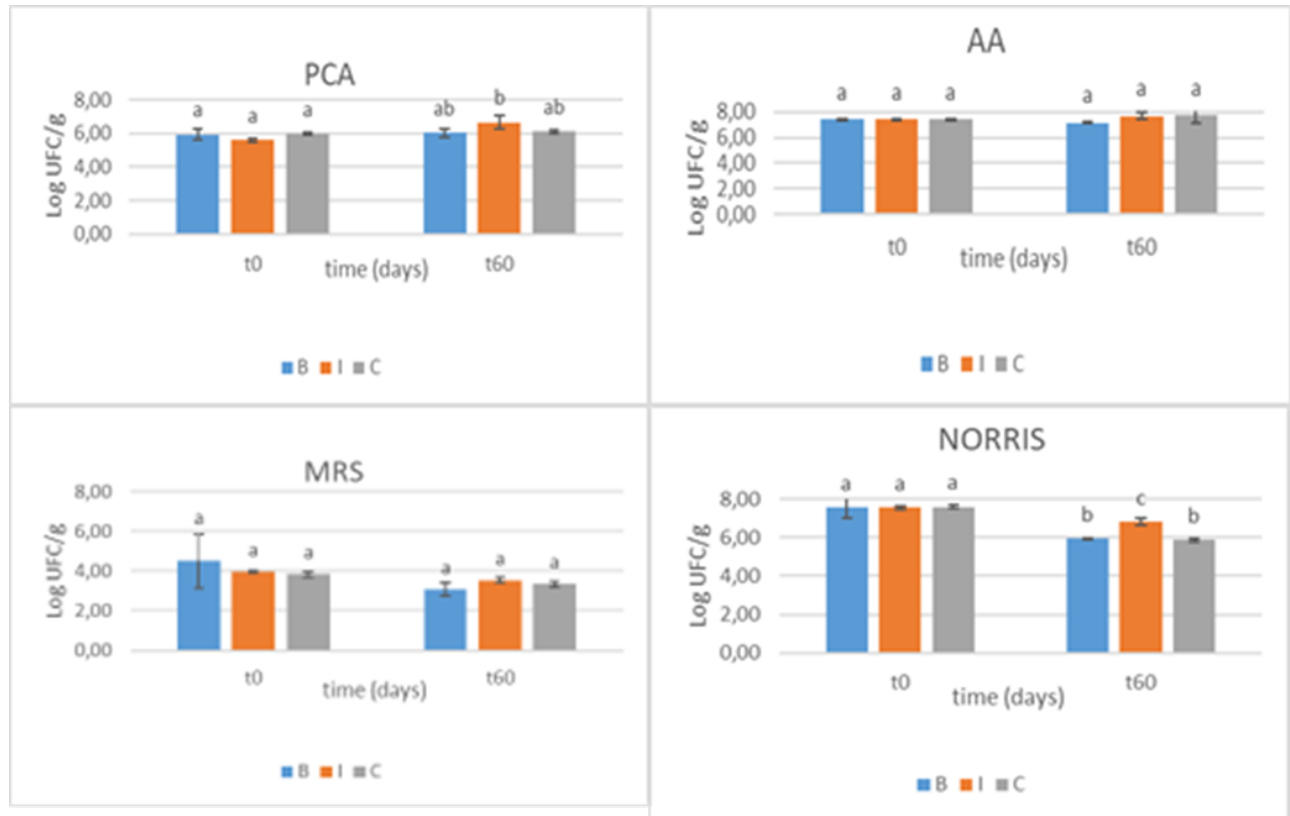
MRS: De Man–Rogosa–Sharpe Agar (lactic acid bacteria)

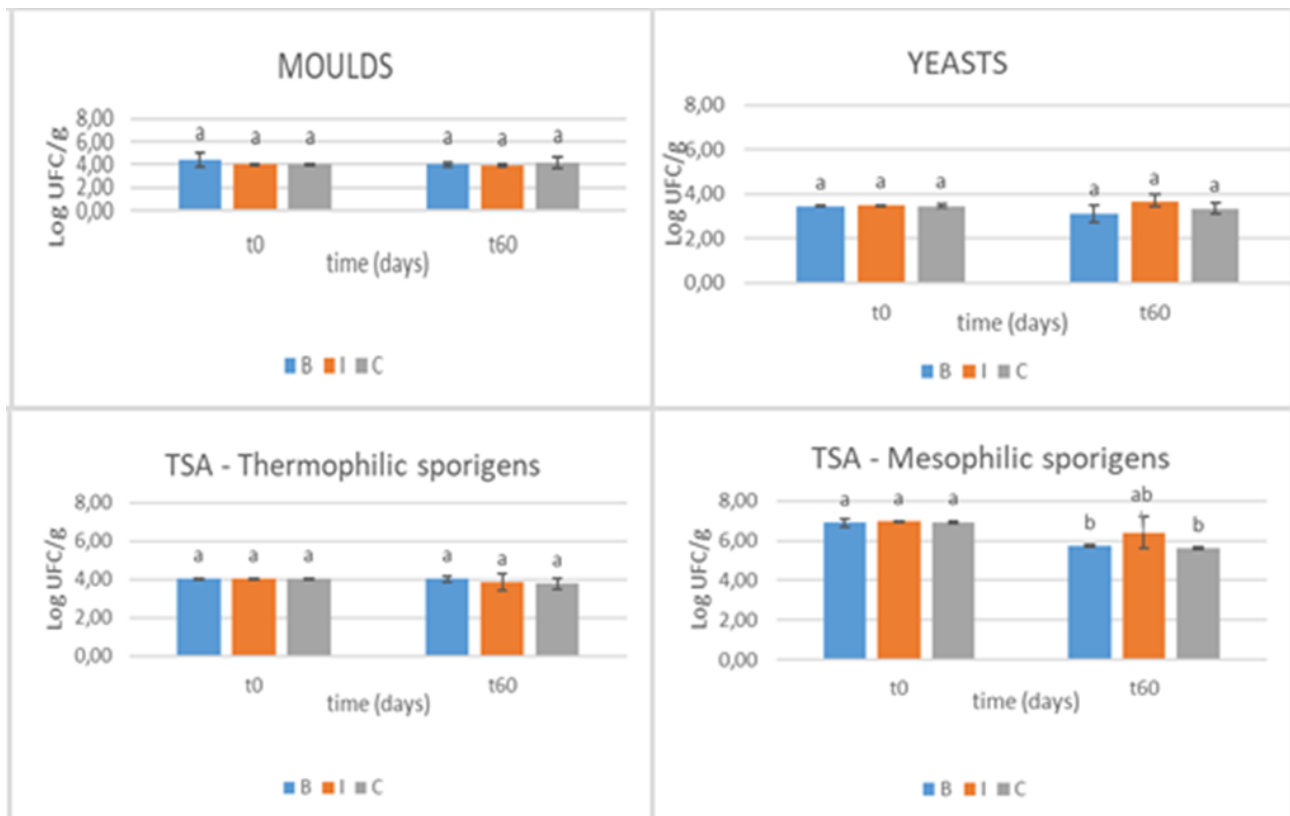
TSA: Tryptic Soy Agar

The PCA analysis of viable counts performed onto viable counts of soil samples suggests that the clustering is driven by sampling time rather than by treatment.

Viable counting of sea fennel rhizosphere soil

Data of the viable counts of sea fennel rhizosphere soil are reported below.





Means $\pm$ standard deviations are shown. For each microbial group and sample, overall means with different small letters are significantly different ($p < 0.05$).

B: Biostimulated

I: Irrigated

C: Control (no treatment)

PCA: Plate count agar (total mesophilic aerobes)

AA: Actinomyces agar (actinomycetes)

NORRIS: Norris Glucose Nitrogen Free Medium (free nitrogen-fixing bacteria)

MRS: De Man–Rogosa–Sharpe Agar (lactic acid bacteria)

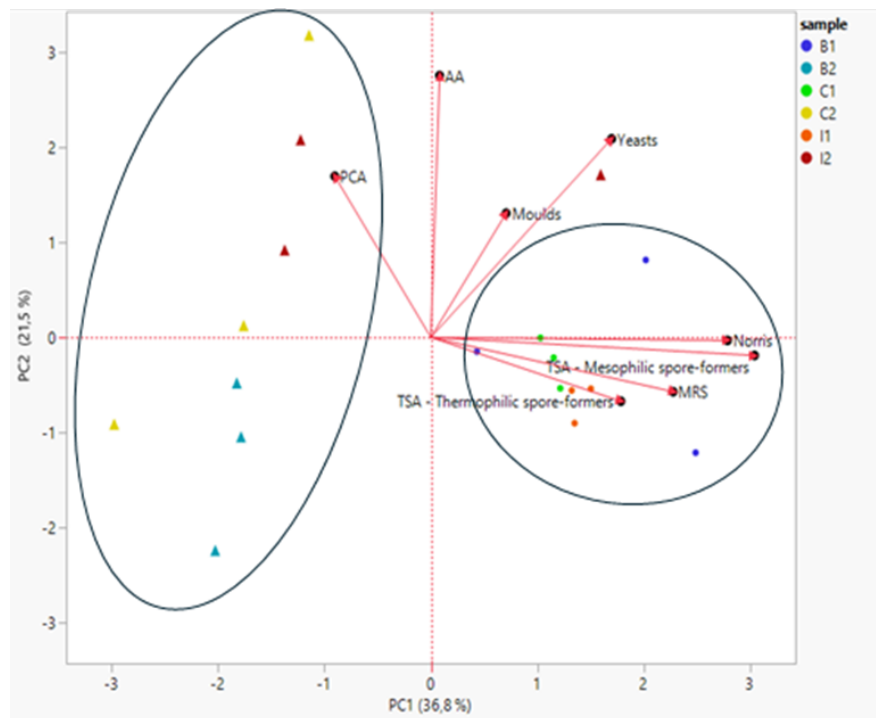
TSA: Tryptic Soy Agar

Similarly to root-free soil samples, the results overall collected from rhizosphere soil samples showed only sporadic statistically significant differences between treatments and time points.

Bi-plot of PCA carried out onto viable counts of rhizosphere soil samples are shown below.

1: 0 days •

2: 60 days ▲



B: Biostimulated

I: Irrigated

C: Control (no treatment)

PCA: Plate count agar (total mesophilic aerobes)

AA: Actinomyces agar (actinomycetes)

NORRIS: Norris Glucose Nitrogen Free Medium (free nitrogen-fixing bacteria)

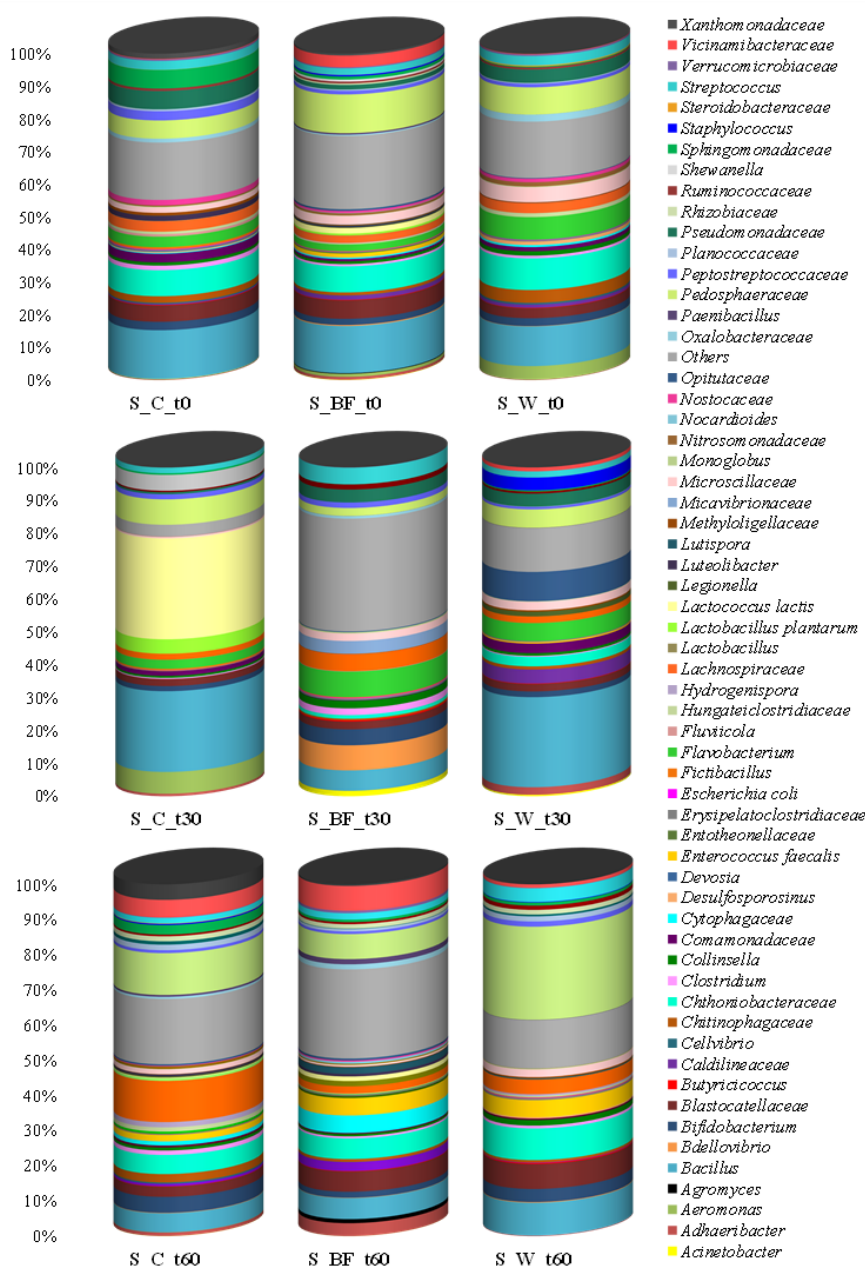
MRS: De Man–Rogosa–Sharpe Agar (lactic acid bacteria)

TSA: Tryptic Soy Agar

Again, the results collected onto rhizosphere soil samples showed only sporadic statistically significant differences between treatments and time points.

NGS

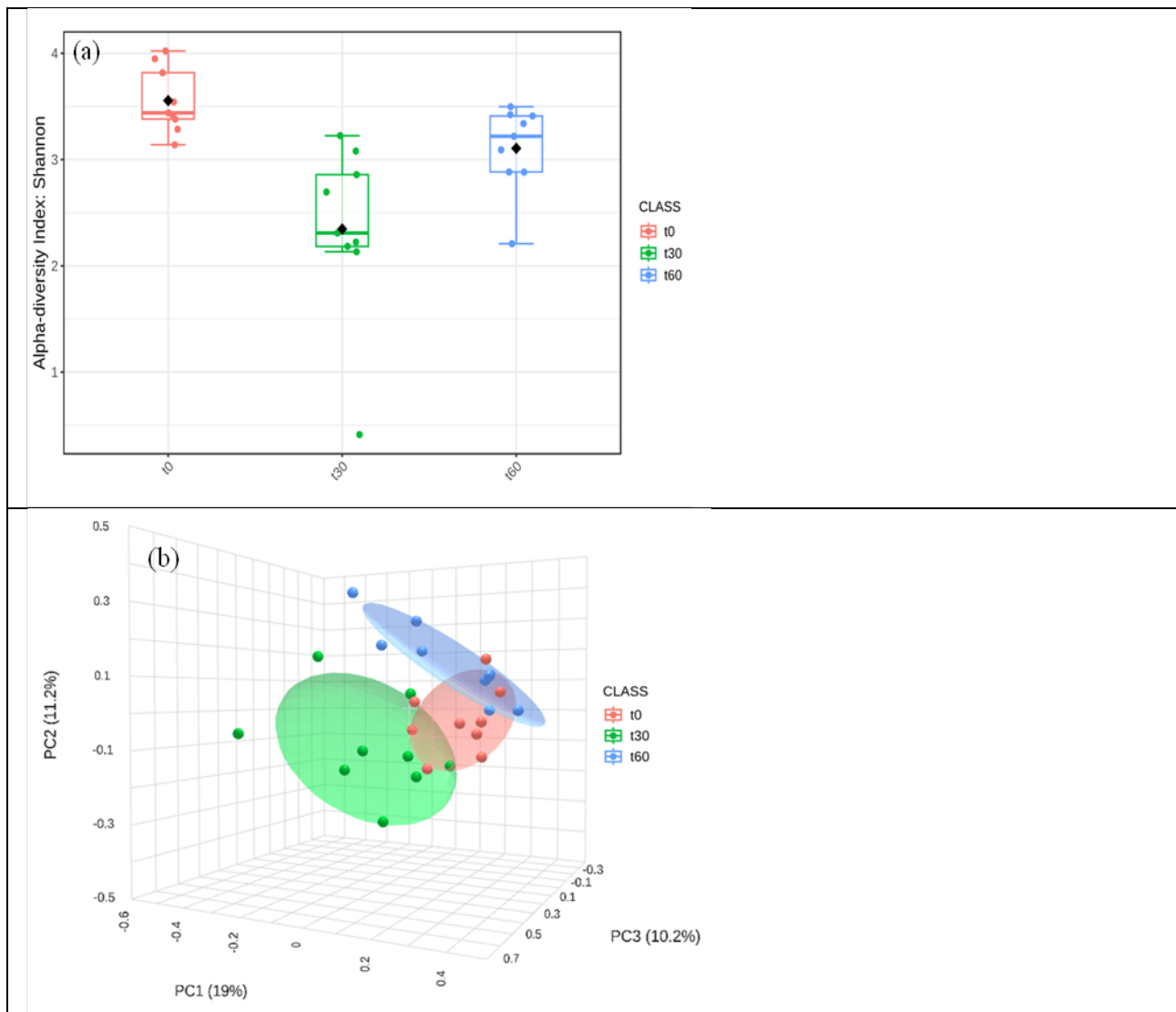
A total of 731,985 denoised reads were obtained for bacteria, with an average of 18,769 reads per sample, and 8,121,046 reads were generated for fungi, averaging 208,232 reads per sample. The bacterial composition of the soil samples is presented in the figure below.



Relative frequency of bacterial ASVs of soil (S) samples at different sampling times (t_0 , t_{30} , t_{60}). C: control; BF: biofertilizer-treated; W: water-treated.

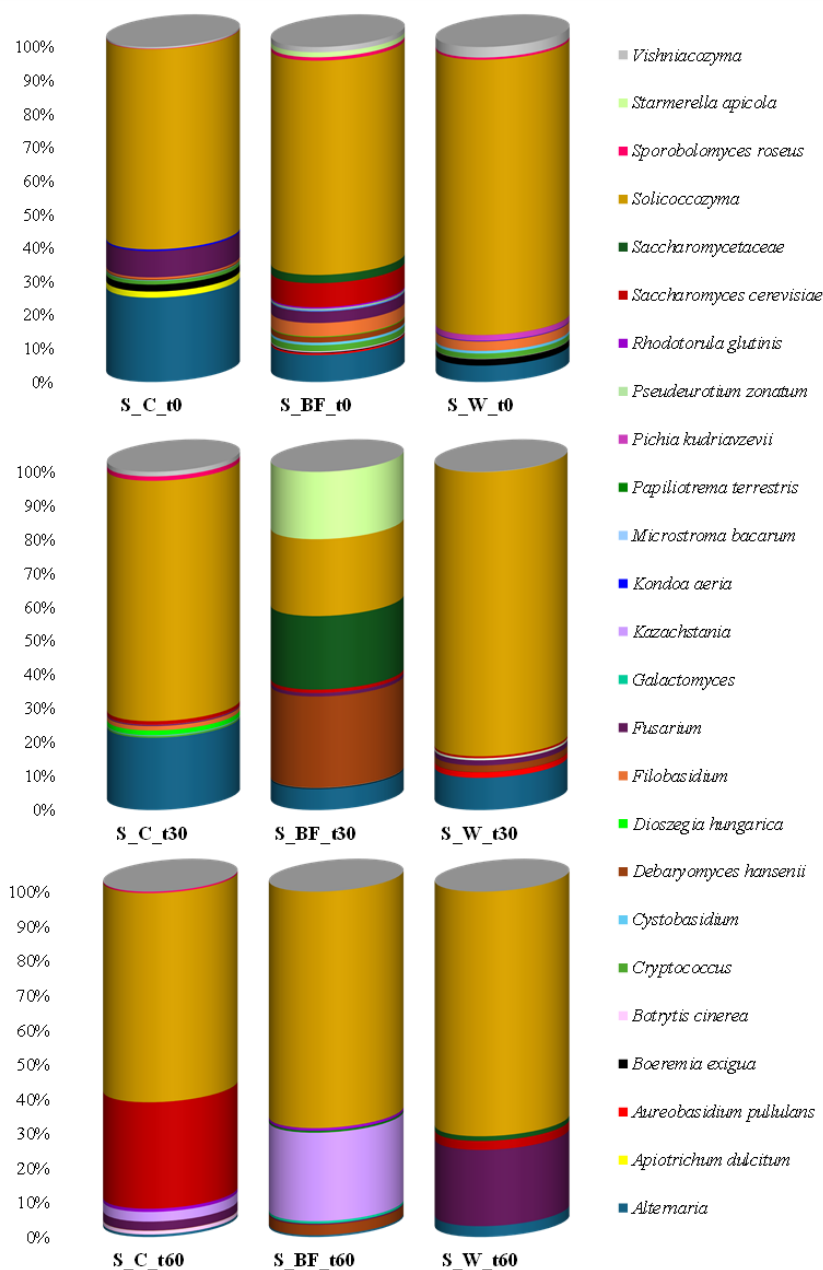
Overall, the most abundant taxa across samples included *Bacillus* spp. (ranging from 5.66% to 26.74% of the relative frequency), *Bifidobacterium* spp. (1.61% – 4.87%), *Blastocatellaceae* (1.99% – 6.80%), *Lachnospiraceae* (1.80% – 12.56%), and *Pedospaeraceae* (2.34% – 25.44%). *Chthoniobacteraceae* displayed higher relative abundances at t_0 and t_{60} (5.54% – 9.36%) compared to t_{30} (< 3%). At t_{60} , *Enterococcus faecalis* ranged between 1.87% and 4.64% of the relative frequency, whereas the relative frequency was < 1% at both t_0 and t_{30} . *Flavobacterium* spp. was found as dominant taxon at t_0 and t_{30} (1.87% – 7.57%), whereas at t_6 the relative frequency was < 1%. Of note, sample S_C_30 showed high relative frequencies of *Lactobacillus plantarum* (reclassified as *Lactiplantibacillus plantarum* subsp. *plantarum*, 4.35%) and *Lactococcus lactis* (30.34%). Samples at t_0 were also characterized by the occurrence of *Nostocaceae* and *Pseudomonadaceae*, while *Rhizobiaceae* and *Vicinamibacteraceae* were more abundant in soil samples at t_{60} .

Diversity indices revealed no significant differences in bacterial diversity related to treatment types (C, W, BF) ($p > 0.05$) (data not shown). By contrast, significant differences ($p < 0.05$) were observed in both alpha-diversity and beta-diversity indices across different sampling times (t_0 , t_{30} , t_{60}), as shown in the figure below.



Alpha- (a) and beta-diversity (b) indices of soil samples based on sampling times.

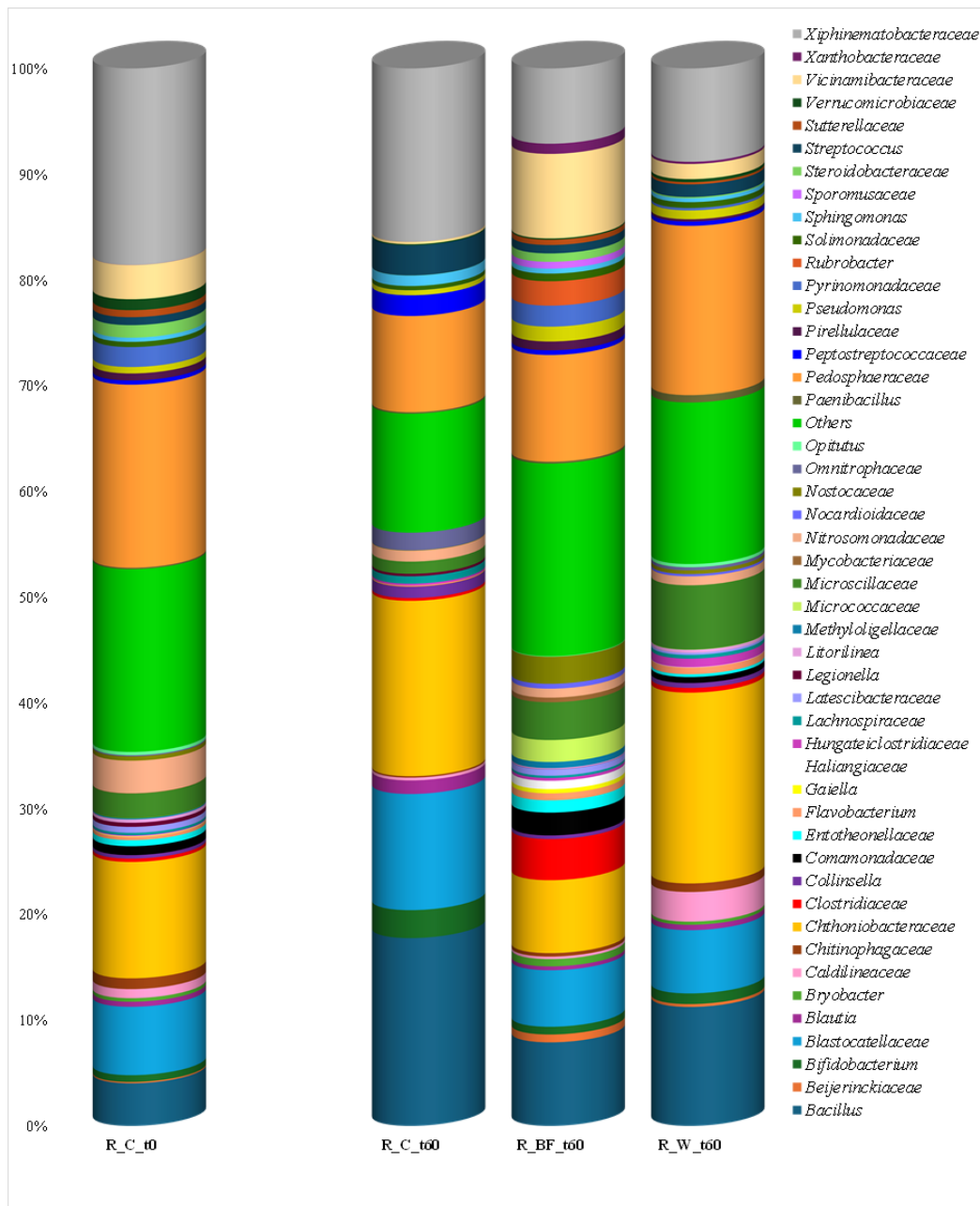
The mycobiota composition of the soil samples is presented in the figure below.



Relative frequency of fungal ASV of soil samples (S) at different sampling times (t0, t30, t60). C: control; BF: biofertilizer-treated; W: water-treated.

In detail, *Solicoccozyma* spp. was identified as the dominant taxon across all soil samples, with relative frequency ranging from 22.72% to 83.67%. At t0 and t30, *Alternaria* spp. showed high relative frequency ranging from 4.83% to 25.08%. Of note, sample S_BF_t30 showed high relative frequency of *Debaryomyces hansenii* (26.97%), *Saccharomycetaceae* (21.72%), and *Starmerella apicola* (19.92%), while sample S_BF_t60 showed high relative frequency of *Kazachstania* spp. (25.64%). Moreover, sample S_W_t60 showed a high relative frequency of *Fusarium* spp. (22.07%), while sample S_C_t60 showed a high relative frequency of *Saccharomyces cerevisiae* (30.54%). Statistical analysis of alpha and beta diversity indices revealed that the mycobiota composition was not significantly affected by either treatment type or time ($p > 0.05$; data not shown).

The bacterial composition of the rhizosphere samples is shown in the figure below.

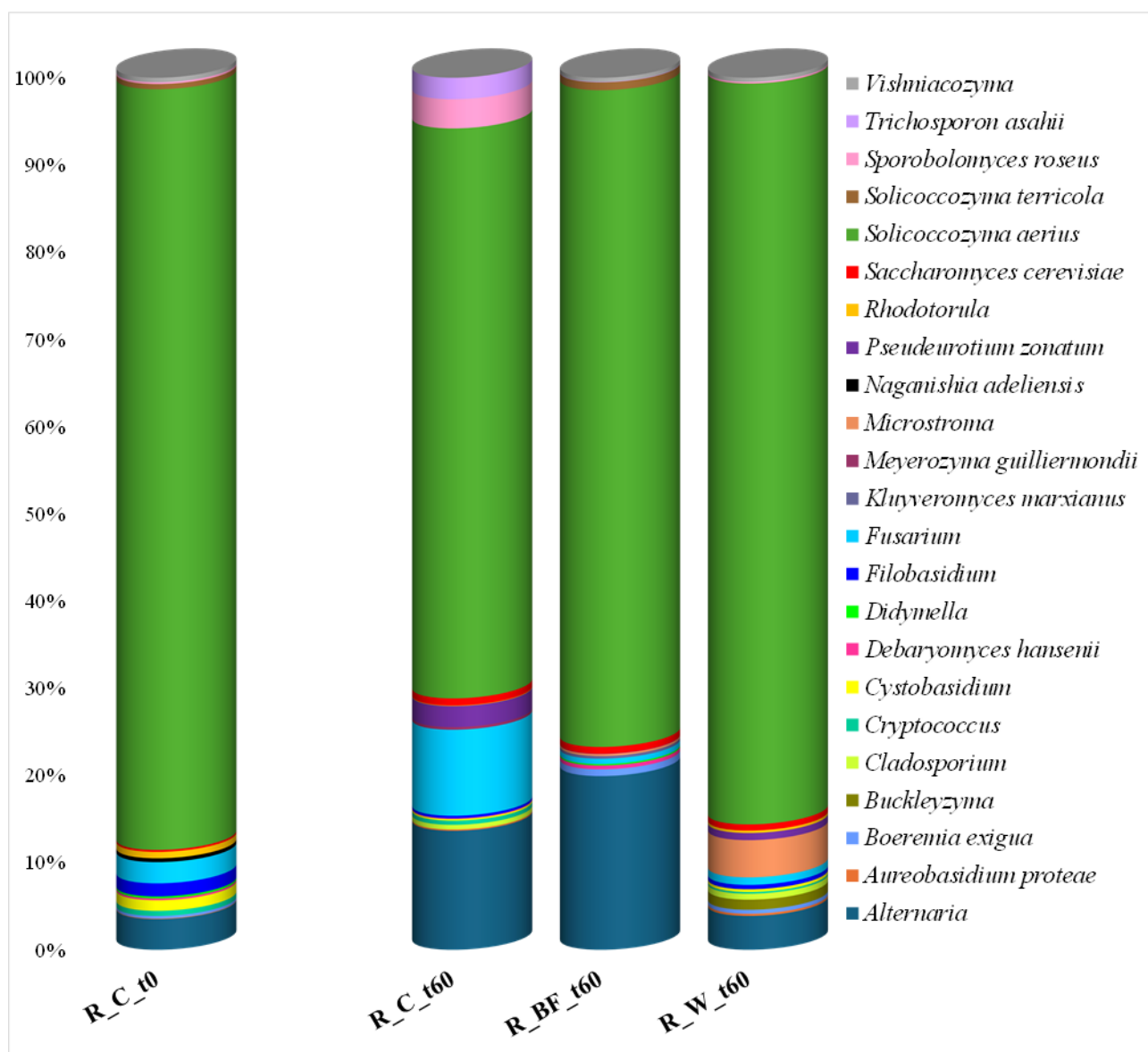


Relative frequency of bacterial ASVs of rhizosphere (R) samples. C: control; BF: biofertilizer-treated; W: water-treated.

Overall, the most abundant taxa across samples included *Bacillus* spp. (ranging from 3.49% to 15.17% of the relative frequency), *Blastocatellaceae* (4.65% – 9.38%), *Chthoniobacteraceae* (6.00% – 15.38%), *Pedosphaeraceae* (7.77% – 15.00%), and *Xiphinematobacteraceae* (6.20% – 16.10%).

No significant differences in alpha- and beta-diversity indices were observed among samples ($p > 0.05$; data not shown).

The mycobiota composition of the rhizosphere samples is shown in the figure below.



Relative frequency of fungal ASVs of rhizosphere (R) samples. C: control; BF: biofertilizer-treated; W: water-treated.

The fungal composition of all rhizosphere samples was dominated by *Solicoccozyma aerius*, ranging between 65.24% and 85.96% of the relative frequency, followed by *Alternaria* spp. (3.47% – 19.80%). Sample R_C_t0 was additionally characterized by the occurrence of *Cystobasidium* spp. (1.19%), *Filobasidium* spp. (1.47%), and *Fusarium* spp. (2.40%). Control rhizosphere sample at t60 (R_C_t60) showed the presence of *Fusarium* spp. (9.84%), *Pseudoeurotium zonatum* (2.41%), *Sporobolomyces roseus* (3.34%), and *Trichosporon asahii* (2.47%), whereas sample R_W_t60 was characterized by the presence of *Buckleyzyma* spp. (1.14%), and *Microstroma* spp. (4.19%).

No significant differences in alpha- and beta-diversity indices were observed among samples ($p > 0.05$; data not shown).

Chemical analysis of soil samples

The soil analysis shows that the biostimulated treatment exhibits statistically significant lower values in inorganic carbon going from t0 to tf as it activated metabolic mechanisms of root uptake, for the other variables measured, there were no statistically significant intra- and inter-treatment differences, probably because the effect of the microbial biostimulant requires several years of administration, according to the pH the soil is classified as sub-alkaline, the table is reported below

	Total inorganic carbon (g/Kg)	Total organic carbon (g/Kg)	Humic and fulvic acid carbon (g/Kg)	pH in H ₂ O	C/N	Organic matter (g/Kg)	Total nitrogen (g/Kg)
B to	42,7 ± 0,2abA	9,95 ± 0,7aA	5,95 ± 0,20aA	7,95 ± 0,03aA	9,1 ± 0,1aA (M)	17,15 ± 1,7aA (L)	1,1 ± 0,2aA (M)
I t0	42,4 ± 0,4abA	9,05 ± 0,8aA	5,45 ± 0,15aA	7,99 ± 0,02aA	9,1 ± 0,1aA (M)	15,6 ± 0,8aA (L)	1 ± 0,15aA (L)
C to	43,6 ± 0,3aA	10,1 ± 1aA	6,65 ± 0,12aA	7,93 ± 0,03aA	8,8 ± 0,1aA (L)	17,4 ± 1,8aA (L)	1,15 ± 0,05aA (M)
B tf	41,77 ± 0,76bA	9,83 ± 2,00aA	5,77 ± 1,16aA	7,99 ± 0,04aA	8,63 ± 0,45aA (L)	16,95 ± 3,45aA (L)	1,13 ± 0,18aA (M)
I tf	42,83 ± 0,74abA	9,12 ± 0,83aA	5,47 ± 0,48aA	7,99 ± 0,02aA	8,57 ± 0,23aB (L)	15,72 ± 1,43aA (L)	1,07 ± 0,10aA (M)
C tf	42,87 ± 0,60abA	10,18 ± 1,73aA	5,72 ± 0,63aA	7,97 ± 0,05aA	8,83 ± 0,59aA (L)	17,57 ± 2,98aA (L)	1,15 ± 0,13aA (M)

Means ± standard deviations are shown. Means followed by different letters are significantly different. Lowercase letters indicate significant differences between all the samples, uppercase letters indicate significant differences between the t0 and tf samples subjected to the same treatment. The letters in brackets indicate whether the associated values are high (H), average (M) or low (L).

B: Biofertilized

I: Irrigated

C: Control (no treatment)

t0: 0 days

tf: 60 days

H: High value

L: Low value

M: Medium value

Physical analysis of soil samples

Soil characterisation was performed before the *C. maritimum* L. growing season. According to the USDA classification, the soil detected is clayey loam, therefore with good water retention but subject to water stagnation.

Soil properties in the 0-30 cm layer in the experimental plots in 2023 (n = 3) are shown below:

Soil properties	Values
Sand (g kg ⁻¹)	125 ± 19
Silt (g kg ⁻¹)	445 ± 12
Clay (g kg ⁻¹)	430 ± 15
Volumetric soil water content (%):	
Field Capacity	44.3 ± 1.1
Permanent Wilting Point	18.4 ± 2.2
Total available water	26.2 ± 1.7

values expressed in mean±standard deviation.

Phenological development

To characterize the phenological development of the crop in the treatments, for the main stem, we adopted the Bundesanstalt, Bundessortenamt, Chemische Industrie (BBCH) scale (Bleiholder et al., 1991) during the entire biological cycle of the crop: the phenological characterization began between the vegetative recovery phase indicated with BBCH 20-29 and continued until the BBCH 51 phase corresponding to the initial emergence of the inflorescence or when the leaves are collected for industrial use. To understand the phenological growth of *Crithmum maritimum* L. and develop the planning of water and biostimulant treatments as well as the sampling of epigeal biomass, we based ourselves on a series of studies concerning *C. maritimum* L. and referring to open-field Mediterranean environments (Zenobi et al., 2021; Zenobi et al., 2022).

Between the phenological phases BBCH 20-29 and BBCH 40-49, 50% of water and biostimulant of the quantity foreseen in each treatment for the 2023 growing season were administered, while within the phenological phase BBCH 51, water and biostimulant were administered. The total volume of water administered on irrigated and biostimulated plots is equal to 150 mm while only on the biostimulated plots 10^8 cells of microbial strains per ml were added.

Fresh and dry epigeal biomass yield

C. maritimum L. production was evaluated at three different phenological stages: side shoots formation (BBCH scale 20-29), vegetative parts development (BBCH scale 40-49) and flower buds visible (BBCH scale 51): the phenological phase BBCH =40-49 occurs after 50% of the inputs have been administered (water and biostimulant "BS2") while the phenological phase BBCH = 51 occurs after all the inputs have been administered (water and biostimulant "BS2") and is the phase that corresponds to leaves harvest of for industrial use.

The epigeal biomass was cut at 5 cm from the ground level and its fresh weight was determined. Then, the biomass was placed in an oven at 105 °C for 48 h, after which its dry weight was determined.

Within the three replications in each plot, the average biomass production in three test areas, for a total of 45 plants in each treatment.

The values obtained from all replications within the same treatment were averaged, and the standard deviation was determined. Production per hectare for each treatment was calculated from the average fresh unit production, considering the actual plant density. Yield per hectare was then calculated using the following formula:

Total yield ($t\ ha^{-1}$) = (Biomass sampled plant weight (g) $\times$ plant density ($n\ plants\ ha^{-1}$)) $\times 10^{-6}$

where Biomass sampled plant weight is the average production per plant for each treatment type [$g\ plant^{-1}$], plant density is the number of plants per ha, and 10^{-6} is the conversion factor from g to t. The means and standard deviations of the total production in the plots within the same treatment were then calculated and compared with the results of other treatments. The table is reported below

Treatment	BBCH	UFW (g plant ⁻¹)	TFW (t ha ⁻¹)	UDW (g plant ⁻¹)	TDW (t ha ⁻¹)
CT	20-29	95.3(± 24.8) b	9.3(± 2.4) a	14.0(± 2.5) b	1.4 (± 0.4) b
BIO	20-29	151.1(± 41.4) a	14.8(± 4.1) a	27.4(± 2.6) a	2.7(± 0.3) a
IRR	20-29	141.6(± 24.1) a	13.9 (± 2.4) a	24.1(± 4.0) a	2.4(± 0.4) a
Mean 20-29		129.3(± 38.9)	12.7(± 3.8)	21.9(± 6.5)	2.1 (± 0.6)
CT	40-49	380.7(± 32.1) c	37.3(± 3.2) c	59.4(± 8.6) b	5.8(± 0.8) b
BIO	40-49	548.6 (± 39.8) b	53.8(± 3.9) b	86.6(± 7.9) a	8.5(± 0.8) a
IRR	40-49	596.8(± 37.9) a	58.4(± 3.7) a	88.0(± 12.0) a	8.6 (± 1.2) a
Mean 40-49		508.7(± 100.8)	49.9(± 9.9)	78.0(± 16.3)	7.6 (± 1.6)
CT	51	523.7(± 28.2) c	51.3(± 2.8) c	88.1(± 7.5) c	8.6(± 0.7) c
BIO	51	936.1(± 70.6) a	91.7(± 6.9) a	153.7(± 19.0) a	15.1(± 1.9) a
IRR	51	820.4(± 35.0) b	80.4(± 3.4) b	122.9(± 9.3) b	12.0(± 0.9) b
Mean 51		760.1(± 183.0)	74.5(± 17.9)	121.6(± 30.0)	11.9 (± 2.9)

Means (+/- standard deviation) within each column followed by different letters are significantly different (ANOVA, $p < 0.05$).

BIO: Biofertilized

IRR: Irrigated

CT: Control (no treatment)

BBCH = phenological phase 20-29 Side shoots formation; 40-49 Vegetative parts development; 51 Flower buds visible

UFW Unit Fresh Weight

TFW Total Fresh Weight

UDW Unit Dry Weight

TDW Total Dry Weight

The microbial biostimulant shows significant effects in terms of production of fresh and dry biomass after the start of administration compared to the untreated control: in the BBCH 51 phenological phase (visible flower buds), corresponding to the leaf harvesting phase for industrial use, the biostimulated plants show significantly higher values than irrigated and control plants for both biomass variables measured

SPAD Units

In order to avoid Soil Plant Analysis Development (SPAD) readings with strong variations, we have performed the sampling during a fully-sunny day. SPAD readings were made by using the chlorophyll meter SPAD Minolta 502 (Konica Minolta Sensing 2003, Osaka, Japan). The functioning of the SPAD Minolta 502 is based on production of light by two silicon photodiodes, with one sensitive to red light (650 nm; peak chlorophyll absorbance) and the other sensitive to infrared radiation (940 nm; non-chlorophyll absorbance). Electrical currents converted from light received by the silicon photodiodes are received by a microprocessor, which linearizes the signal and calculates a SPAD (unit less) value according to equation $SPAD\ readings = A \times [\log(I_{or} \times I_{r^{-1}}) - \log(I_{of} \times I_{f^{-1}}) + B]$

Where:

A = constant;

B = constant;

I_{or} = current from red detectors with sample in place;

I_{r} = current from red infrared detectors with sample in place;

I_{of} = currents from red detectors with no sample;

I_{f} = currents from infrared detectors with no sample.

We have randomly selected two test areas for each sub-plot. At each test areas we have chosen 10 full expanded and intact leaves on which SPAD readings were made. The readings were taken on the central portion of the leaves, at the same time slot, around midday (11:00–13:00 a.m.). Moving from one plot to the next one, we carefully cleaning the optical sensor with an alcohol solution and we performed the calibration activity as suggested by the SPAD Minolta 502 instruction manual. The 10 leaves for each test area, after the SPAD readings, were cut and in the same bag containing the plant of the same test area, placed in a portable refrigerator and transferred to the laboratory where the biomass was analyzed.

The table is reported below

Treatment	BBCH	SPAD
CT	20-29	35.5(±3.6) a
BIO	20-29	35.4(±7.0) a
IRR	20-29	35.1(±3.6) a
Mean 20-29		35.3(±4.8)
CT	40-49	44.9(±2.5) b
BIO	40-49	53.1 (±3.9) a
IRR	40-49	50.9(±2.8) a
Mean 40-49		49.6(±4.6)
CT	51	41.8(±2.3) c
BIO	51	58.7(±2.6) a
IRR	51	52.5(±1.8) b
Mean 51		51.0(±7.4)

BIO: Biofertilized

IRR: Irrigated

CT: Control (no treatment)

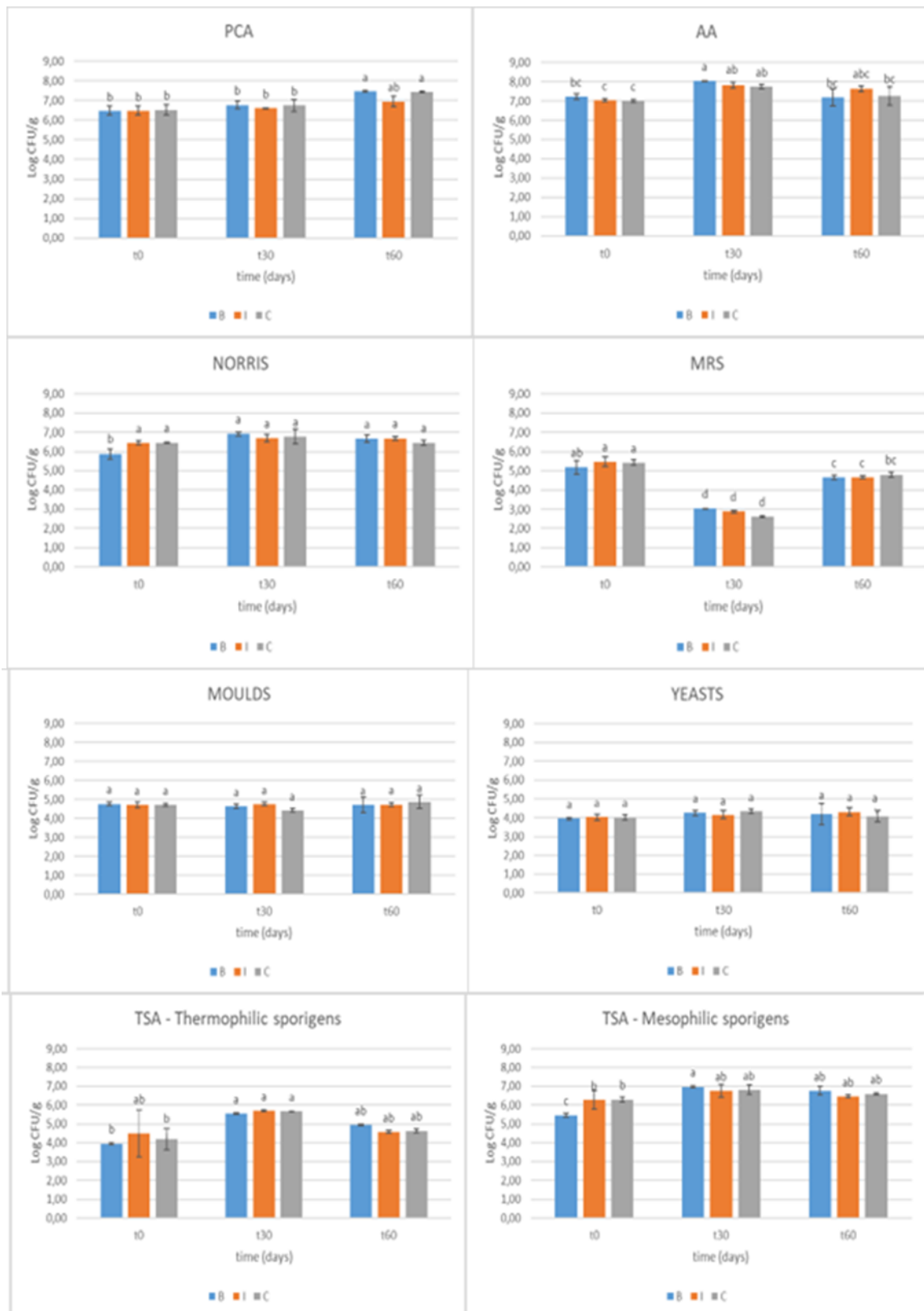
BBCH = phenological phase 20-29 Side shoots formation; 40-49 Vegetative parts development; 51 Flower buds visible

The first effects of the treatments begin to appear in the phenological phase Vegetative parts development in which the irrigated and biostimulated plants show significantly higher values than the untreated control. In the flower buds visible phenological phase corresponding to the harvest of leaves for industrial use, the biostimulated plants show significantly higher values than irrigated plants and control plants.

Site AGUGLIANO - demo field with transplanted sea fennel seedlings

Viable counting of root-free soil

Data of the viable counts of root-free soils are reported below.



Means $\pm$ standard deviations are shown. For each microbial group and sample, overall means with different small letters are significantly different ($p < 0.05$).

B: Biostimulated

I: Irrigated

C: Control (no treatment)

PCA: Plate count agar (total mesophilic aerobes)

AA: Actinomyces agar (actinomycetes)

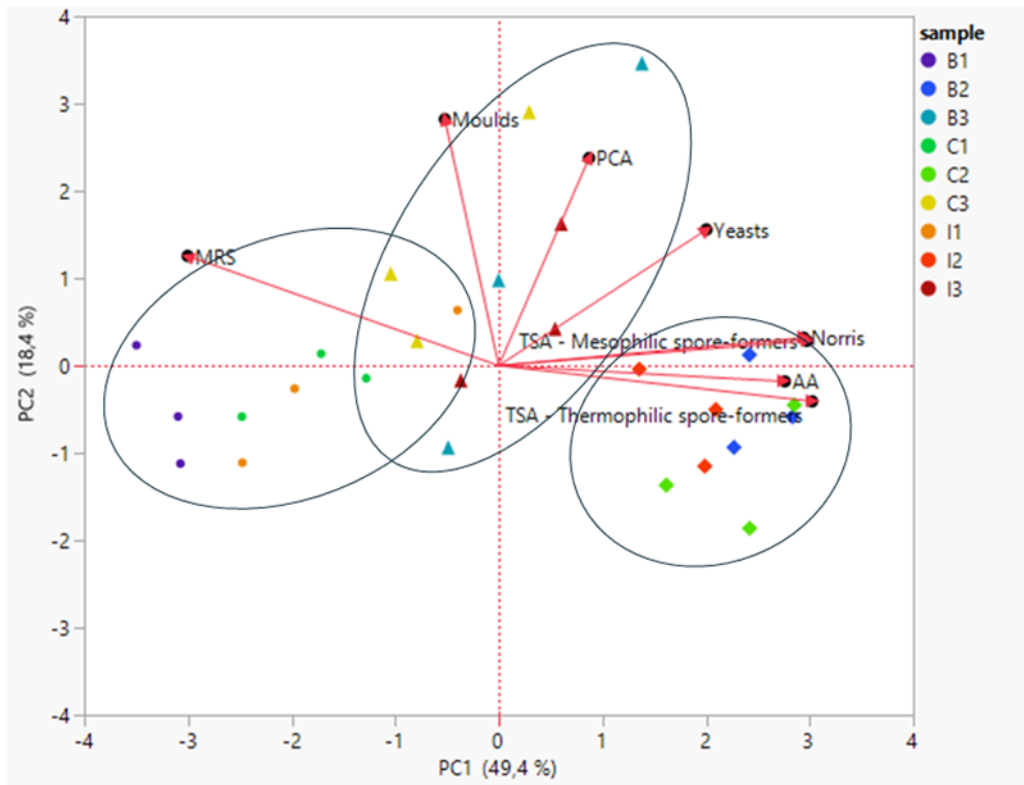
NORRIS: Norris Glucose Nitrogen Free Medium (free nitrogen-fixing bacteria)

MRS: De Man–Rogosa–Sharpe Agar (lactic acid bacteria)
TSA: Tryptic Soy Agar

The results overall collected showed only sporadic statistically significant differences between the treatments and within them, between the six points (t0, t30 and t60).

Bi-plot of PCA carried out onto viable counts of root-free soil samples are shown below.

- 1: 0 days •
- 2: 30 days ♦
- 3: 60 days ▲

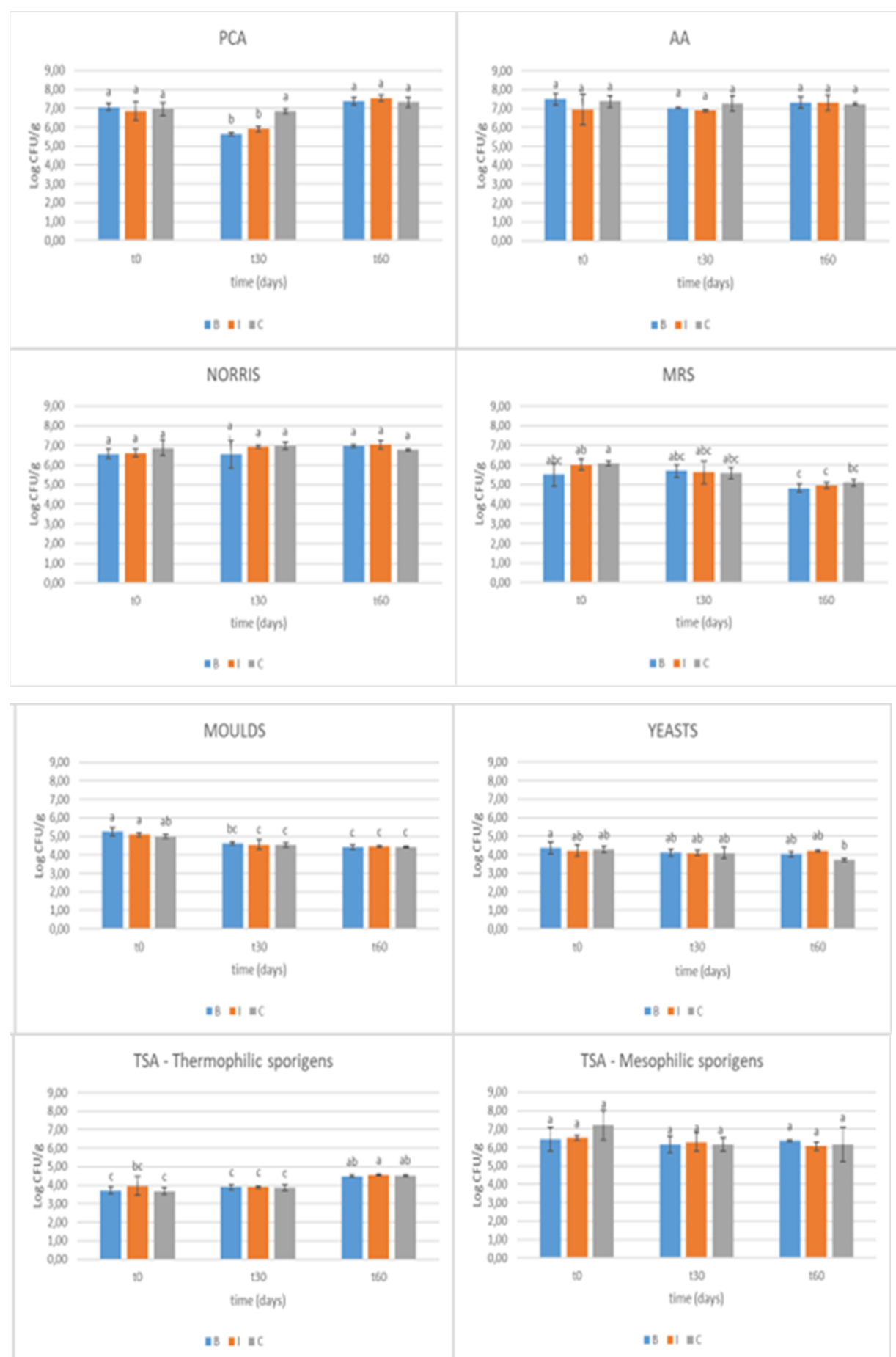


B: Biostimulated
I: Irrigated
C: Control (no treatment)
PCA: Plate count agar (total mesophilic aerobes)
AA: Actinomyces agar (actinomycetes)
NORRIS: Norris Glucose Nitrogen Free Medium (free nitrogen-fixing bacteria)
MRS: De Man–Rogosa–Sharpe Agar (lactic acid bacteria)
TSA: Tryptic Soy Agar

The PCA analysis of viable counts performed on root-free soil samples suggests a statistically significant difference between samples related to the sampling time, rather than to the different treatments under investigation.

Viable counting of sea fennel rhizosphere soil

Data of the viable counts of sea fennel rhizosphere soil samples are reported below.



Means $\pm$ standard deviations are shown. For each microbial group and sample, overall means with different small letters are significantly different ($p < 0.05$).

B: Biostimulated

I: Irrigated

C: Control (no treatment)

PCA: Plate count agar (total mesophilic aerobes)

AA: Actinomyces agar (actinomycetes)

NORRIS: Norris Glucose Nitrogen Free Medium (free nitrogen-fixing bacteria)

MRS: De Man–Rogosa–Sharpe Agar (lactic acid bacteria)

TSA: Tryptic Soy Agar

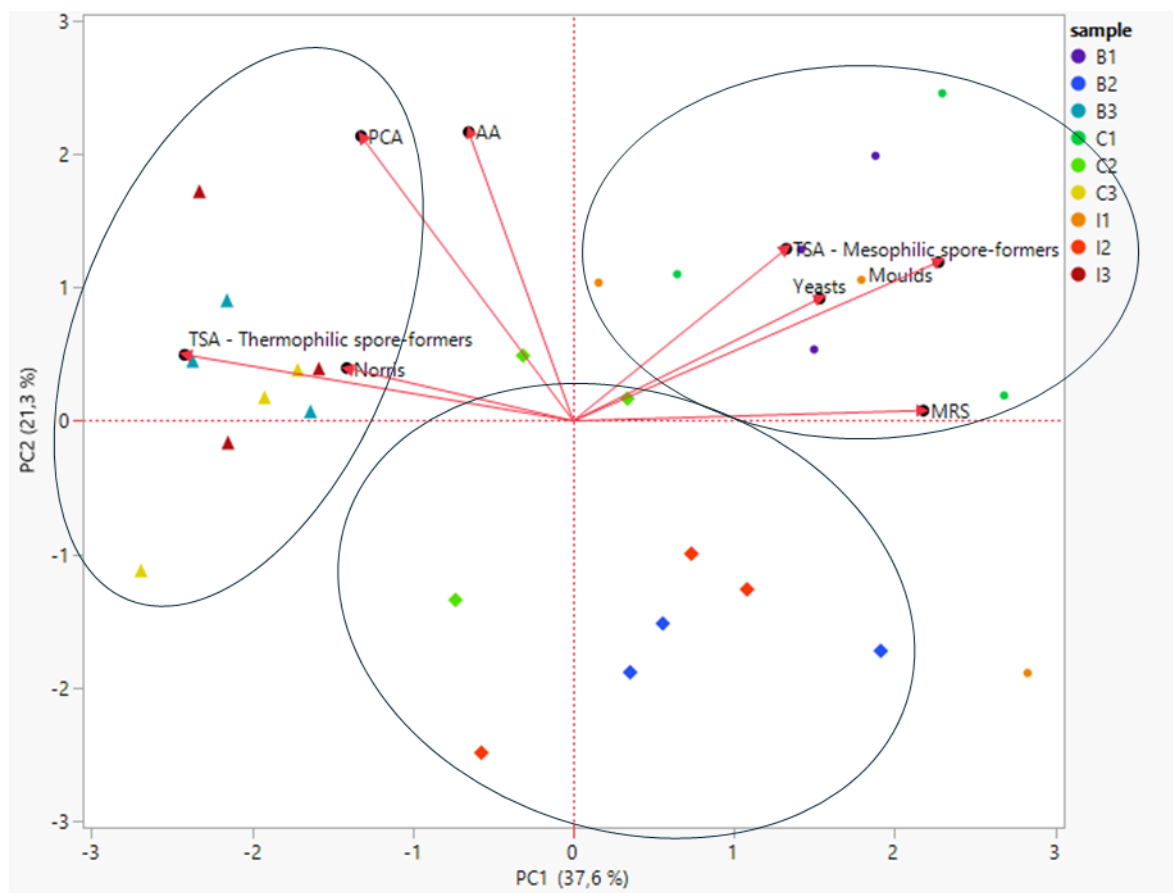
Similarly to root-free soil samples, the results showed only sporadic statistically significant differences between the treatments and no clear correlation with the application of the biofertilizer was observed.

Bi-plot of PCA carried out onto viable counts of rhizosphere samples are shown below.

1: 0 days •

2: 30 days ♦

3: 60 days ▲



B: Biostimulated

I: Irrigated

C: Control (no treatment)

PCA: Plate count agar (total mesophilic aerobes)

AA: Actinomyces agar (actinomycetes)

NORRIS: Norris Glucose Nitrogen Free Medium (free nitrogen-fixing bacteria)

MRS: De Man–Rogosa–Sharpe Agar (lactic acid bacteria)

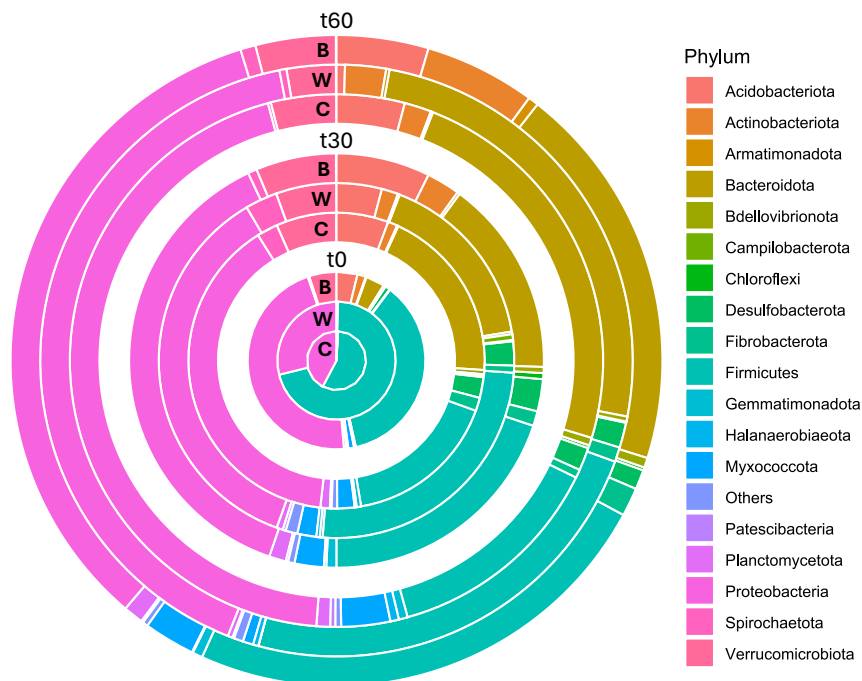
TSA: Tryptic Soy Agar

The PCA analysis of viable counts performed on rhizosphere samples suggests.

NGS

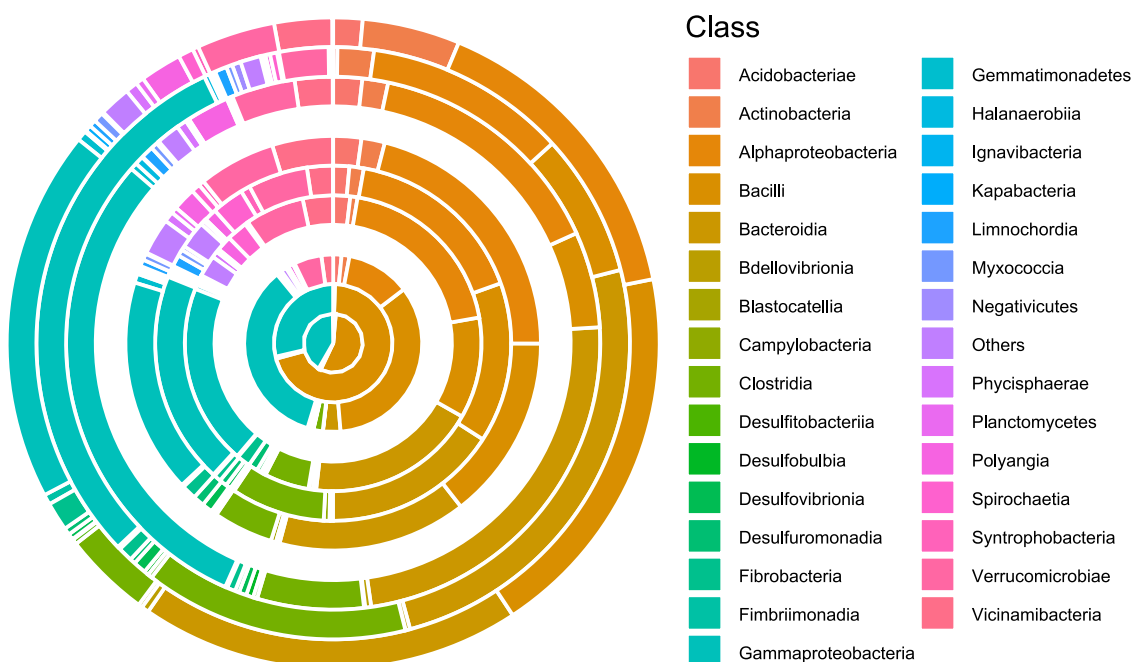
Microbiota composition

Concerning the bacterial biota of soil, a total of 235,577 reads were analyzed, with an average of 9,008 reads per sample. The bacterial composition at different taxonomic levels is presented in the following figures.



Relative frequency of bacterial ASVs at the phylum level in soil samples. C: control; W: water-irrigated; B: biofertilizer-treated.

Notably, the phyla Proteobacteria and Firmicutes were consistently dominant across all samples, followed by Bacteroidota, Acidobacteriota, Actinobacteriota, and Verrucomicrobiota.



Relative frequency of bacterial ASVs at the class level in soil samples

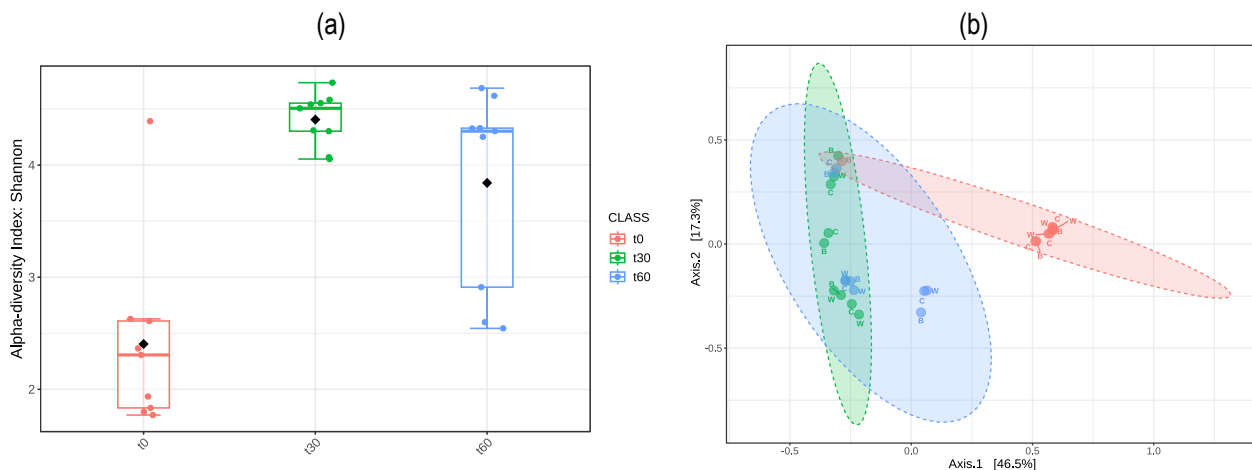


Relative frequency of bacterial ASVs at the order level in soil samples.

Across all samples, the microbial communities were predominantly composed of the classes Alphaproteobacteria, Bacilli, Bacteroidia, and Gammaproteobacteria.

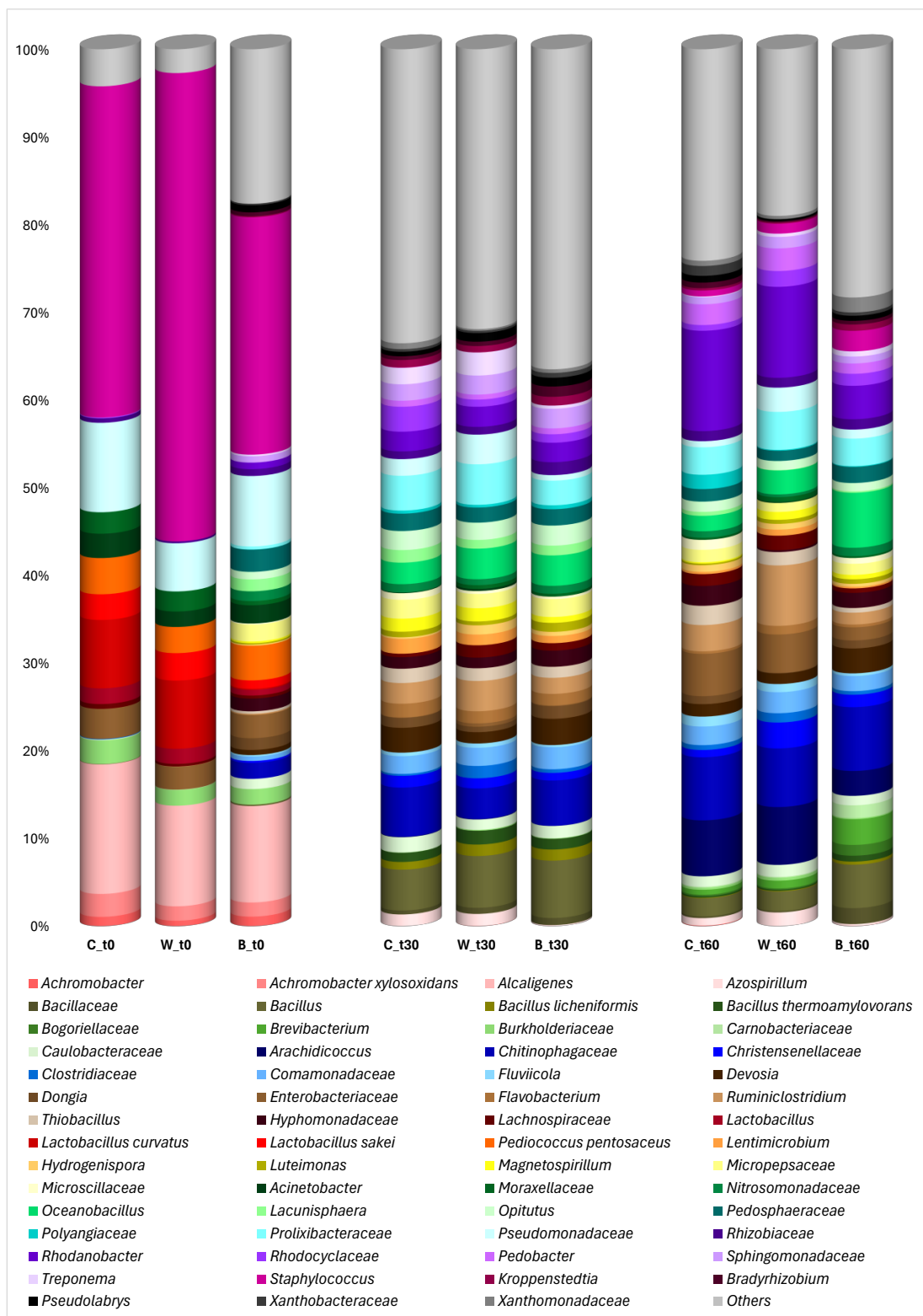
A total of 66 bacterial orders were found in soil samples. At t0, bio-fertilized soil samples showed a higher diversity compared to control and water-irrigated samples. However, Staphylococcales, Burkholderiales, Lactobacillales, Pseudomonadales, and Enterobacterales were consistently detected in all soil samples at t0. A higher complexity was observed at both t30 and t60 in all samples. At these sampling times, the most prevalent orders included Bacillales, Burkholderiales, Rhizobiales, Bacteroidales, and Xanthomonadales.

At the finest taxonomic resolution, a total of 111 bacterial taxa were identified. No statistically significant differences in diversity indices were observed among the different treatment groups (C, B, and W). However, time had a significant effect on both alpha and beta diversity. Specifically, samples collected at t0 differed significantly from those collected at t30 and t60, as shown in the following figure.



Alpha- (a) and Beta-diversity (b) indices of bacterial ASVs in soil samples.

The bacterial biota composition at the finest taxonomic resolution is shown in the following figure. At t0, *Staphylococcus* spp. was the dominant taxon across all samples, accounting for at least 27% of the relative abundance. This was followed by *Alcaligenes* spp. ($\geq 11\%$) and members of the *Pseudomonadaceae* family ($\geq 5.5\%$). In addition, several low-abundance taxa were consistently detected in all samples, including *Achromobacter xylosoxidans*, *Burkholderiaceae*, *Enterobacteriaceae*, *Lactobacillus* spp., *Pediococcus pentosaceus*, *Acinetobacter* spp., and *Moraxellaceae*. By t30, a significant shift in community structure was observed. *Staphylococcus* spp. nearly disappeared in all samples. At this sampling time, the bacterial community was composed by a plethora of taxa, including *Bacillus* spp. (approximately 10% of the relative abundance), *Chitinophagaceae* (approx. 5% of the relative abundance), *Comamonadaceae* (2–3%), *Devosia* spp. (1–3%), *Ruminiclostridium* spp. (1–3.5%), *Micropepsaceae* (1.5–2.5%), *Oceanobacillus* spp. (2.5–3.5%), *Opitutus* spp. (approx. 2%), *Prolixibacteraceae* (2.5–5%), *Rhodanobacters* spp. (approx. 2.2%), and *Sphingomonadaceae* (approx. 2%). At t60, a similar bacterial distribution was found. *Rhodanobacter* spp. reached approximately 11% of the relative frequency in the control and water-irrigated soil samples. Samples at t60 also showed a significant presence of *Arachidicoccus* spp. and *Pedobacter* spp.

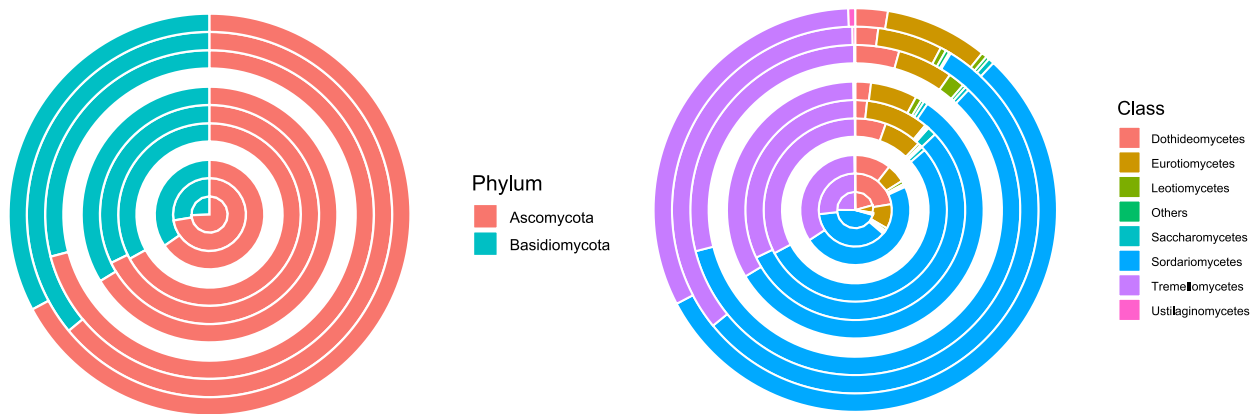


Relative abundance of bacterial ASVs in soil samples, presented at the finest taxonomic resolution. Only ASVs with at least 0.5% in two samples are shown.

Concerning the fungal biota of soil, a total of 3,553,942 reads were analyzed, with an average of 59,974 reads per sample. The fungal composition at different taxonomic levels is presented in the following figures.

(a)

(b)



Relative frequency of fungal ASVs at the phylum (a) and class (b) level in soil samples.

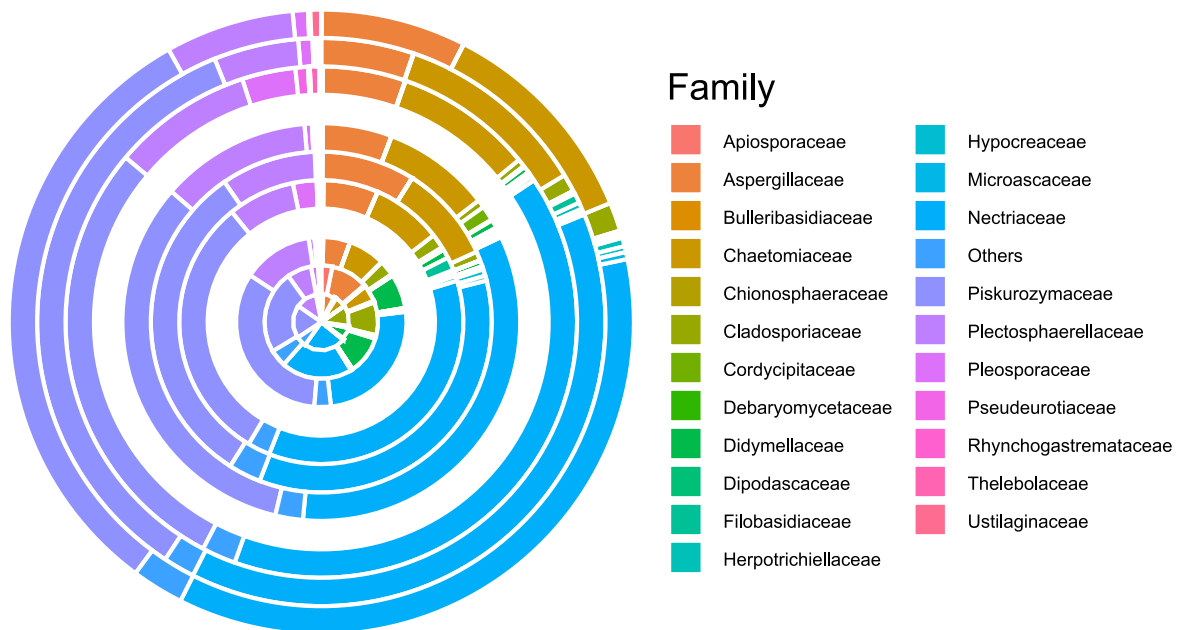
Overall, the fungal biota was composed of Ascomycota and Basidiomycota, accounting for approximately 68% and 32% of the relative frequency, respectively. Sordariomycetes was the most abundant class in all samples, followed by Tremellomycetes. The class Dothideomycetes was particularly abundant in samples at t0.



Relative frequency of fungal ASVs at the order level in soil samples.

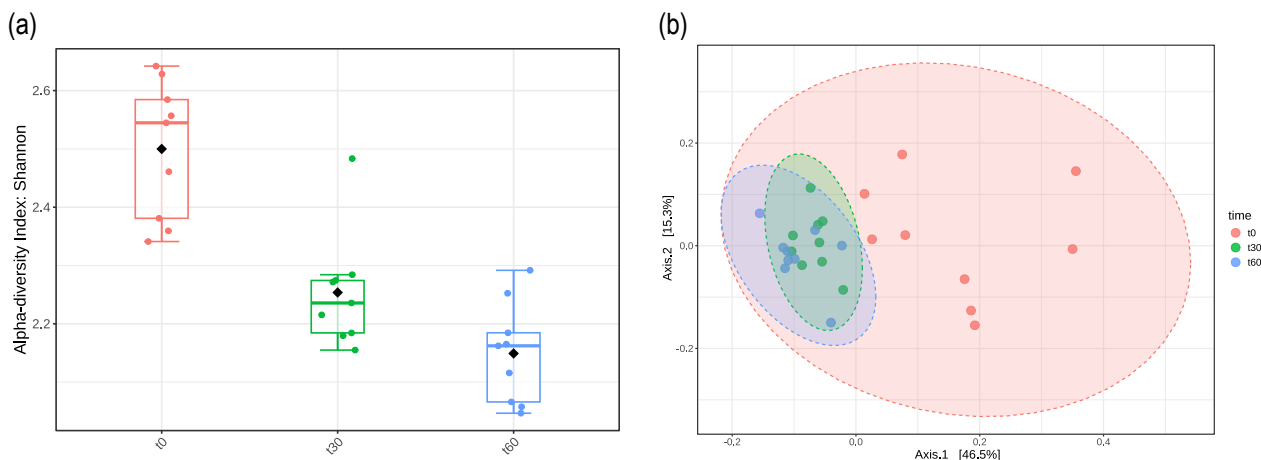
Hypocreales and Filobasidiales were the most abundant fungal orders across the soil samples, followed by Eurotiales, Glomerellales, and Sordariales. Capnodiales and Pleosporales were particularly abundant in samples at t0.

Piskurozymaceae and Nectriaceae were the dominant fungal families in the soil samples. Aspergillaceae, Plectosphaerellaceae, and Chaetomiaceae also constituted a significant portion of the fungal community. Notably, samples collected at t0 showed higher relative abundances of Cladosporiaceae and Didymellaceae compared to those from later sampling times.



Relative frequency of fungal ASVs at the family level in soil samples.

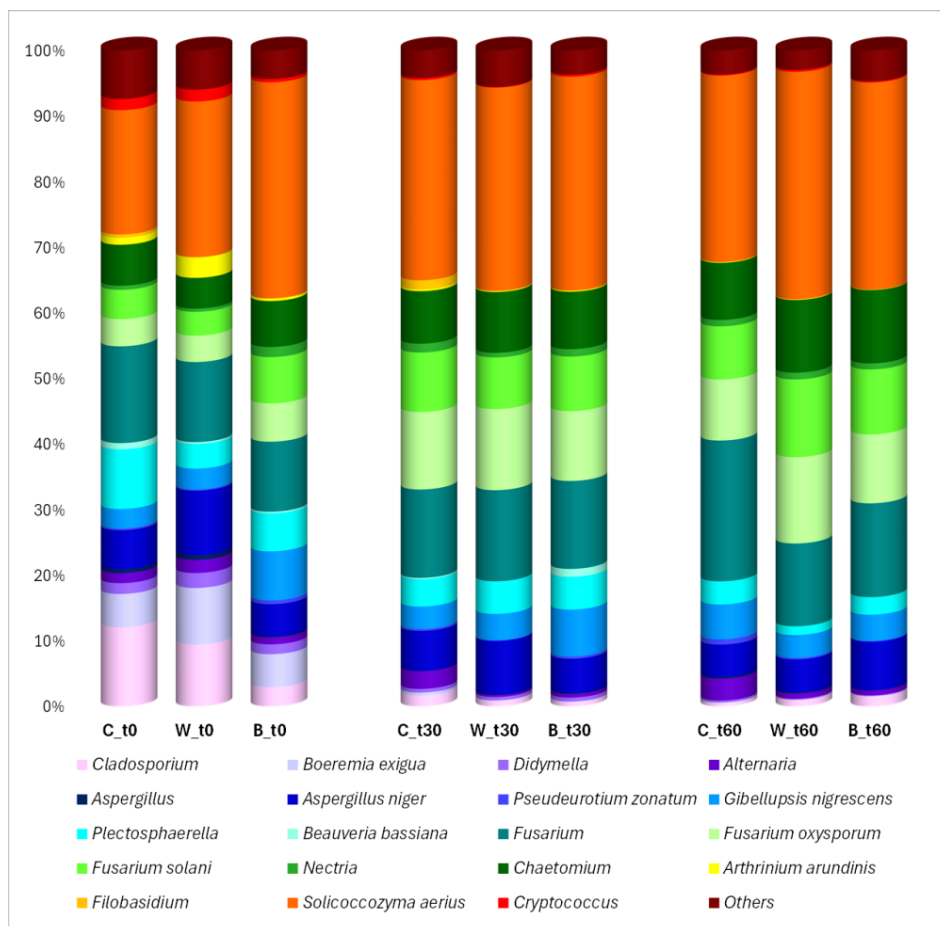
At the finest taxonomic resolution, a total of 30 fungal taxa were identified. No statistically significant differences in diversity indices were observed among the different treatment groups (C, B, and W). However, time had a significant effect on both alpha and beta diversity. Specifically, samples collected at t0 differed significantly from those collected at t30 and t60, as shown in the following figure.



Alpha- (a) and Beta-diversity (b) indices of fungal ASVs in soil samples.

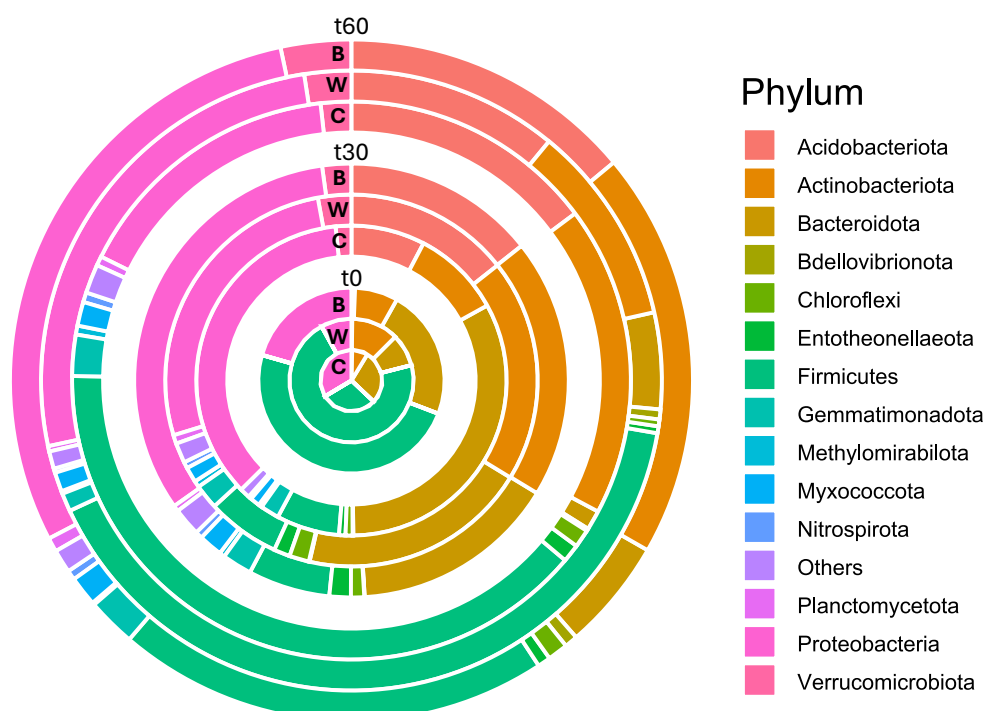
The fungal biota composition at the finest taxonomic resolution is presented in the following figure.

Solicoccozyma aerius was the dominant taxon across all soil samples, with relative abundances ranging from 18.95% to 33%. *Fusarium* spp. was also consistently detected in all soil samples, ranging between 10.67 and 21.46% of the relative frequency. *Fusarium oxysporum* and *Fusarium solani* were additionally present in all samples, ranging between 3.64% and 13.16% of the relative frequency. Other taxa, including *Aspergillus* spp., *Gibellupsis nigrescens*, *Plectosphaerella* spp., and *Chaetomium* spp., were also consistently represented across all conditions. Notably, soil samples collected at t0 exhibited higher relative abundances of *Cladosporium* spp. (3–12%), *Boeremia exigua* (5–9%), *Didymella* spp. (1.5–2.3%), and *Cryptococcus* spp. (0.5–1.85%).

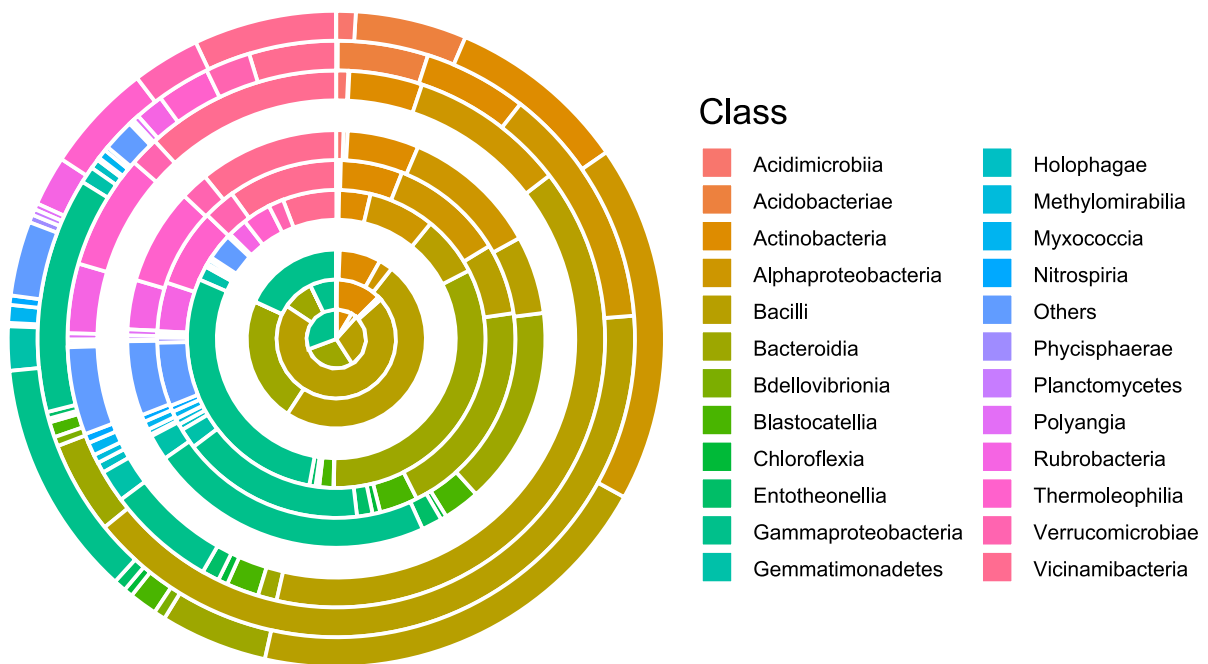


Relative abundance of fungal ASVs in soil samples, presented at the finest taxonomic resolution. C: control; W: water-irrigated; B: biofertilizer-treated. Only ASVs with at least 0.5% in two samples are shown.

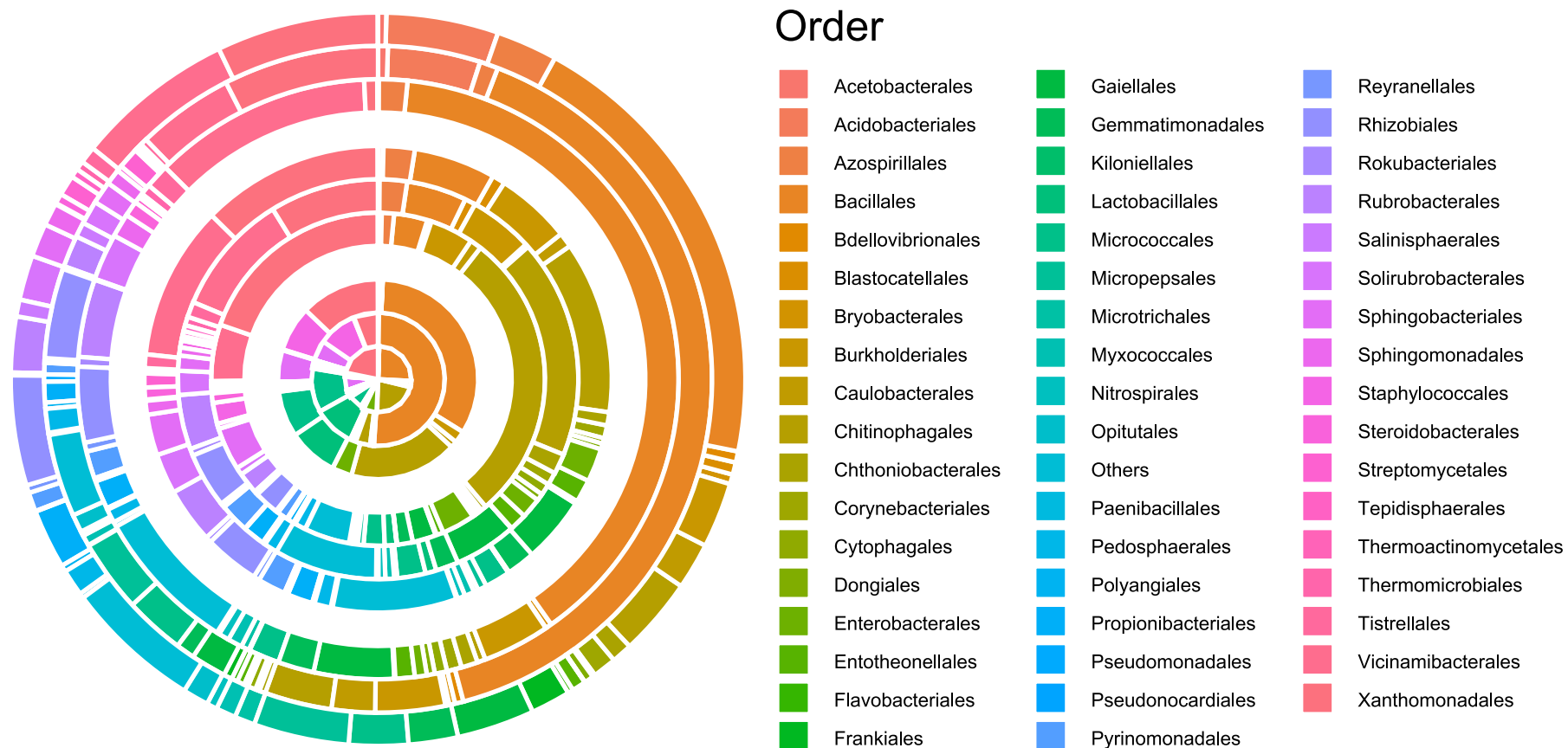
Concerning the bacterial biota of rhizosphere samples, a total of 228,125 reads were analyzed, with an average of 8,449 reads per sample. The bacterial composition at different taxonomic levels is presented in the following figures.



Relative frequency of bacterial ASVs at the phylum level in rhizosphere samples. C: control; W: water-irrigated; B: biofertilizer-treated.



Relative frequency of bacterial ASVs at the class level in rhizosphere samples.

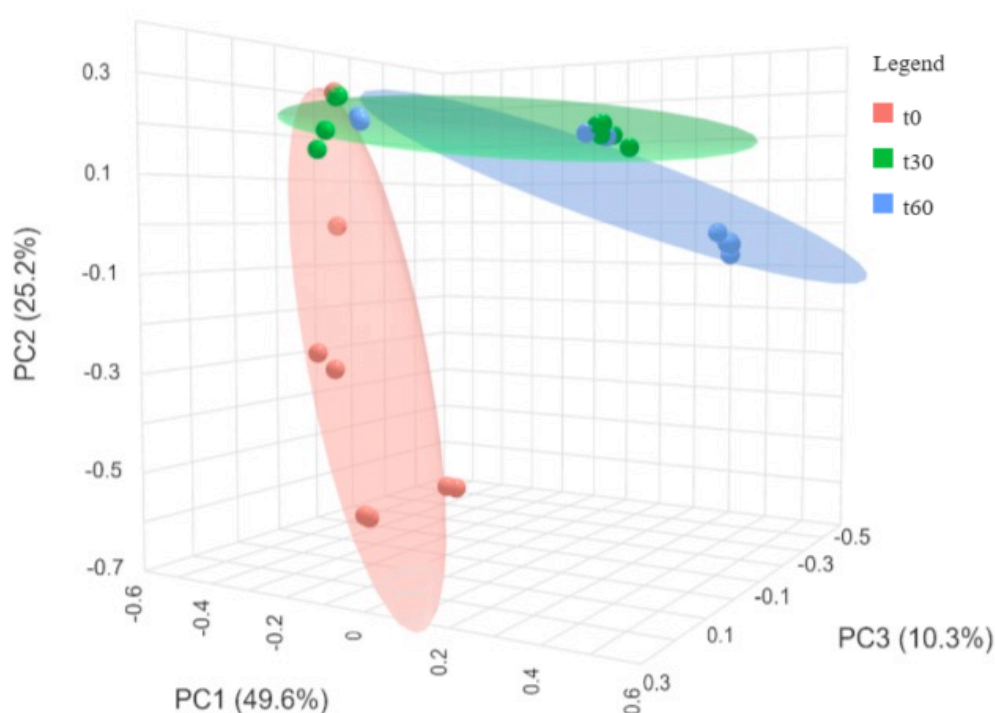


Relative frequency of bacterial ASVs at the order level in rhizosphere samples.

The phyla Bacteroidota, Actinobacteriota, Firmicutes, and Proteobacteria were consistently detected across all rhizosphere samples. Notably, samples collected at t30 and t60 exhibited increased relative abundances of Acidobacteriota, Verrucomicrobiota, Myxococcota, and Gemmatimonadota compared to those collected at t0. At the class level, Actinobacteria, Bacteroidia, Bacilli, and Gammaproteobacteria were the most prevalent across all sampling times. Additionally, samples from t30 and t60 displayed higher relative abundances of Blastocatellia, Vicinamibacteria, Rubrobacteria, Thermoleophilia, Gemmatimonadetes, and Alphaproteobacteria compared to t0 samples.

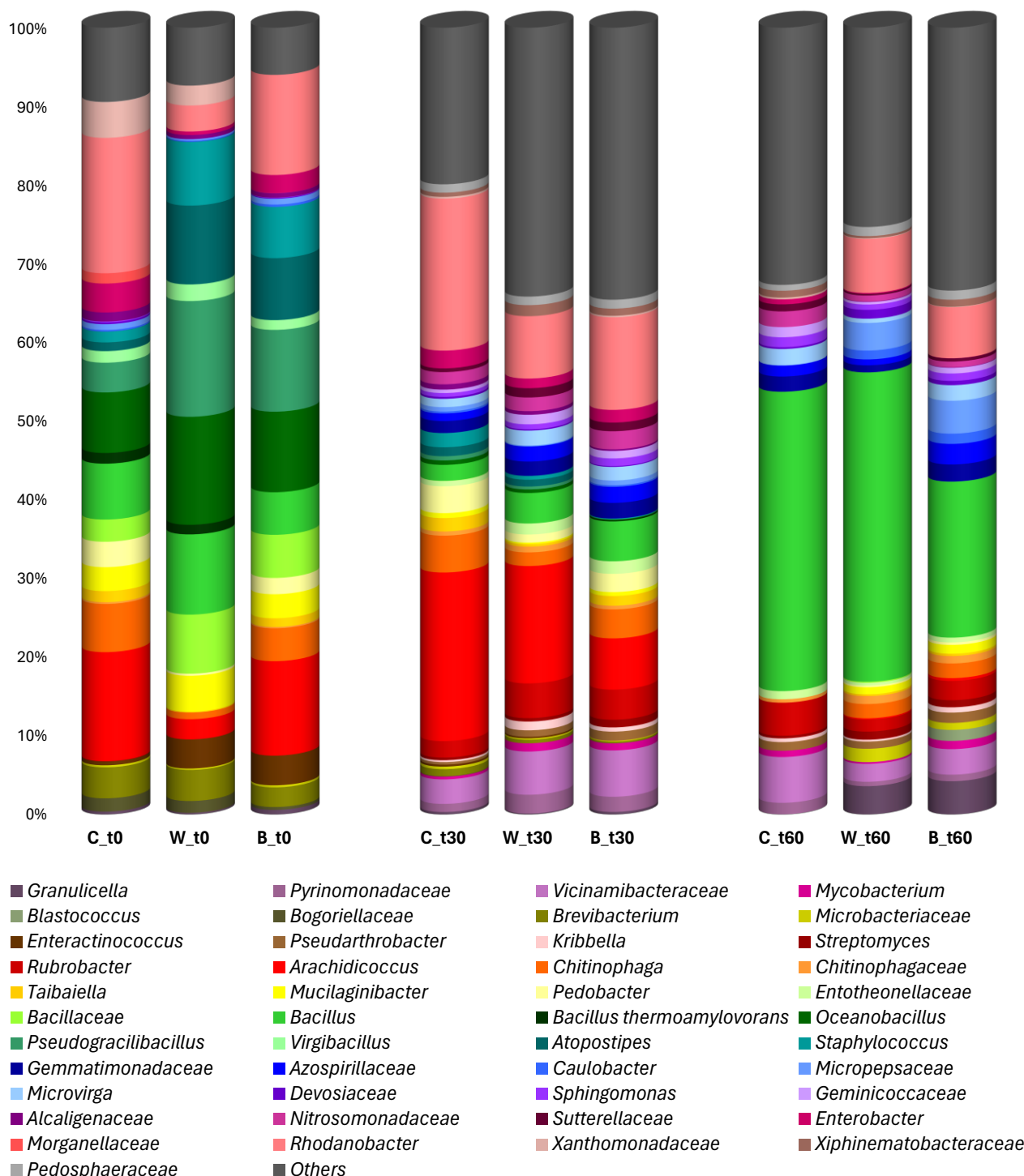
At the order level, Micrococcales, Bacillales, Burkholderiales, and Xanthomonadales were consistently present in all samples. In contrast, Lactobacillales and Staphylococcales were more abundant in t0 samples compared to later sampling times. Similarly, Chitinophagales, Enterobacterales, and Sphingobacteriales were more prevalent in samples from t0 and t30 than in those from t60. Despite these differences, samples from t30 and t60 exhibited greater microbial diversity compared to t0, including the emergence of orders such as Pyrinomonadales, Vicinamibacterales, Propionibacterales, Rubrobacterales, Gaiellales, Gemmatimonadales, Rhizobiales, and Pedosphaerales.

At the finest taxonomic resolution, a total of 95 bacterial taxa were identified across all samples. No statistically significant differences in alpha diversity, as measured by the Shannon index, were observed among the different treatment groups (C, B, and W) or across sampling times (t0, t30, t60). However, sampling time had a significant effect on beta diversity. Specifically, the microbial community composition at t0 differed significantly from that at t30 and t60, as illustrated in the following figure.



Principal Coordinates Analysis (PCoA) plot based on Bray-Curtis dissimilarity showing the beta diversity of bacterial ASVs in rhizosphere samples.

The bacterial biota composition at the finest taxonomic resolution is shown in the following figure.

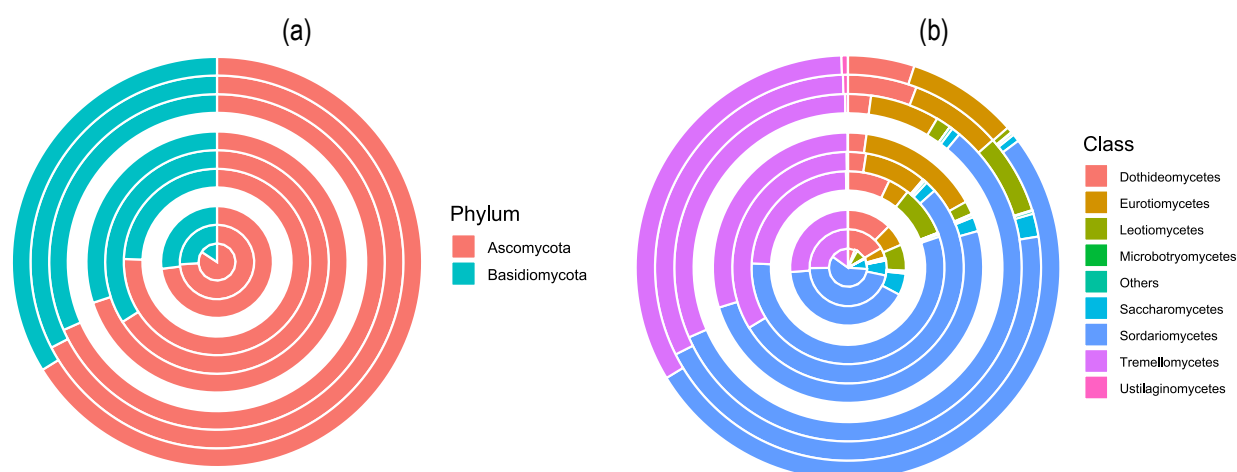


Relative frequency of bacterial ASVs in rhizosphere samples. C: control; W: water-irrigated; B: biofertilizer-treated. Only ASVs with at least 1% in one sample are shown.

Overall, bacterial ASVs in rhizosphere samples exhibited high variability, with no single taxon consistently detected across all samples. Rhizosphere samples collected at t0 were characterized by a distinct microbial profile, notably marked by the

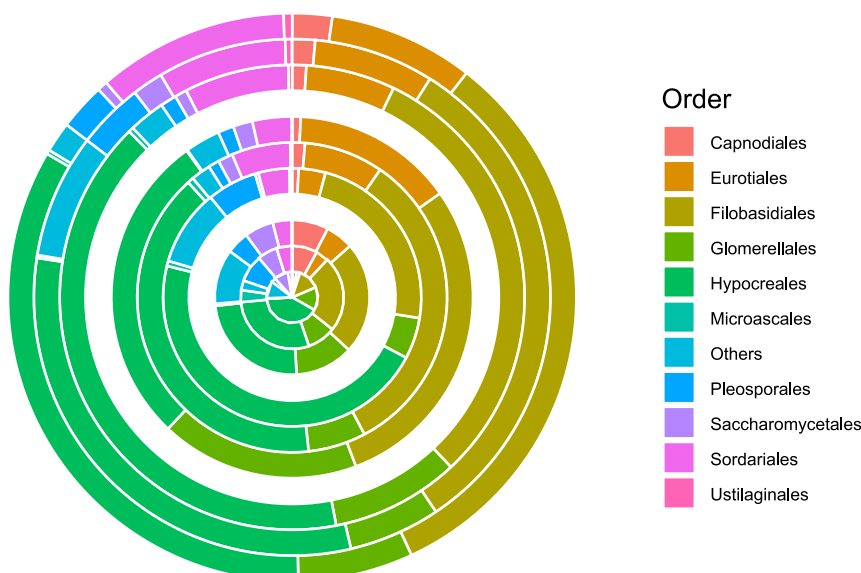
presence of *Brevibacterium* spp., *Enteractinococcus* spp., *Mucilaginibacter* spp., *Oceanobacillus* spp., *Pseudogracilibacillus* spp., *Virgibacillus* spp., *Atopostipes* spp., and *Staphylococcus* spp. Samples at t0 and t30 shared the common presence of *Arachidococcus* spp. (ranging between 2.57% and 21.43% of the relative frequency) and *Rhodanobacter* spp. (ranging between 3.31% and 19.32% of the relative frequency). Rhizosphere samples collected at t30 and t60 showed the common presence of *Vicinamibacteraceae* (2.28%–5.89% of the relative frequency), *Rubrobacter* spp. (1.54%–4.42%), *Gemmatimonadaceae* (0.90%–2.17%), *Azospirillaceae* (0.75%–2.62%), *Microvirga* spp. (0.55%–2.07%), *Nitrosomonadaceae* (0.73%–2.30%), *Xiphinematobacteraceae* (0.29%–1.40%), and *Pedosphaeraceae* (0.74%–1.16%). Of note, *Bacillus* spp. was particularly abundant in rhizosphere samples collected at t60, with values ranging between 19.82% and 39.32% of the relative frequency.

Concerning the fungal biota of rhizosphere, a total of 3,459,050 reads were analyzed, with an average of 128,113 reads per sample. The fungal composition at different taxonomic levels is presented in the following figures.



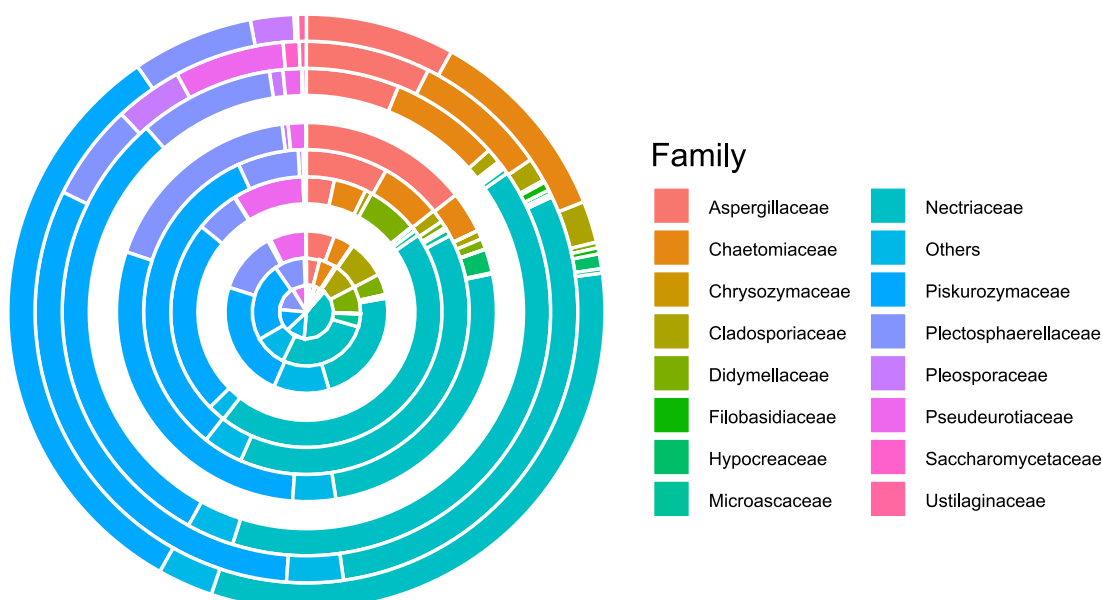
Relative frequency of fungal ASVs at the phylum (a) and class (b) level in rhizosphere samples.

Overall, the fungal biota was composed of Ascomycota and Basidiomycota, accounting for approximately 72% and 28% of the relative frequency, respectively. Sordariomycetes was the most abundant class in all samples, followed by Tremellomycetes, Dothideomycetes, Eurotiomycetes, and Leotiomyces. The relative frequency of the class Saccharomycetes was higher in samples at t0 compared to those collected at t30 and t60.



Relative frequency of fungal ASVs at the order level in rhizosphere samples.

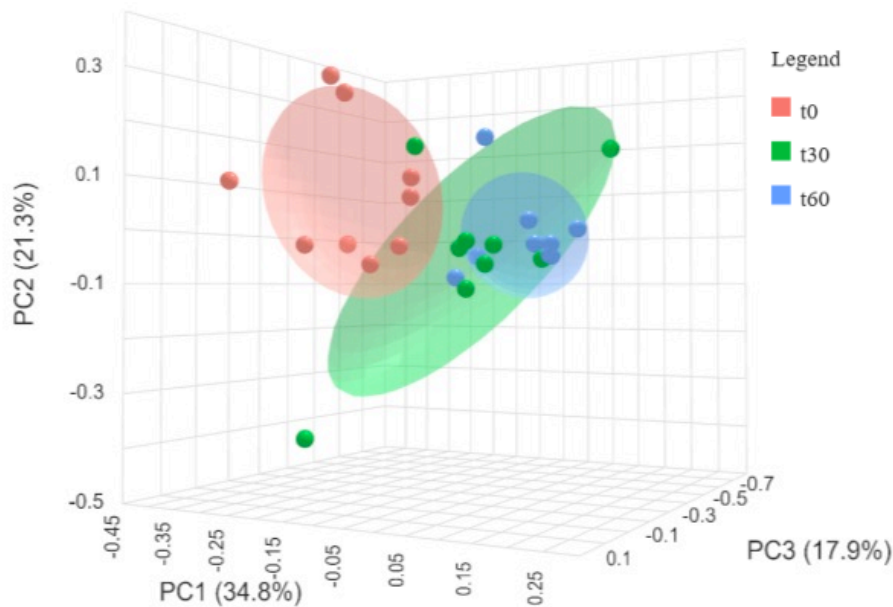
Hypocreales and Filobasidiales were the most abundant fungal orders across all rhizosphere samples, followed by Pleosporales, Eurotiales, Glomerellales, and Sordariales. The relative frequency of Capnodiales and Saccharomycetales was higher in samples at t0 compared to those collected at t30 and t60.



Relative frequency of fungal ASVs at the family level in rhizosphere samples.

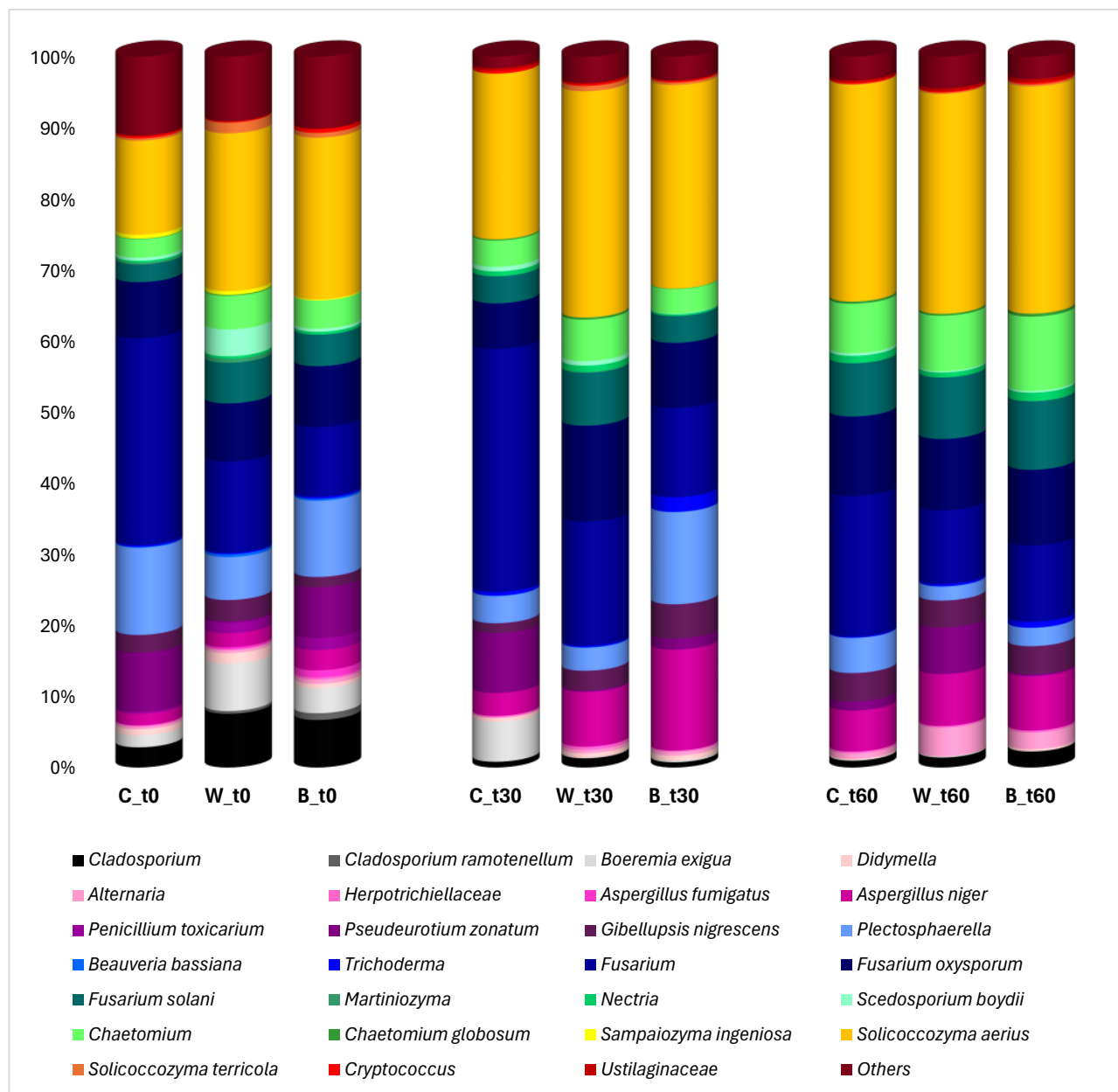
At the family level, Nectriaceae and Piskurozymaceae dominated the fungal composition of rhizosphere samples. Several taxa were consistently found in all samples, including Aspergillaceae, Pseudeurotiaceae, Plectosphaerellaceae, and Chaetomiaceae. The relative frequency of Cladosporiaceae and Didymellaceae was higher in samples at t0 compared to those collected at t30 and t60.

At the finest taxonomic resolution, a total of 27 fungal taxa were identified across all samples. No statistically significant differences in alpha diversity, as measured by the Shannon index, were observed among the different treatment groups (C, B, and W) or across sampling times (t0, t30, t60). However, sampling time had a significant effect on beta diversity ($p < 0.05$), as illustrated in the following figure.



Principal Coordinates Analysis (PCoA) plot based on Bray-Curtis dissimilarity showing the beta diversity of fungal ASVs in rhizosphere samples.

The fungal biota composition at the finest taxonomic resolution is presented in the following figure.



Relative abundance of fungal ASVs in soil samples, presented at the finest taxonomic resolution.
C: control; W: water-irrigated; B: biofertilizer-treated. Only ASVs with at least 0.5% in two samples are shown.

Solicoccozyma aerius and *Fusarium* spp. were the dominant taxa across all rhizosphere samples, with relative abundances ranging from 13.24% to 31.98% and 9.82% to 34.18%, respectively. *Fusarium oxysporum* and *Fusarium solani* were additionally present in all samples, ranging between 2.50% and 13.51% of the relative frequency. Other taxa, including *Aspergillus* spp., *Pseudeurotium zonatum*, *Plectosphaerella* spp., *Gibellupsis nigrescens*, and *Chaetomium* spp., were also consistently represented across all conditions. Notably, soil samples collected at t0 exhibited higher relative abundances of *Cladosporium* spp. (2.80–7.56%), *Boeremia exigua* (1.67–6.67%), and *Penicillium toxicarium* (0.26–1.65%).

Chemical analysis of soil

The soil analysis showed relatively stable values across treatments and sampling times, with only minor variations in some parameters. According to the pH the soil is classified as sub alkaline.

Sample	Total inorganic carbon (g/Kg)	Humic and fulvic acid carbon (g/Kg)	pH in H ₂ O	C/N	Organic matter (g/Kg)	Total nitrogen (g/Kg)	Exchangeable potassium (mg/Kg)	Total organic carbon (g/Kg)
B t0	36,13 ± 6.94 ^a	5,60 ± 0.79 ^a	7,94 ± 0.06 ^b	7,77 ± 0.35 ^a (L)	18,42 ± 6.98 ^a (L)	1,37 ± 0.46 ^a (M)	312,33 ± 54.37 ^a (H)	10,67 ± 4.05 ^a
I t0	35,83 ± 6.98 ^a	5,62 ± 0.58 ^a	7,97 ± 0.03 ^b	7,80 ± 0.36 ^a (L)	17,80 ± 4.70 ^a (L)	1,25 ± 0.30 ^a (M)	314,67 ± 45.36 ^a (H)	10,30 ± 2.70 ^a
C t0	35,50 ± 6.93 ^a	5,63 ± 0.58 ^a	7,99 ± 0.02 ^{ab}	7,83 ± 0.4 ^a (L)	17,15 ± 2.43 ^a (L)	1,27 ± 0.12 ^a (M)	315,67 ± 34.30 ^a (H)	9,93 ± 1.40 ^a
B tf	31,57 ± 7.24 ^a	5,60 ± 0.90 ^a	8,11 ± 0.07 ^a	9,33 ± 0.35 ^b (M)	17,78 ± 3.49 ^a (L)	1,10 ± 0.17 ^a (M)	239,00 ± 11.00 ^a (H)	10,32 ± 2.03 ^a
I tf	31,30 ± 6.14 ^a	5,37 ± 1.91 ^a	8,10 ± 0.04 ^a	9,10 ± 0.7 ^{ab} (M)	17,60 ± 3.33 ^a (L)	1,12 ± 0.13 ^a (M)	228,67 ± 14.43 ^a (H)	10,20 ± 1.93 ^a
C tf	30,20 ± 6.25 ^a	5,85 ± 1.57 ^a	8,09 ± 0.06 ^a	9,23 ± 0.58 ^b (M)	18,40 ± 3.36 ^a (L)	1,15 ± 0.13 ^a (M)	252,33 ± 36.25 ^a (H)	10,67 ± 1.95 ^a

Means ± standard deviations are shown. Means followed by different letters are significantly different. The letters in brackets indicate whether the associated values are high (H), average (M) or low (L).

B: Biofertilized

I: Irrigated

C: Control (no treatment)

t0: 0 days

tf: 60 days

H: High value

L: Low value

M: Medium value

Physical analysis of soil samples

According to the USDA classification, the soil detected is clayey loam, therefore with good water retention but subject to water stagnation.

Soil properties in the 0-30 cm layer in the experimental plots in 2024 (n = 3) are shown below

Soil properties	Values
Sand (g kg ⁻¹)	65 ± 18
Silt (g kg ⁻¹)	504 ± 17
Clay (g kg ⁻¹)	431 ± 20
Volumetric soil water content (%):	
Field Capacity	35.2 ± 1.2
Permanent Wilting Point	18.0 ± 2.4
Total available water	26.0 ± 1.8

values expressed in mean ± standard deviation.

Materials and methods

The experimental site was laid out in a randomized block design with three replicates. We compared two populations, Atlantic (AT) and Mediterranean (ME), both subjected at three treatments: control rainfed treatment without fertilization (CT), irrigation with water (IR) and biostimulation with microbial strains and water (BS) at point level within irrigated plots.

A drip irrigation system was installed with the tubes positioned on the ground, below the plastic mulching sheet, so that the water is diffused by means of low-pressure sprays directly next to the plants and their root systems, so that it can be easily absorbed, thus avoiding irrigation with large volumes of water and maintaining low pressure use. Irrigation planning was carried out considering the rainfall regime and the soil moisture level, which was continuously monitored by a probe: at 65% of total available water (TAW), the water supply started. Biostimulation contains four microbial strains: *Azospirillum brasilense* (strain DSMZ 1690), *Azotobacter chroococcum* (strain DSM 2286) used for atmospheric nitrogen fixation, *Priestia megaterium* (strain DSMZ 339) as phosphorus-dissolving bacteria, and *Niallia circulans* (strain DSMZ 30598) as potassium-solubilizing bacteria, at a ratio of 1:1:1:1 (v/v/v). A dose of 2 L of biostimulant (10⁸ cells mm⁻¹) was diluted in 10 L of water at each biostimulation, and this solution passed from a sprinkler in each date of treatment. During the growing season, the amount of water provided for both biostimulation and irrigation treatments was 20,000 L corresponding to about 200 mm.

Measured variables

Biomass production and SPAD Unit were evaluated at three different phenological stages: side shoots formation (BBCH scale 20-29), vegetative parts development (BBCH scale 40-49) and flower buds visible (BBCH scale 51): the phenological phase BBCH = 40-49 occurs after 50% of the inputs have been administered (water and biostimulant) while the phenological phase BBCH = 51 occurs after all the inputs have been administered (water and biostimulant) and is the phase that at company level corresponds to the harvest of leaves for industrial use. The epigeal biomass was cut at 5 cm from the ground level and its fresh weight was determined. Then, the biomass was placed in an oven at 105 °C for 48 h, after which its dry weight was determined. In order to avoid Soil Plant Analysis Development (SPAD) readings with strong variations, we have performed the sampling during a fully sunny day. SPAD readings were made by using the chlorophyll meter SPAD Minolta 502 (Konica Minolta Sensing 2003, Osaka, Japan).

Results

Fresh and dry epigeal biomass

Analysis of variance and quantification of *Crithmum maritimum* L. fresh and dry biomass per plant and per unit area in the different phenological phases (BBCH scale 20-29, 40-49 and 51) for each treatment in both population. The table is reported below:

BBCH	Treatment	Population	UFB g pt ⁻¹	TFB t ha ⁻¹	UDB g pt ⁻¹	TDB t ha ⁻¹
20-29 (side shoots formation)	Biostimulated		1.76(±0.44) a	0.172(±0.043) a	0.26(±0.07) a	0.025(±0.007) a
		AT	1.88(±0.51)	0.184(±0.050)	0.28(±0.06)	0.028 (±0.006)
		ME	1.64(±0.32)	0.161(±0.031)	0.24(±0.07)	0.023 (±0.007)
	Irrigated		1.77(±0.50) a	0.173(±0.049) a	0.28(±0.10) a	0.029 (±0.010) a
		AT	1.82(±0.53)	0.179(±0.052)	0.28(±0.10)	0.029 (±0.010)
		ME	1.71(±0.48)	0.168(±0.047)	0.29(±0.11)	0.029 (±0.011)
	Control		1.67(±0.40) a	0.163(±0.039) a	0.29(±0.09) a	0.028 (±0.008) a
		AT	1.79(±0.45)	0.176(±0.044)	0.29(±0.10)	0.028 (±0.009)
		ME	1.54(±0.33)	0.151(±0.032)	0.30(±0.08)	0.028 (±0.008)
Mean 20-29			1.73(±0.44)	0.170(±0.043)	0.28(±0.09)	0.027(±0.009)
40-49 (vegetative parts development)	Biostimulated		23.80(±3.62) a	2.332(±0.355) a	3.43(±0.64) a	0.336(±0.063) a
		AT	23.64(±3.36)	2.317 (±0.329)	3.19(±0.40)	0.313(±0.039)
		ME	23.96(±4.01)	2.348(±0.393)	3.66(±0.77)	0.359(±0.075)
	Irrigated		23.01(±4.31) a	2.255(±0.423) a	3.27(±0.68) a	0.320(±0.066) a
		AT	23.00(±5.07)	2.254(±0.497)	3.31(±0.83)	0.324(±0.082)
		ME	23.01(±3.63)	2.255(±0.355)	3.23(±0.51)	0.317(±0.050)
	Control		14.28(±4.94) b	1.399(±0.484) b	2.43(±0.97) a	0.238(±0.095) a
		AT	14.03(±4.39)	1.375(±0.430)	2.32(±0.71)	0.228(±0.070)
		ME	14.52(±5.62)	1.423(±0.551)	2.54(±1.19)	0.249(±0.117)
Mean 40-49			20.36(±6.09)	1.995(±0.597)	3.04(±0.88)	0.298(±0.086)
51 (visible flower buds)	Biostimulated		107.05(±35.03) a	10.491(±3.433) a	16.35(±5.46) a	1.602(±0.535) a
		AT	110.25(±32.03)	10.804(±3.139)	15.10(±4.42)	1.479(±0.434)

	ME	103.86(±40.62)	10.178(±3.980)	17.61(±6.49)	1.725(±0.636)
Irrigated		89.49(±21.40) a	8.770(±2.097) a	13.29(±3.28) ab	1.303(±0.322) ab
	AT	89.87(±17.79)	8.807(±1.744)	12.78(±2.39)	1.253(±0.234)
	ME	89.11(±25.31)	8.732(±2.481)	13.80(±4.03)	1.352(±0.395)
Control		42.89(±13.36) b	4.203(±1.309) b	7.64(±3.16) b	0.749(±0.310) b
	AT	40.30(±13.27)	3.949(±1.300)	7.18(±3.06)	0.704(±0.300)
	ME	45.48(±13.51)	4.457(±1.324)	8.10(±3.33)	0.794(±0.326)
Mean 51		74.36(±34.40)	7.287(±3.372)	11.64(±5.08)	1.141(±0.498)

AT = Atlantic population

ME = Mediterranean population,

UFB = Unit Fresh Biomass,

TFB = Total Fresh Biomass,

UDB = Unit dry biomass,

TDB = Total Dry Biomass,

Signif. codes: 0 '****' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Values are shown as mean ± standard deviation

Concerning unitary and total fresh biomass in terms of absolute values, a fourteen-fold increase in value was recorded between the side shoot formation phase (BBCH scale 20-29) and that of vegetative parts development (BBCH scale 40-49) in the biostimulated treatment, thirteen-fold in irrigated treatments, while the control treatment increased its value eightfold. From the phenological phase BBCH scale 40-49 to that of flowering buds visible (BBCH scale 51), the biostimulated treatment increased the biomass value fivefold, the irrigated one fourfold, while the control one threefold. For the unit and total fresh weight, the significant differences between the treatments, with values in the biostimulated and irrigated statistically higher than those detected in the control (zero input), start from the phenological phase BBCH scale 40-49 (vegetative parts development) and are maintained also in the last phenological phase investigated BBCH scale 51 (flower buds visible).

As regards the unit and total dry biomass in terms of absolute values, a thirteen-fold increase in the value was recorded between the phase of lateral shoot formation (BBCH scale 20-29) and that of vegetative parts development (BBCH scale 40-49) in the biostimulated treatments, twelve times for the irrigated, while the control treatment increased the value eight times. From the phenological phase BBCH 40-49 to that of visible flowering buds (BBCH scale 51), the biostimulated treatment increased the biomass value five times, the irrigated one four times, while the control one three times. At the time of leaves harvesting (BBCH scale 51), the biostimulated treatment is significantly higher than the control, indicating the effects of the microbial inoculum and water inputs. while in the two previous phenological phases (BBCH scale 20-29 and 40-49) there are no significant differences between treatments



(A)

(B)

(C)

Example of compared treatments at flower buds visible phenological stage (BBCH 51): Control (A), Irrigated (B) and Biostimulated (C).

As for the four variables measured for epigeal biomass, the two intra-treated populations do not differ significantly in the non-destructive measurement of the chlorophyll content of leaves.

SPAD units

Analysis of variance and quantification of *Crithmum maritimum* L. SPAD units in the three different phenological phases (BBCH scale 20-29, 40-49 and 51) for compared treatments on both populations. The table is reported below:

BBCH	Treatment	Population	SPAD Unit
20-29 (side shoots formation)	Biostimulated		32.2(±6.72) a
		AT	32.0(±8.89)
		ME	32.3(±4.50)
	Irrigated		34.3(±7.47) a
		AT	35.1(±8.36)
		ME	33.5(±7.16)
	Control		32.6(±7.91) a
		AT	34.2(±10.27)
		ME	31.0(±5.91)
Mean 20-29			33.0(±7.23)
40-49 (vegetative parts development)	Biostimulated		76.7(±2.88) a
		AT	76.5(±2.31)
		ME	76.9(±3.45)
	Irrigated		71.9(±2.38) b
		AT	71.9(±2.67)
		ME	71.8(±2.17)
	Control		65.1(±4.24) c
		AT	66.0(±3.87)
		ME	64.2(±4.58)
Mean 40-49			71.2(±5.76)
51 (visible flower buds)	Biostimulated		81.3(±2.62) a
		AT	81.4(±2.27)
		ME	81.3(±3.16)
	Irrigated		75.2(±4.24) b
		AT	74.5(±4.49)

	Control	ME	75.8(±4.07)
		AT	56.4(±4.38) c
		ME	55.9(±3.18)
		ME	56.9(±5.44)
Mean 51			68.9(±11.26)

AT = Atlantic population,

ME = Mediterranean population,

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Values are shown as mean ± standard deviation.

Moving from the BBCH scale 20-29 phenological phase (formation of lateral shoots) to the BBCH scale 40-49 phase (development of vegetative parts) the biostimulated treatment sees an increase in SPAD units of 138%, the irrigated one of 109% and the control one of 99% while from the BBCH scale 40-49 phase to the BBCH scale 51 phase (flower buds visible) the biostimulated treatment sees an increase in SPAD units of 6%, the irrigated one of 5% while in the control one records a decrease of 15%. Differences between treatments begin to appear at the vegetative parts development phase (BBCH scale 40-49). Even with just half the total dose of biostimulant distributed throughout the year, statistically higher SPAD values were observed compared to both the irrigated and the control treatments. The same dynamic is also confirmed in the BBCH scale 51 phase (flower buds visible) i.e. at the time of harvesting for industrial uses and once all the water and biostimulant "BS2" has been administered for the 2024 growing season.

Multispectral imagery captured with unmanned aerial vehicles (UAVs) (drones) equipped with multispectral cameras

The epigeal biomass and SPAD sampling points were georeferenced, and the drone equipped with multispectral cameras was subsequently flown in correspondence with the three phenological phases:

"Side shoots formation" (BBCH 20-29), "Vegetative parts development" (BBCH 40-49) and "Flower buds visible" (BBCH 51). At the end of each drone flight, the images were downloaded using the Qgis software, thus in each georeferenced point the vegetation indices were calculated in the three phenological phases in 2024

Exist several Vis (Vegetation indices), to select the most relevant VIs applied for precision agriculture application, we compared some VI categories.

The most important and widely used VI calculated from multispectral information is the NDVI which is a normalized ratio between the red and NIR, which can obtain a value between 0 and 1. NDVI has been used to estimate LAI, chlorophyll content, biomass, and yield. However, NDVI is sensitive to the effects of soil brightness, soil color, atmospheric noise and cloud shadow.

To reduce the soil background effect, were proposed several VIs which introduce a soil adjustment factor into the formula. One of the widely used to analyze plant growth, desertification research, grassland yield estimation, LAI, analysis of soil organic matter, drought monitoring and analysis of soil erosion is the Modified Secondary Soil-Adjusted Vegetation Index (MSAVI2).

Another category of VI is those can be extracted by Red-Blu-Green (RGB) camera, which can acquire images in the visible spectral bands. They are high resolution cameras at low price and low weight. One recent VI developed is the Visible-Band Difference Vegetation Index (VDVI). The values of VDVI are between +1 and -1, and the peculiarity of this index compared to those previously mentioned is that it is the only one to introduce the green band in its formula.

To reduce the NDVI's tendency to quickly saturate its response with a LAI greater than 2, it has proposed the Wide Dynamic Range Vegetation Index (WDRVI) which is based by applying to the NIR band a weighting coefficient to reduce the saturation trend). When the biomass is low (LAI<1) the NDVI is still the best choice for plant classification.

The Normalized Difference Red Edge Index (NDRE) is used for crop canopy evaluations. The Red Edge (RE) band penetrates deep into canopy and is sensitive to crop canopy chlorophyll at higher canopy biomass, overcoming the saturation inherent to the NDVI

Categories of vegetational indices, and related formulas analyzed in this study are reported below

Vegetation index Category	Index	Formula
Adjusted-soil vegetation index	MSAVI2 (Modified Soil Adjusted Vegetation Index 2)	$\text{MSAVI2} = \frac{2 * \text{NIR} + 1 - \sqrt{(2 * \text{NIR} + 1)^2 - 8(\text{NIR} - \text{Red})}}{2}$
Basic vegetation index	NDRE (Normalized Vegetation Red Edge Index)	$\text{NDRE} = \frac{\text{NIR} - \text{RE}}{\text{NIR} + \text{RE}}$
Basic vegetation index	NDVI (Normalized Difference Vegetation Index)	$\text{NDVI} = \frac{\text{NIR} - \text{Red}}{\text{NIR} + \text{Red}}$
Vegetation index in the visible spectra region	VDVI (visible-band difference vegetation index)	$\text{VDVI} = \frac{2 * \text{Green} - \text{Red} - \text{Blue}}{2 * \text{Green} + \text{Red} + \text{Blue}}$
Vegetation indexes related to vegetation status	WDRVI (Wide Dynamic Range Vegetation Index)	$\text{WDRVI} = \frac{a * \text{NIR} - \text{Red}}{a * \text{NIR} + \text{Red}}$ Where: $a = 0,2$

The correlations were calculated between the biomass parameters obtained with the destructive method, the SPAD unit as a proximal sensing instrument and the processed vegetation indices. The correlations were calculated with the data from the three phenological phases of the two years, the matrix is reported below

	Fresh biomass	Dry biomass	Mean SPAD	MSAVI2	NDRE	NDVI	VDVI	WDRVI
Fresh biomass	1.00							
Dry biomass	0.91	1.00						
Mean SPAD	0.67	0.57	1.00					
MSAVI2	0.05	-0.01	0.14	1.00				
NDRE	-0.21	-0.13	-0.09	0.46	1.00			

NDVI	-0.04	-0.06	0.08	0.96	0.60	1.00			
VDVI	-0.13	-0.21	0.12	0.73	0.22	0.73	1.00		
WDRVI	0.05	-0.03	0.15	0.98	0.43	0.96	0.74	1.00	

The correlations between biomass and mean SPAD are high, however, the same cannot be confirmed with the vegetation indices, this may be due to the underdevelopment of the plants, as it is only the first year of growth, and the presence of the black mulching which may have destabilised the measurement of the indices.

1.2.2 Field experiments: open field

Objective

Sustainable production of sea fennel crop in demo field (Task 4.1)

Materials and methods

The new sea fennel crops produced through the open field cultivation trials at RINCI Sr (PARTY 3) have been analysed using destructive analyses (biomass sampling) for the determination of:

- o phenological development
- o fresh and dry epigeal biomass yield;
- o multispectral imagery captured with unmanned aerial vehicles (UAVs) (drones) equipped with multispectral cameras.

Phenological development

To characterize the phenological development of the crop in the treatments, for the main stem, we adopted the Bundesanstalt, Bundessortenamt, Chemische Industrie (BBCH) scale (Bleiholder et al., 1991) during the entire biological cycle of the crop: the phenological characterization began between the vegetative recovery phase indicated with BBCH 20-29 and continued until the BBCH 51 phase corresponding to the initial emergence of the inflorescence or when the leaves are collected for industrial use. The portion of the farm under study is divided into three zones based on the ecotypes used and the year of transplantation: Atlantic transplanted in 2017, Mediterranean transplanted in two years, 2018 and 2020.

Results:

Fresh and dry epigeal biomass

The table of the epigeal biomass of open field is reported below:

Year	Ecotype	TY	BBCH	UFW	TFW	UDW	TDW
2022	ME	2020	20-29	105.4(±20.4)a	10.3(±2.0)a	13.6(±3.5)a	1.3(±0.3)a
2022	ME	2018	20-29	59.4(±6.6)b	5.8(±0.7)b	8.7(±0.7)b	0.9(±0.1)b
2022	AT	2017	20-29	48.9(±16.6)b	4.8(±1.6)b	6.9(±2.2)b	0.7(±0.2)b
2022		Mean 20-29		71.2(±29.3)	7.0(±2.9)	9.7(±3.6)	1.0(±0.4)
2022	ME	2020	40-49	308.7(±95.5)a	30.3(±9.4)a	45.3(±13.5)a	4.4(±1.3)a
2022	ME	2018	40-49	221.7(±7.1)a	21.7(±0.7)a	33.7(±3.0)a	3.3(±0.3)a
2022	AT	2017	40-49	215.2(±11.7)a	21.1(±1.2)a	34.0(±2.5)a	3.3(±0.3)a
2022		Mean 40-49		248.5(±66.1)	24.4(±6.5)	37.7(±9.1)	3.7(±0.9)

2022	ME	2020	51	383.2(±58.2)a	37.6(±5.7)a	62.1(±3.5)a	6.1(±0.3)a
2022	ME	2018	51	374.4(±79.8)a	36.7(±7.8)a	60.7(±9.6)a	6.0(±0.9)a
2022	AT	2017	51	321.0(±29.5)a	31.5(±2.9)a	52.7(±9.7)a	5.2(±1.0)a
2022		Mean 51		359.5(±59.2)	35.2(±5.8)	58.5(±8.3)	5.7(±0.8)
2023	ME	2020	20-29	89.1(±5.4)b	8.7(±0.5)b	15.6(±1.3)b	1.5(±0.1)b
2023	ME	2018	20-29	98.7(±3.5)a	9.7(±0.3)a	18.8(±1.1)a	1.8(±0.1)a
2023	AT	2017	20-29	92.3(±2.4)ab	9.1(±0.2)ab	18.5(±0.8)a	1.6(±0.1)ab
2023		Mean 20-29		93.4(±5.5)	9.2(±0.5)	17.6(±1.8)	1.7(±0.2)
2023	ME	2020	40-49	359.6(±4.4)ab	35.2(±0.4)a	55.0(±2.8)a	5.4(±0.3)ab
2023	ME	2018	40-49	373.4(±45.1)a	36.6(±4.4)a	56.4(±5.5)a	5.5(±0.5)a
2023	AT	2017	40-49	313.0(±20.1)b	30.7(±2.0)b	47.5(±5.3)b	4.7(±0.5)b
2023		Mean 40-49		348.7(±37.0)	34.2(±3.6)	53.0(±5.8)	5.2(±0.6)
2023	ME	2020	51	498.7(±18.1)b	48.9(±1.8)a	80.3(±2.3)a	7.9(±0.2)a
2023	ME	2018	51	408.4(±6.7)b	40.0(±0.7)b	69.4(±2.4)b	6.8(±0.2)b
2023	AT	2017	51	519.1(±24.8)a	50.9(±2.4)a	80.9(±2.2)a	7.9(±0.2)a
2023		Mean 51		475.4(±53.4)	46.6(±5.2)	76.9(±5.9)	7.5(±0.6)

Means (+/- standard deviation) within each column followed by different letters are significantly different (ANOVA, $p < 0.05$).
BBCH = phenological phase 20-29 Side shoots formation; 40-49 Vegetative parts development; 51 Flower buds visible; ME =Mediterranean Ecotype; AT = Atlantic ecotype; TY = Transplanting year; UFW Unit Fresh Weight; TFW Total Fresh Weight; UDW Unit Dry Weight; TDW Total Dry Weight

The results show an increase in epigeal biomass with advancing phenological phase, with higher average values in 2023 compared to 2022. The Mediterranean ecotype (ME) accumulated more biomass in the early growth stages (BBCH 20-29), while the Atlantic ecotype (AT) reached comparable values in the later stages (BBCH 51). The overall increase in biomass in 2023 suggests more favorable environmental conditions or better plant adaptation.

SPAD units

The table of the SPAD unit of open field is reported below:

Year	Ecotype	TY	BBCH	SPAD
2022	ME	2020	20-29	50.4(±2.6) a
2022	ME	2018	20-29	44.5(±1.7) b
2022	AT	2017	20-29	37.4(±2.4) c
2022		Mean 20-29		44.8(±6.0)
2022	ME	2020	40-49	56.9(±1.5) a
2022	ME	2018	40-49	45.2 (±1.5) b
2022	AT	2017	40-49	45.2(±2.2) b
2022		Mean 40-49		49.1(±6.1)
2022	ME	2020	51	55.7(±1.3) a
2022	ME	2018	51	50.8(±2.0) b
2022	AT	2017	51	49.5(±1.3) b

2022		Mean 51		52.0(±3.2)
2023	ME	2020	20-29	45.2(±1.8) a
2023	ME	2018	20-29	43.3(±1.2) a
2023	AT	2017	20-29	36.0(±2.1) b
2023		Mean 20-29		41.5(±4.5)
2023	ME	2020	40-49	55.5(±0.6) a
2023	ME	2018	40-49	46.6(±0.6) b
2023	AT	2017	40-49	47.6(±0.8) b
2023		Mean 40-49		49.9(±4.3)
2023	ME	2020	51	58.6(±0.5) a
2023	ME	2018	51	51.3(±0.5) b
2023	AT	2017	51	51.0(±0.7) b
2023		Mean 51		53.6(±3.8)

BBCH = phenological phase 20-29 Side shoots formation; 40-49 Vegetative parts development; 51 Flower buds visible; ME =Mediterranean Ecotype; AT = Atlantic ecotype; TY = Transplanting year

The portion of the farm with transplanted which took place in 2020 and containing the Mediterranean ecotype shows a statistically higher nutritional status than the other two portions of the company in the phenological phase corresponding at the leaves harvest both two years.

Multispectral imagery captured with unmanned aerial vehicles (UAVs) (drones) equipped with multispectral cameras

The correlations were calculated between the biomass parameters obtained with the destructive method, the SPAD unit as a proximal sensing instrument and the processed vegetation indices. The correlations were calculated in 2022 and 2023 with the data from the three phenological phases of the two years. Correlation with epigeal biomass, proximal and remote sensing parameters is reported below.

	Mean SPAD	Fresh biomass	Dry biomass	MSAVI2	NDRE	NDVI	VDVI	WDRVI
Mean SPAD	1.00							
Fresh biomass	0.72	1.00						
Dry biomass	0.71	0.98	1.00					
MSAVI2	0.22	0.01	0.02	1.00				
NDRE	0.03	0.27	0.32	0.10	1.00			
NDVI	0.20	0.06	0.07	0.93	0.35	1.00		
VDVI	0.27	0.06	0.03	0.83	-0.09	0.76	1.00	
WDRVI	0.25	0.02	0.01	0.96	0.02	0.92	0.85	1.00

The matrix shows that there is a high correlation between biomass and SPAD, however, this cannot be said of the correlation between biomass and SPAD with the vegetation indices, this may be due to the presence of the black mulching which may have caused some problems in the measurement.

Content in lipophilic (tocopherols, carotenoids) and hydrophilic (polyphenols) antioxidant compounds

Carotenoids, expressed as mg/g dry weight (DW). Data are reported as the mean of three analytical replicates $\pm$ standard deviation. Different letters in the column mean statistical difference ($p < 0.05$) after one-way Anova Tukey test.

mg/kg DW	Neoxanthin		Violaxanthin		Zeaxanthin		Lutein		β -carotene		Total carotenoids	
M												
AR							10				14	
-L-	11,	0,7	1.5	0.0	6.1	0.3	2.5	6.2	26.	1.6	8.5	9.0
C	80	$\pm$ 2	3	$\pm$ 9	1	$\pm$ 7	4	$\pm$ 2	59	$\pm$ 1	7	$\pm$ 2

Tocopherols

Tocopherols, expressed as mg/g dry weight. Data are reported as the mean of three analytical replicates $\pm$ standard deviation. Different letters in the column mean statistical difference ($p < 0.05$) after one-way Anova Tukey test.

mg/kg DW	γ -T		α -T		Total tocopherols	
MAR-L-C	23.65	$\pm$	1.44	$\pm$	11.79	$\pm$
					217.94	$\pm$ 13.23

Hydroxycinnamic acids

Hydroxycinnamic acids, expressed as mg/g dry weight (DW). Data are reported as the mean of three analytical replicates $\pm$ standard deviation. Different letters in the column mean statistical difference ($p < 0.05$) after one-way Anova Tukey test.

mg/g DW	Neochlorogenic acid		Chlorogenic acid		Cryptochlorogenic acid		Total chlorogenic acids		3,5-di-O-caffeoylquinic acid	
MAR			38.9				41.6		47.9	
-L-C	1.68	$\pm$ 0.14	3	$\pm$ 3.33	1.04	$\pm$ 0.09	5	$\pm$ 3.56	7	$\pm$ 4.10

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1.3 By-products (old leaves and fibrous stems) from the new organic sea fennel crops

Materials and methods

The epigeal biomass was cut at a height of 5 cm from the ground level, and each by-product of plant was separated from marketable yield (corresponding to edible leaves). then was weighed individually using a laboratory balance to determine the weight. A total of 27 plants were sampled in a randomized manner, with 9 samples taken for each of the 3 replications. These results were calculated with the biomass at the moment of the harvest

Results

Total fresh biomass (g/plant) and marketable yield/fresh biomass (g/plant) for the Camerano experimental site in 2023

Treatment	Total fresh biomass (g/plant)	Marketable Yield (g/plant)	Marketable Yield / Total fresh biomass (g/plant)	% of refuse
CT	523.7	219.95	0.42	58%
BIO	936	439.92	0.47	53%
IRR	820.4	377.38	0.46	54%
Mean	750.75	337.83	0.45	55%

CT = Control

BIO = Biostimulated

IRR = Irrigated

Total fresh biomass (g/plant) and marketable yield/fresh biomass (g/plant) for the Agugliano experimental site in 2024

Treatment	Population	Total fresh biomass (g/plant)	Marketable Yield (g/plant)	Marketable Yield / Total fresh biomass (g/plant)	% of refuse
CT	AT	40.30	27.4	0.68	32%
	MED	45.48	31.8	0.7	30%
BIO	AT	110.25	73.86	0.67	33%
	MED	103.86	73.74	0.71	29%
IRR	AT	89.87	60.21	0.67	33%
	MED	89.11	58.81	0.66	34%
Mean		79.81	54.3	0.68	31%

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CT = Control

BIO = Biostimulated

IRR = Irrigated

AT = Atlantic ecotype

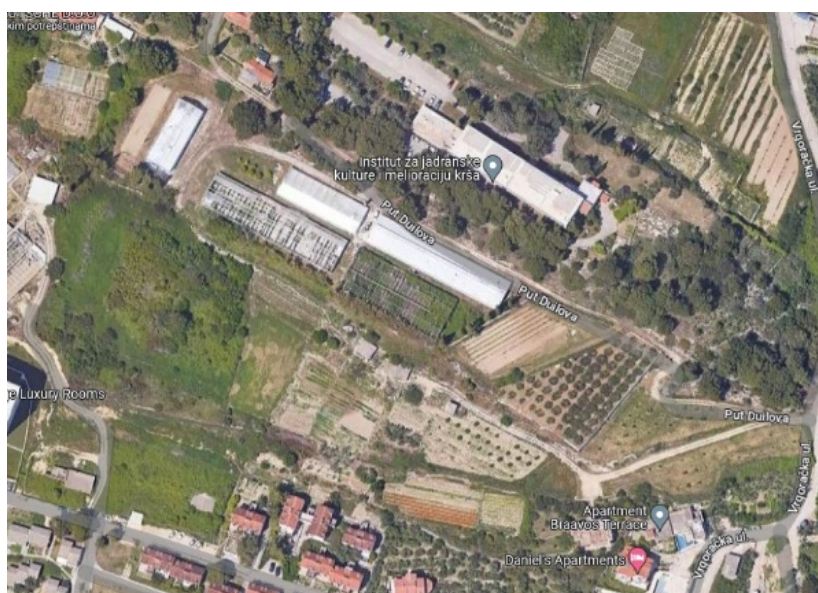
MED = Mediterranean ecotype

One-year-old plants have a lower % of refuse due to the lower lignification

2 Croatian site

2.1 Experimental plan design of cultivation trials

Site: Institute for Adriatic Crops, Split (Croatia)



Experimental site in Split, Croatia

Soil characteristics

pH _{H2O}	pH _{KCl}	CaCO ₃	CaO	TOC	N	P available*	K available*	Texture
		g kg ⁻¹				mg kg ⁻¹		

8.51	7.57	484	188	14.9	0.13	2.6	25.9	Silt loam
------	------	-----	-----	------	------	-----	------	-----------

*available P was determined after extraction with $(\text{NH}_4)_2\text{SO}_4$ – Troug method

*available K was determined after extraction with NH_4 -acetate

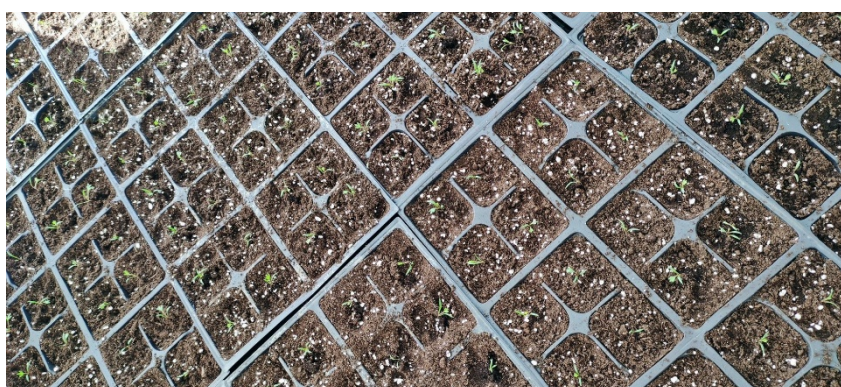
Compared ecotypes: *Crithmum maritimum* L ecotype Korčula, *Crithmum maritimum* L ecotype Atlantic coast

Seedbed preparation: sowing on 27th January 2023, in polystyrene plug trays filled with commercial peat (Brill 7, Austria), 3-4 seeds per plug was used, tray were put in heated germination tables inside unheated greenhouse, irrigation as needed.



Polystyrene plug trays used for sowing of sea fennel seeds.

Seeds started to germinate 5 days after sowing and 45 days after plants were transplanted into PE trays with 24 places, one plant per pot were transplanted. Plants were irrigated every 2-3 days.



PE trays used for first transplantation of sea fennel seedlings.

Fertilization: Fertilizers or bio-stimulants were not applied at seedling stage.

Plant Transplanting: On the third week of May 2023, seedlings with four- five true leaves have been transplanted at IACKR site.

Compared Plant Density 40 × 60 cm

Field experiment dimension: The total area of the experimental field is 700 m².

Experimental design: Complete randomized blocks with 3 replications (R).

Experimental field was prepared with deep ploughing and motocultivator to obtain fine soil surface before transplanting. Before cultivation organic pelletized bovine and equine manure – NPK 2-2-2 (Fertildung, Fomet-IT) were applied at half of the experimental field at rate of 20 kg/100 m².



Motocultivator used for soil bed preparation.



Scheme of fertilized and unfertilized plots of experimental fields

2.1.1 Experiments with seedlings production

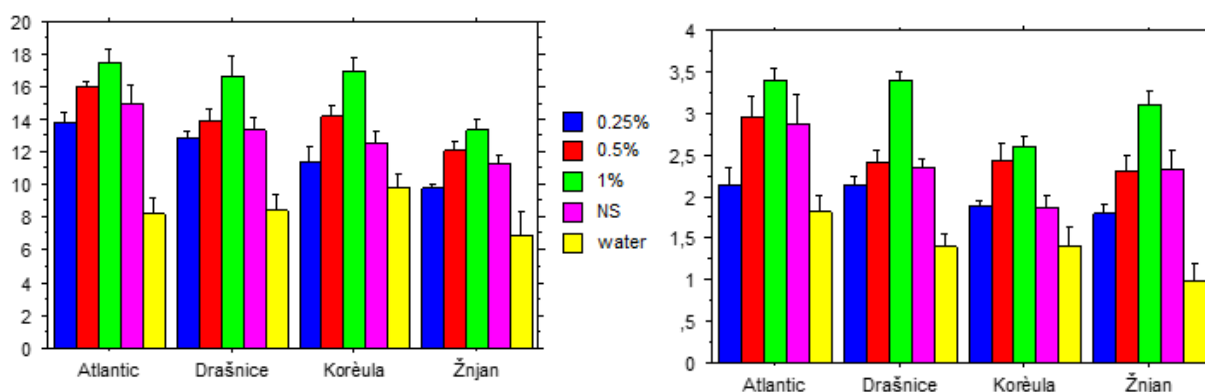
Experiment 1.

Different ecotypes seeds were sown on 27th January 2023, in polystyrene plug trays filled with commercial peat (Brill 7, Austria), 3-4 seeds per plug was used, tray were put in heated germination tables inside unheated greenhouse, irrigation as needed. Four ecotypes were used in this experiment: Atlantic, Drasnica, Korcula and Split (Znjan).

Seeds started to germinate 5 days after sowing and 45 days after, plants were started to irrigate with different solutions. Solutions were: NS – nutrient solution as half Hoagland nutrient solution, three solutions made with pelletized manure soaked in water for one day (0.25, 0.5 and 1% of manure in water) and water as control. After two months of cultivation and use of mentioned solutions plants were harvested and main growth traits were determined (plant height and weight, number of leaves) as shown in Figure X.



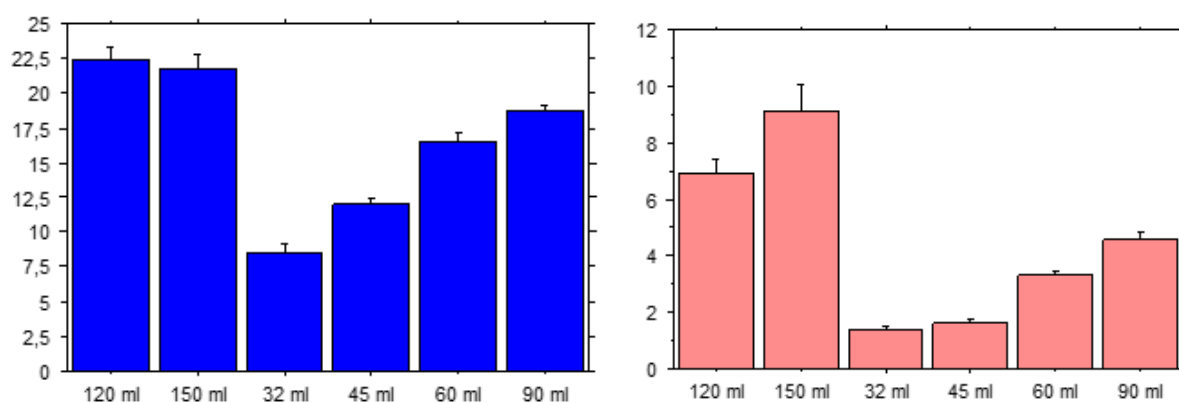
Results showed that all ecotypes had best growth parameters when fertirigated with 1% manure solution.



The height (cm) and weight (g) of four ecotypes seedlings cultivated with different nutrient solutions

Experiment 2

Same as in the previous Experiment 1, 45 days after germination plants were transplanted in trays and pots of different volumes: 32, 45, 60, 90, 120 and 150 ml. Plants were irrigated only with water and after 60 days of cultivation were harvested for measurements. Only one ecotype was used for sowing, Drašnice.



The height (cm) and weight (g) of seedlings cultivated in trays and pots of different volumes

2.2 Fresh biomass (edible aerial parts) from the new organic sea fennel crops

Materials and methods

After transplanting in May 2023 plants were irrigated once a week over the summer, but overall growth was slow until September, so harvest was not done until end of October 2023.

Harvest was done on two ecotypes/ populations, French - Atlantic and Croatian - Korčula. The edible biomass of the plant was collected at phenological stage of full umbell development for only one treatment studied: unfertilized (control). Plants were divided in 3 parts: leaves, stem and flower, weighed fresh and dried at 70 C to calculate dry mass percentage of each plant organ/parts.



Harvest of sea-fennel plant

Results

Results are shown in the following Table. Differences in fresh and dry weight, as also dry weight percentage were not significant between ecotypes due to high variability of plants.

Ecotype	Plant		Leaves		Stem		Flover	
	Fresh weight (g/plant)							
Atlantik	216,4	± 19,1	120,2	± 10,4	62,1	± 6,7	34,1	± 6,9
Korčula	198,4	± 18,4	93,2	± 11,2	73,9	± 7,5	31,2	± 6,7
	Dry weight (g/plant)							
Atlantik	41,8	± 4,5	21,8	± 1,9	12,5	± 1,8	7,5	± 1,6
Korčula	39,1	± 3,8	17,3	± 2,1	15,4	± 1,6	6,3	± 1,3
	Dry weight percentage (%)							
Atlantik	19	± 0,7	18,2	± 0,6	19,3	± 0,9	22,1	± 1,7
Korčula	19,6	± 0,4	18,5	± 0,3	20,5	± 0,8	19,9	± 1,1

*Sea fennel plants harvested in October 2023; fresh and dry weight, and dry weight percentage
Means are shown as +/- standard error*

Next harvest was done in July 2024 what can be consider as first standard harvest in period when sea fennel plants leaves were collected for future use and processing.

Ecotype Korčula had bigger plants than ecotype Atlantic, but no differences were found in dry weight percentage between ecotypes. Differences were found for weight of flowers as Atlantic ecotype started earlier with flowering phase and flowers with stems were more developed in harvest time. That can be explained due to differences in climatic conditions in Croatian site with warm, hot Mediterranean climate comparing from conditions in north-west part of France where is origin of Atlantic ecotype.

Seafennel plants harvested in July 2024 - fresh and dry weight, and dry weight percentage

	Plant		Leaves		Stem		Flower	
Ecotype								
	Fresh weight (g/plant)							
Atlantik	212	± 22,1	85,3	± 8,46	67,4	± 9,1	59,4	± 7,1
Korčula	374	± 29,0	189	± 18,2	159	± 11,9	24,5	± 4,8
	Dry weight (g/plant)							
Atlantik	58,1	± 5,7	23,5	± 2,1	18,8	± 2,4	15,7	± 1,6
Korčula	97,5	± 7,5	48,1	± 4,5	42,9	± 3,4	6,5	± 1,3
	Dry weight percentage (%)							
Atlantik	27,7	± 0,4	28,1	± 0,5	28,3	± 0,5	26,9	± 0,5
Korčula	26,1	± 0,3	25,6	± 0,3	26,8	± 0,4	27,1	± 0,6

2.3 By-products (old leaves and fibrous stems) from the new organic sea fennel crops

Objective

Measurement of biomass for 2 sea fennel ecotypes (Korčula and Atlantik) cultivated at demo field in summer season

Materials and methods

The whole plant was cut epigeal at a height of 5 cm above the ground level, and each sample was divided on leaves used for determination of marketable yield, flowers and stems with damaged leaves. Each subsample was weighed to get fresh weight and then dried in oven on 70 C for few days to get dry weight. Harvest was done in July 2024 and total of 18 plants were sampled in a randomized manner, with 6 samples taken for each of the 3 replications.

Seafennel plants harvested in July 2024 - fresh and dry weight, and dry weight percentage (data from unfertilized plots)

Ecotype	Plant		Leaves		Stem		Flover	
	Fresh weight (g/plant)							
Atlantik	212	± 22,1	85,3	± 8,46	67,4	± 9,1	59,4	± 7,1
Korčula	374	± 29,0	189	± 18,2	159	± 11,9	24,5	± 4,8

	Dry weight (g/plant)			
	Atlantik	Korčula	Atlantik	Korčula
Atlantik	58,1 ± 5,7	23,5 ± 2,1	18,8 ± 2,4	15,7 ± 1,6
Korčula	97,5 ± 7,5	48,1 ± 4,5	42,9 ± 3,4	6,5 ± 1,3
	Dry weight percentage (%)			
	Atlantik	Korčula	Atlantik	Korčula
Atlantik	27,7 ± 0,4	28,1 ± 0,5	28,3 ± 0,5	26,9 ± 0,5
Korčula	26,1 ± 0,3	25,6 ± 0,3	26,8 ± 0,4	27,1 ± 0,6

Means are shown as +/- standard error

First harvest of full developed plants was done in July 2024, because in summer 2023 plants were small for harvesting due to late planting. No differences were found between unfertilized or fertilized plots.

Ecotype Korčula had bigger plants than ecotype Atlantic and all plant parts had similar dry weight percentage for both ecotypes.

3 Tunisian sites

3.1 Experimental plan design of cultivation trials



Site: INRGREF experimental station in Nabeul-Tunisia (36.464325, 10.705955)

Compared ecotypes: *Crithmum maritimum* L. ecotype Tabarka (Tunisia), *Crithmum maritimum* L. ecotype Atlantic coast (France)

Seedbed preparation: seed were sown on December 2024, in germination plates at the rate of two-three seeds per well, regular irrigation, greenhouse (controlled conditions).

Fertilization: NPK fertilizer (20-20-20) was used

Plant Transplanting: In May, plants with three-four true leaves were transplanted in the site.

Compared Plant Density 45 x 45 cm

Plot dimension: 9.8 plant m⁻² corresponding 98,000 plants ha⁻¹

Field experiment dimension: 400 m²

Experimental design: Complete randomized blocks with 3 replications.

Before planting, tillage was carried out by ploughing of depth between 35-40 cm and seed beds were prepared.

To assess the influence of soil composition and salinity on plant crop yield, 5 soil samples (0-30 cm depth) were taken from the experimental site to determine its physicochemical properties. In April 2024 all seedlings had only 1-2 true leaves. According to the protocol prepared by the international Consortium of Project partners, transplantation in the field was done when the seedlings had 3-4 true leaves (May 2024). Prior to transplantation, soil was prepared in order to have fine soil surface. Hand weeding was performed in order to control weed abundance and avoid competition with the crops.



Collection of soil samples for physicochemical analyses (Left picture), and transplantation of sea fennel in the field (May 2024) (Right picture)

Plant material was collected randomly in September 2024, January 2025 and April 2025.

Soil analysis

Soil analysis showed that the experimental station is installed on a sandy neutral soil with a pH level of 7. The soil analysis reveals a moderate level of organic matter at 2.167%, beneficial for enhancing soil structure, promoting microbial activity, and supporting overall soil health. The organic carbon content of 1.259% is consistent with the organic matter value, as carbon typically makes up about 58% of organic matter. This indicates a moderately fertile soil, capable of supporting healthy plant growth.

The soil pH is neutral at 7.0, which could optimize the availability of essential nutrients and encourage beneficial microbial activity. The electrical conductivity (EC) is measured at 300 μ S/cm, which indicates low salinity.

Regarding macronutrients, the total nitrogen content is 2.14 g/kg, a moderate to high level that supports vigorous vegetative growth. The phosphorus content, at 0.93 g/kg, is also sufficient, contributing to healthy root development and reproductive processes like flowering and fruiting. Similarly, the potassium level of 1.13 g/kg is adequate to support various physiological processes.

Among the secondary nutrients, calcium is notably high at 8.17 g/kg, which is beneficial for plant structural integrity. The magnesium level, at 2.05 g/kg, is satisfactory, and Sodium is very low (<0.1 g/kg).

Finally, the chloride content of 73.67 mg/kg is within a normal range and should not pose a toxicity risk. The sulfate level, at 579.78 mg/kg, is relatively high, ensuring an adequate supply of sulfur for protein synthesis and enzymatic functions in plants.

Soil chemical analysis of experimental site

Variable	Value	Unit	Method
Organic Matter	2.167	% of dry matter	Calcination at 525°C
Organic carbon	1.259	% of dry matter	ISO 14235 (1998)
pH	7.0 (at20.1°C)		NF EN 12176 (1998)
Electric conductance	300	µS/cm	NF ISO 11265 (1995)
Total nitrogen	2.14	g/Kg of dry matter (DM)	NF ISO 11261 (1995)
Total phosphorus	0.93	g/Kg DM	ISO 11885 (2007)
Potassium	1.13	g/Kg DM	ISO 11885 (2007)
Sodium	<0.1	g/Kg DM	ISO 11885 (2007)
Calcium	8.17	g/Kg DM	ISO 11885 (2007)
Magnesium	2.05	g/Kg DM	ISO 11885 (2007)
Chloride	73.67	mg/Kg DM	ISO 10304-1 (2007)
Sulfate	579.78	mg/Kg DM	ISO 10304-1 (2007)

3.1.1 Germination tests in greenhouse

Materials and methods

Sowing of seeds from Tunisian and Atlantic ecotype in greenhouse took place on December 2024.

Seeds were placed in alveolated plates containing peat sowing substrate. A total of 1000 seeds were planted for each ecotype. Irrigation was carried out almost daily.



Sowing of seeds from Tunisian and Atlantic ecotypes

3.2 Fresh biomass (edible aerial parts) from the new organic sea fennel crops

Materials and methods

The harvest of sea fennel started from september 2024. Harvest was done on two ecotypes, French - Atlantic and Tunisian-Tabarka. After harvest, the leaves and branches were separated, cleaned and than weighed using a balance to determine the fresh weight. The plant material was then introduced into an oven until reaching a constant weight for the determination of the dry matter content.

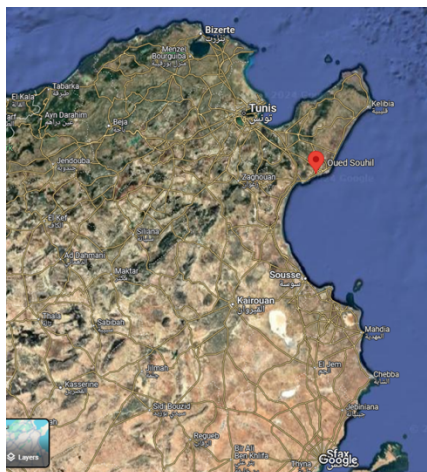


Sea fennel in the field (Left picture), weight of fresh biomass (right picture)

3.2.1 Field experiments: demo field

Materials and methods

The experimental site was set on Nabeul locality as shown in the following picture:

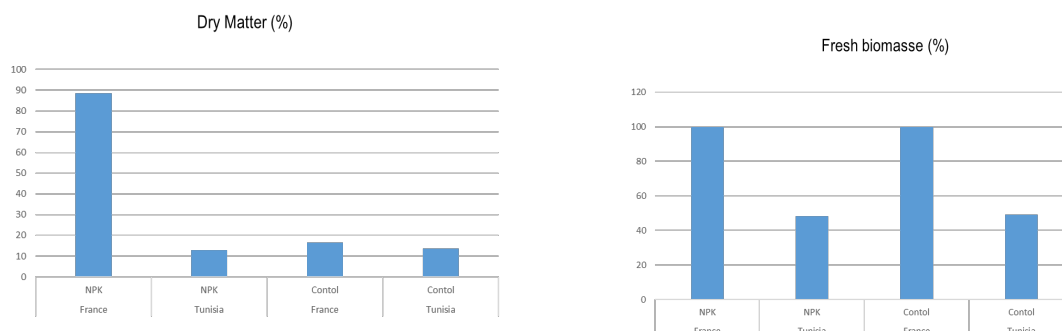


Before planting, tillage was carried out by ploughing of depth between 35-40 cm and seed beds were prepared. To assess the influence of soil composition and salinity on plant crop yield, 5 soil samples (0-30 cm depth) were taken from INRGREF experimental site to determine its physicochemical properties. The transplantation was done in May 2024. Plants were treated using a commercial biofertilizer (NPK) from August 2024. The first sample harvest was done in September 2024.



Transplantation of Sea Fennel plants in the field

Results



Recent agronomic trials on sea fennel (*Crithmum maritimum* L.) conducted across two harvesting periods—September 2024 and January 2025—demonstrate clear variations in biomass production and edible yield across ecotypes and treatments.

In the September 2024 harvest, the Atlantic ecotype performed uniquely: both NPK-treated and control plants showed 100% edible fresh biomass, with NPK-treated samples averaging 3.36 g/plant. In contrast, the Tunisian ecotype presented significantly lower edible-to-total mass ratios, with NPK-treated plants yielding 3.36 g of edible biomass (48% of total), and controls producing 2.6 g (49%). Despite similar edible yields between treated and untreated Tunisian plants, total biomass was higher in NPK-treated samples.

By January 2025, a substantial overall biomass increase was recorded, particularly for the Tunisian ecotype. NPK-treated Tunisian plants reached an average total fresh biomass of 179.11 g/plant, though only 9% (16.21 g) was edible. In the control group, total biomass was lower (103.36 g/plant), but the edible fraction slightly increased to 14% (13.34 g).

The Atlantic ecotype followed a similar trend, albeit with lower yields. NPK-treated plants produced 24.78 g total biomass with 11% edible parts, while controls had just 4.06 g total biomass but a slightly higher edible proportion of 19%.

These findings highlight two key observations:

1. Fertilization enhances overall biomass, especially in the Tunisian ecotype.
2. The proportion of edible material is significantly higher in early harvests (September), particularly for the Atlantic ecotype, suggesting timing is crucial for optimizing harvest quality and usability.

Averages of Fresh-Biomass (g/plant) of edible parts of sea fennel samples

Ecotype	Treatment	Harvest period	Total plant fresh biomass (g)	Edible fresh mass (g)	% of edible (FW)
Tunisia	NPK	Sep-24	15.38±10.80	3.36±1.51	48%±39%
Tunisia	Control	Sep-24	8.03±9.60	2.6±2.13	49%±28%
Atlantic	NPK	Sep-24	3.36±1.63	3.36±1.63	100%
Atlantic	Control	Sep-24	1.92±1.17	1.92±1.17	100%
Tunisia	NPK	Jan-25	179.11±148.83	16.21±11.86	9%±3%
Tunisia	Control	Jan-25	103.36±98.33	13.34±12.33	14%±6%
Atlantic	NPK	Jan-25	24.78±7.20	2.56±0.66	11%±2%
Atlantic	Control	Jan-25	4.06±3.38	0.61±0.42	19%±8%

3.3 By-products (old leaves and fibrous stems) from the new organic sea fennel crops

The cultivation of sea fennel for last year (2023) failed due to high temperatures, the cultivation was renewed in the year 2024.

Materials and methods

Two harvests of sea fennel aerial parts were conducted; September 2024 and January 2025. Sampled plants were separated into “edible portion” and “refuse” categories. A total of 24 plants were harvested randomly with 6 samples taken in every treatment*ecotype combination. The plant material was dried in an oven until reaching a constant weight for the determination of the dry matter content, which was expressed in % of fresh weight (FW).

Results

Averages of Fresh-Biomass (g/plant) of refused parts of sea fennel populations

Ecotype	Treatment	Harvest period	Total plant fresh biomass (g)	Refused parts mass (g)	% of refuse
Tunisia	NPK	Sep-24	15.38±10.80	12.02±10.62	52%±39%
Tunisia	Control	Sep-24	8.03±9.60	5.43±7.53	51%±28%
Atlantic	NPK	Sep-24	3.36±1.63	0.00	0%
Atlantic	Control	Sep-24	1.92±1.17	0.00	0%
Tunisia	NPK	Jan-25	179.11±148.83	162.90±137.65	91%±3%
Tunisia	Control	Jan-25	103.36±98.33	90.02±86.76	86%±6%
Atlantic	NPK	Jan-25	24.78±7.20	22.22±6.70	89%±2%
Atlantic	Control	Jan-25	4.06±3.38	3.45±2.99	81%±8%

Newly collected data on refused biomass portions—non-edible or discarded parts—of sea fennel plants across two ecotypes and seasonal harvests reveal important insights into crop efficiency and processing yield.

During the September 2024 harvest, the Atlantic ecotype showed exceptional performance, with zero biomass refuse across both NPK-treated and control plants. This suggests that during early growth, Atlantic plants yield entirely edible material, representing ideal efficiency for fresh use.

Conversely, the Tunisian ecotype showed significant waste at this stage: NPK-treated plants had 52% refuse, while controls had 51%, indicating that over half the biomass was deemed inedible or unsuitable for processing. Although the absolute biomass values were higher in fertilized samples, the proportion of unusable material remained similar.

In the January 2025 harvest, refuse percentages increased dramatically across both ecotypes. The Tunisian plants under NPK treatment reached 91% refuse, while controls reached 86%, suggesting that winter-harvested plants grow bulkier but produce a much smaller proportion of edible material.

The Atlantic ecotype, while producing less overall biomass, followed a similar trend: NPK-treated samples had 89% refuse, and controls 81%.

These results underscore a key agronomic challenge: although later harvests boost overall biomass, the proportion of usable material drops sharply. The Atlantic ecotype appears more efficient for early harvests with minimal waste, while the Tunisian variety benefits more from fertilization but generates higher refuse.

4 Turkish site

4.1 Experimental plan design of cultivation trials

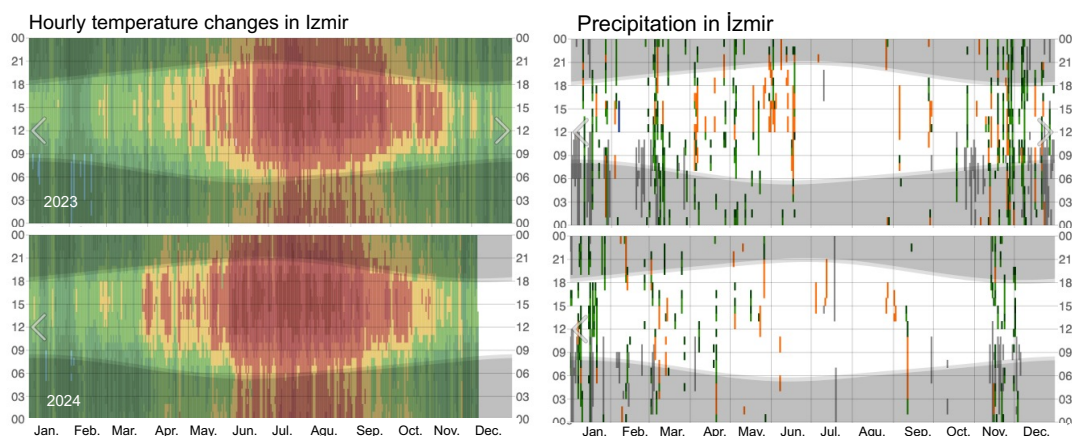
Site: Experimental field of Ege University, Faculty of Agriculture, Department of Field Crops, Bornova-Izmir/Turkey (38°27'06.0"N 27°13'31.9"E)

Soil samples (0–30 cm depth) were collected from the experimental site before transplanting. The samples were analyzed for various physicochemical properties, including soil texture, pH, organic matter content, electrical conductivity, total nitrogen (N), available phosphorus (P), exchangeable calcium (Ca), potassium (K), sodium (Na), iodine (I), magnesium (Mg), and major anions. Throughout the growing period, irrigation was carried out using a drip irrigation system. Field observations confirmed that sea fennel plants were unable to survive under rainfed conditions at the experimental site. Weed management was performed through mechanical removal, targeting *Sorghum halepense* L., the predominant weed species in the field. Hourly average temperature and total precipitation data for 2023 and 2024 at the experimental site (İzmir-Bornova) are presented in the Figure below. Mean temperatures in 2024 were recorded as 1.2°C (7.2%) higher than in 2023. Total precipitation in 2023 was 780 mm higher than long-term averages, while precipitation levels in 2024 were projected to be at least 20% lower by the end of the year.

Soil

pH	7.88
Org.(%)	1.99
Salinity (%)	0.09
CaCO ₃ (%)	14.2
Structure	Clay-loamy
Sand (%)	42
Silt (%)	21
Clay (%)	37
N (%)	0.05
P (ppm)	6.75
K (ppm)	506
Fe (ppm)	0.18
Cu (ppm)	1.10
Mg (ppm)	367
Zn (ppm)	1.95
Na (ppm)	73
Mn (ppm)	1.17

Physicochemical properties of the soil sampled from the experimental site in Izmir. The samples were collected before the sea fennel seedlings transplanted to the demo field trail (2023).



Hourly average temperature and total precipitation during 2023 and 2024 at the experimental site (İzmir-Bornova).

Compared ecotypes: *Crithmum maritimum* L ecotype AC-1 (Aegean Coast 1), *Crithmum maritimum* L ecotype AC-2 (Aegean Coast 2) and *Crithmum maritimum* L ecotype ATL (Atlantic Coast).

Seedbed preparation: As part of work package 3, fresh sea fennel samples and seeds were collected from four different locations in the Turkish flora and stored under suitable conditions in work package 4. In addition, sea fennel seeds of the French Ecotype were provided by Dr. Christian Magne, our project partner from France. The seeds of French Ecotype "Atlantis" were received later than scheduled, on March 18. Immediately upon receipt of the seeds, on March 20, Atlantis was planted as a control genotype along with seeds collected from the four different locations of the Turkish flora. These seeds were planted in plastic seedbed trays filled with sterilized organic pits, with approximately 2,000 seeds planted from each ecotype, for a total of 8,000 seeds. The vials were irrigated with a sprinkler every day at 9:00-10:00 AM.



Planting of sea fennel seeds to the plastic viols filled with organic pit in 20th of March (left picture). Irrigation by a sprinkler head fixed garden pipe (right picture).

The first seeds germinated on April 11th 2023 and 57-66% of the seedlings had 1-2 true leaves by April 25th (Fig 2). According to the protocol prepared by the international consortium, the seedlings were transplanted into demo field after the appearance of 2-4 true leaves (May 2023).



Germinated seedlings in vials filled with organic peats. The pictures were taken on 25th April 2023.

Biostimulation: A bio-stimulant was applied to the seedlings at the beginning of the seeding process in the greenhouse. Half of the plots in the field trial will be treated with bio-fertilizer, while the other half will not receive any fertilizer application.

Plant Transplanting: The seedlings are scheduled to be transplanted to the field between May 8 and May 12. The field has already been prepared, soil samples have been collected, plots have been arranged, and the irrigation system has been installed.

Compared Plant Density: The experimental layout consisted of rows spaced 45 cm apart, with plants within each row spaced 22.5 cm apart (9.8 plants/m²).

Plot dimension: Each plot has a size of 1.8 x 5.0 meters, and there are a total of 36 plots.

Field experiment dimension: The total area of the experimental field is 390 m², of which 324 m² was used for planting.

Experimental design: The study incorporated two main treatment factors: (1) ecotype (ATL, CAN, and URL) and (2) fertilization (bio-fertilizer application versus control with no fertilization). A split-plot experimental design based on a randomized complete block (RCB) design with three replications was implemented. A biostimulant was applied to the seedlings at the early growth stage in the greenhouse. In the field trial, half of the plots received bio-fertilizer, while the other half remained untreated. The bio-fertilizer treatment consisted of a commercial organic fertilizer (Organomineral®) containing 12% N, 12% P₂O₅, and 12% K₂O, applied in comparison with a no-fertilization control.

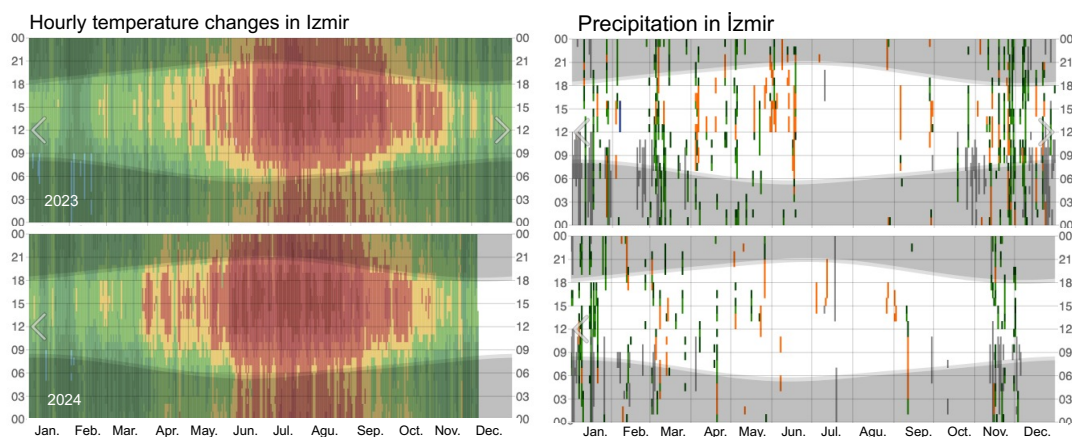


Transplanting of the sea fennel (*Crithmum maritimum* L.) seedlings to the field between 8-12 May 2023.

4.2 Fresh biomass (edible aerial parts) from the new organic sea fennel crops

Materials and methods

Plant sampling and harvesting were conducted on October 5. The aerial parts of sea fennel were separated into two categories: edible portions (sprouts, tender young leaves, and stems) and refuse (older leaves and fibrous stems). Both fractions were stored at +4°C immediately after harvesting for subsequent analysis and utilization.



Hourly average temperature and total precipitation during 2023 and 2024 at the experimental site (İzmir-Bornova).

Some plant samples were stored in liquid nitrogen and subsequently transferred to -70°C for further chemical analysis. For dry matter determination, plant material was dried in a forced draft oven at 105°C until a constant weight was achieved. Dry matter content was expressed as g per 100 g fresh weight (FW). Additionally, leaf area (m²) and color measurements were performed using the CIE Lab* color scale to assess lightness (L*), redness (a*), and yellowness (b*).

Several biochemical properties of sea fennel samples were analyzed using spectrophotometric methods (UV-VIS Cary 50 spectrophotometer), as high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS) were not available in the laboratory.



*Sampling of sea fennel (*Crithmum maritimum* L.) plants on October 5th (left photo). Storing plant samples in liquid nitrogen (right-down photo).*

Antioxidant activity was determined using the Ferric Reducing Antioxidant Power (FRAP) assay as described by Benzie and Strain (2009). Plant samples (0.5 g) were extracted with 20 mL of 80% (v/v) ethanol and centrifuged for 20 min at 4°C. The reaction was initiated by adding 0.05 mL of the extract to 1.50 mL of FRAP reagent and 0.15 mL of distilled water. Absorbance was measured spectrophotometrically at 593 nm, and results were expressed as μ moles FRAP per gram of dry matter.

Total flavonoid content was measured using the aluminum chloride colorimetric assay (Zhinsel et al., 1999). Extracts were treated with NaNO_2 , AlCl_3 , and NaOH solutions before incubation in darkness for 30 min. Absorbance was measured at 510 nm, with results expressed as mg rutin per gram of dry matter.

Antiradical activity was determined following the method of Brand-Williams et al. (1995). A 0.1 mL extract was mixed with 3.9 mL of DPPH• solution, and absorbance at 515 nm was recorded over 10 min. The efficient concentration (EC_{50}) was calculated as the amount of antioxidant required to reduce the initial DPPH• concentration by 50%.

Carotenoid analysis of sea fennel leaves was performed using a solvent extraction method followed by UV-Vis spectrometric detection, according to Biswas et al. (2011). All analyses were conducted using validated spectrophotometric techniques, ensuring reliable quantification of the biochemical parameters assessed in this study.

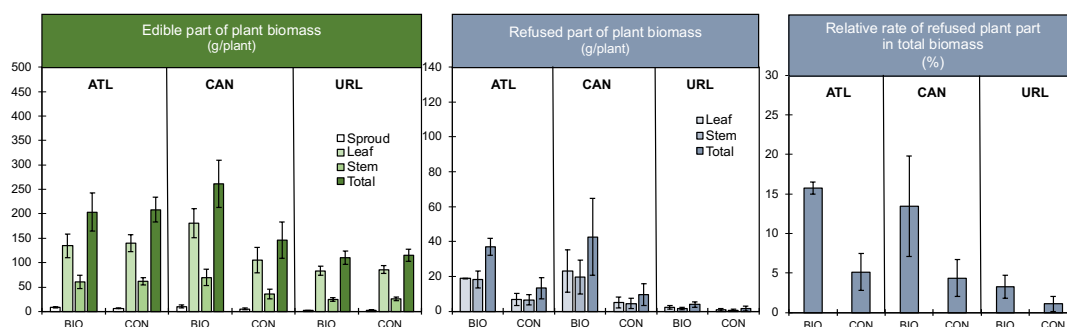
4.3 By-products (old leaves and fibrous stems) from the new organic sea fennel crops

Materials and methods

Sea fennel aerial parts were separated into “edible portion” (sprouts, tender young leaves, and stems) and “refuse” (older leaves and fibrous stems) categories, which were both stored at +4°C immediately after harvesting, prior to their analysis/use. Liquid nitrogen were used to store some of the sea fennel plant samples for further chemical analysis. Then the samples were stored in -70 °C. For each sample, a portion of the plant material was dried in a forced draft oven at 105 °C until reaching a constant weight for the determination of the dry matter content, which was expressed as g 100 g⁻¹ fresh weight (FW). Leaf area (m²) and CIE Lab colour was performed and the CIE L (lightness) a* (redness) b* (yellowness) color scale.

Results

Effect of bio-fertilizer application versus no fertilization on the biomass distribution of sea fennel in 2023, including edible and refused (leaf, stem, and total) plant fractions, as well as the relative proportion of refused plant parts in the total biomass of three populations, are shown in Figure below. Considering the average biomass values of the edible parts of all three populations, a 19% increase was observed in the plots with biological fertilizer application, yielding 191 g/plant, compared to the 160 g/plant from the control plots. When considering the refused parts of the plant, the effect of biological fertilization was more pronounced, resulting in a 3.5-fold increase compared to control plots. The biomass of the refused parts was 28 g/plant in the biologically fertilized plots, compared to 8 g/plant in the unfertilized plots.

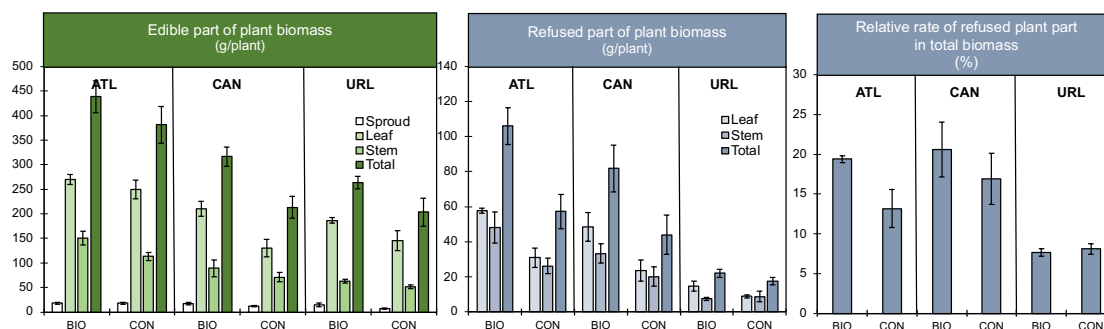


Effect of bio-fertilizer application versus no fertilization on the biomass distribution of sea fennel (*Crithmum maritimum* L.) in 2023, including edible (sprout, leaf, stem, and total) and refused (leaf, stem, and total) plant fractions, as well as the relative proportion of refused plant parts in the total biomass of three populations (ATL: Atlantis, CAN: Çandarlı, and URL: Urla).

The highest biomass values were recorded in the ATL and CAN populations, with 206 g/plant and 204 g/plant, respectively, followed by the URL population with 130 g/plant. The lowest proportion of refused parts was observed in the URL population (2%). Moreover, biological fertilization increased the ratio of refused plant parts in the total biomass by an average of 7%.

In the second year of the field trial (2024), an average 68% increase in the edible biomass was recorded. The effect of biological fertilizer application was further enhanced, with 340 g/plant of edible biomass obtained in fertilized plots and 266 g/plant in unfertilized plots, resulting in a 28% increase due to fertilization. On the other hand, the biomass of the refused plant parts increased by 75% in the fertilized plots, reaching 70 g/plant, compared to 40 g/plant in the unfertilized plots.

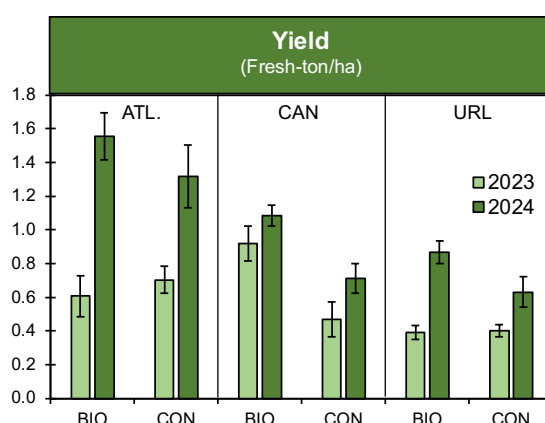
The highest biomass value in 2024 was recorded in the ATL population, with 410 g/plant, followed by the CAN and URL populations with 265 g/plant and 234 g/plant, respectively. The lowest proportion of refused parts was observed in the URL population (8%). Additionally, biological fertilization increased the ratio of refused plant parts in total biomass by an average of 3%.



Effect of bio-fertilizer application versus no fertilization on the biomass distribution of sea fennel (*Crithmum maritimum* L.) in 2024, including edible (sprout, leaf, stem, and total) and refused (leaf, stem, and total) plant fractions, as well as the relative proportion of refused plant parts in the total biomass of three populations (ATL: Atlantis, CAN: Çandarlı, and URL: Urla).

According to the results from the field trials, the average fresh yield in 2023 was recorded at 0.58 tons/ha, with a 76% increase in 2024, reaching 1.02 tons/ha. When considering the average yields over both trial years, the ATL population exhibited the highest yield at 1.05 tons/ha, followed by the CAN and URL populations with yields of 0.80 tons/ha and 0.57 tons/ha, respectively.

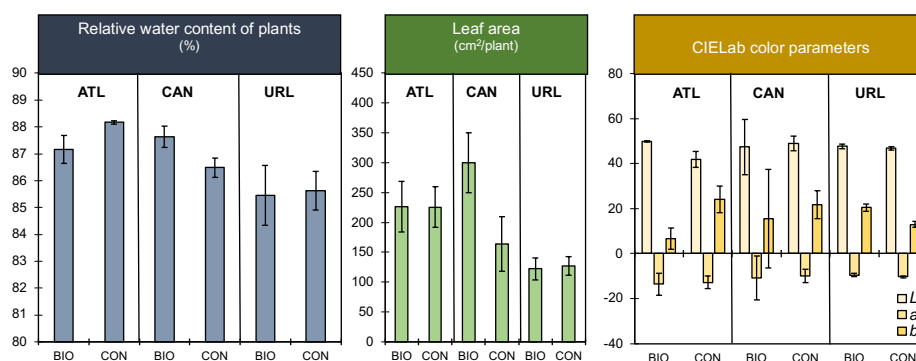
In 2023, biological fertilization resulted in a significant yield increase of 96% in the CAN population, whereas no notable effect was observed in the other populations. In 2024, biological fertilization positively impacted all populations, with an average yield increase of 32%.



Effect of bio-fertilizer application versus no fertilization on the fresh yield of three sea fennel (*Crithmum maritimum* L.) populations (ATL: Atlantis, CAN: Çandarlı, and URL: Urla) during the 2023 and 2024 growing seasons.

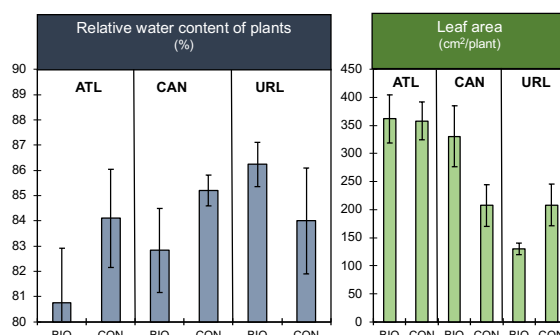
The average relative water content (RWC) of the harvested sea fennel plants in 2023 was 87%, with no statistically significant differences observed between the populations in terms of RWC. In the same year, the average leaf area did not exhibit significant variation between the ATL and CAN populations, which measured 226 cm² and 232 cm², respectively. However, the leaf area of the URL population was significantly lower than the other two, at 125 cm². Additionally, bio-

fertilization led to an 83% increase in the leaf area of the CAN population. According to the results obtained from the CIELab color analysis, the average L (lightness) value of sea fennel plants was recorded as 47.1. The highest L value was observed in the ATL population treated with biological fertilizer (BIO), at 49.9, while the lowest value was obtained from the ATL population under control conditions (CON), at 41.9.



Effect of bio-fertilizer application versus no fertilization on relative water content of plants, leaf area and CIELab color of three sea fennel (*Crithmum maritimum* L.) populations (ATL: Atlantis, CAN: Çandarlı, and URL: Urla) in 2023.

Regarding the a (redness/greenness) values, the ATL population with BIO treatment exhibited the lowest value (-13.5), indicating greater greenness, whereas the URL population with BIO treatment showed the highest value (-9.5). As for the b (yellowness) values, the highest measurement (24.1) was obtained from the ATL population under control conditions (CON), whereas the lowest measurement (6.7) was recorded for the ATL population under BIO treatment. Overall, biological fertilizer application did not result in consistent or significant changes across the CIELab color parameters in sea fennel populations.



Effect of bio-fertilizer application versus no fertilization on relative water content of plants and leaf area of three sea fennel (*Crithmum maritimum* L.) populations (ATL: Atlantis, CAN: Çandarlı, and URL: Urla) in 2023.

In the second year of the study (2024), the relative water content (RWC) of sea fennel plants decreased by 3.5% compared to the first year, reaching 84%. However, greater variation was observed between populations and treatments. The highest RWC value was recorded in the URL population treated with biological fertilizer at 86%, while the lowest RWC was observed in the ATL population under the same treatment at 81%. Leaf area measurements showed a similar trend to the first year, with the highest values recorded in the ATL and CAN populations, reaching 360 cm² and 270 cm², respectively. The leaf area of the URL population was lower, measured at 169 cm².

According to the biochemical analyses of sea fennel populations, total antioxidant activity (FRAP) values ranged from 112 to 177 $\mu\text{mol FRAP/g DW}$ (Table 2). The highest antioxidant activity was recorded in the flowers samples of URL population (URL-F) (177 $\mu\text{mol FRAP/g DW}$), while the lowest was in leaf samples of CAN population (CAN-L) (112 $\mu\text{mol FRAP/g DW}$). Total flavonoid content varied significantly among samples, with the highest value observed in leaf samples of ATL population (ATL-F) (11.2 mg/g DW) and the lowest in CAN-L (2.71 mg/g DW).

Antiradical activity (DPPH) ranged from 66 to 265 $\mu\text{g/mL}$. The lowest IC₅₀ value, indicating the highest antiradical activity, was observed in leaf samples of URL population (URL-L) (66 $\mu\text{g/mL}$), while the highest IC₅₀ was recorded in URL-F (265 $\mu\text{g/mL}$). Total carotenoid content was highest in URL-L (113.1 mg/g DW) and lowest in flower samples of CAN population (CAN-F) (28.6 mg/g DW).

Total antioxidant activity, total flavonoids, antiradical activity, total carotenoids, total phenolic compounds, total tocopherol and vitamin C content of leaves (L) and flowers (F) of three sea fennel (Crithmum maritimum L.) populations (ATL: Atlantis, CAN: Çandarlı, and URL: Urla).

Ecotypes	[1] Total antioxidant activity $\mu\text{mol FRAP/g DW}$	[2] Total flavonoids mg/g DW	[3] Antiradical activity DPPH as IC ₅₀ , $\mu\text{g/mL}$	[4] Total carotenoids mg/g DW	[5] Total phenolic compounds mg/g DW	[6] Total tocopherol mg/kg DW	[7] Vitamin C mg/g DW
ATL-L	172.5 $\pm$ 28.2	2.89 $\pm$ 0.88	136.0 $\pm$ 23.3	86.2 $\pm$ 22.5	6.12 $\pm$ 2.13	369 $\pm$ 19.4	1.72 $\pm$ 0.19
ATL-F	146.5 $\pm$ 32.1	11.2 $\pm$ 1.66	243.8 $\pm$ 40.6	47.7 $\pm$ 13.6	16.2 $\pm$ 3.18	23.9 $\pm$ 8.8	1.80 $\pm$ 0.09
CAN-L	112.3 $\pm$ 24.1	2.71 $\pm$ 1.04	87.7 $\pm$ 18.2	48.3 $\pm$ 17.4	2.10 $\pm$ 0.98	218 $\pm$ 15.7	1.19 $\pm$ 0.24
CAN-F	154.0 $\pm$ 17.0	6.03 $\pm$ 1.12	220.1 $\pm$ 27.0	28.6 $\pm$ 12.7	10.8 $\pm$ 2.44	44.0 $\pm$ 3.45	0.92 $\pm$ 0.16
URL-L	163.8 $\pm$ 18.8	3.44 $\pm$ 1.29	66.3 $\pm$ 12.4	113.1 $\pm$ 33.6	2.84 $\pm$ 0.34	143 $\pm$ 16.6	2.04 $\pm$ 0.37
URL-F	177.2 $\pm$ 27.3	3.82 $\pm$ 0.78	265.1 $\pm$ 31.5	50.4 $\pm$ 12.7	8.79 $\pm$ 2.05	14.9 $\pm$ 3.02	1.86 $\pm$ 0.11

[1]AO-Benzie and Strain (2009), [2]FLV-Zhishen et al. (1999), [3]AR-Brand-Williams et al. (1995), [4]TC-Arnon and Copper (1949), [5]TPC-Singleton et al.(1999), [6]TTC-Biswas et al (2011), [7]VC-Asghari et al. (2015)

Total phenolic compounds ranged from 2.10 to 16.2 mg/g DW . ATL-F had the highest phenolic content (16.2 mg/g DW), whereas CAN-L had the lowest (2.10 mg/g DW). Tocopherol content varied significantly, with ATL-L showing the highest level (369 mg/kg DW) and URL-F the lowest (14.9 mg/kg DW). Vitamin C content ranged from 0.92 to 2.04 mg/g DW . The highest vitamin C content was detected in URL-L (2.04 mg/g DW), while the lowest was in CAN-F (0.92 mg/g DW).

Overall, biochemical profiles varied notably across ecotypes and plant parts, indicating both genetic and treatment-related differences in the accumulation of bioactive compounds.

4.3.1 Contributions on objectives of the work package

Within the scope of WP4, the Turkish research team contributed to developing a sustainable production protocol by establishing a demonstration field trial with sea fennel. Their efforts focused on a detailed evaluation of yield and quality parameters across different plant parts and the formulation of recommendations and guidelines for potential growers. In line with the objectives of the work package, the Turkish partners supported tasks related to the sustainable production of sea fennel in the demonstration field, the analysis of newly cultivated sea fennel crops, and statistical evaluations (Tasks 1, 2, and 4).

4.3.2 Research activities

Research activities were conducted in the experimental field and greenhouse of Ege University, Faculty of Agriculture, Department of Field Crops, located in Bornova-Izmir, Türkiye (38°27'06.0"N 27°13'31.9"E). The study focused on the sustainable cultivation of sea fennel (*Crithmum maritimum* L.), utilizing the Atlantis (ATL) ecotype as the reference genotype, along with two Turkish ecotypes, Çandarlı (CAN) and Urla (URL), selected based on seedling performance. The French ecotype ATL was provided by Dr. Christian Magne, a project partner from France. On March 20, ATL was sown as the control genotype, alongside seeds collected from four different locations in Türkiye. The seeds were planted in plastic seedbed trays filled with sterilized organic peat, with approximately 2,000 seeds per ecotype, totaling 8,000 seeds. The seedlings were irrigated daily using a sprinkler system between 9:00 and 10:00 AM (Fig. 1).



Figure 1. Planting of sea fennel seeds to the plastic viols filled with organic pit in 20th of March (left picture). Irrigation by a sprinkler head fixed garden pipe (right picture).

Prior to transplantation, seedling emergence rates were recorded as follows: 1,115 ATL seedlings (56.9%), 1,395 CAN seedlings (66.4%), and 1,224 URL seedlings (63.5%). The first germination was observed on April 11, 2023, and by April 25, 57–66% of the seedlings had developed 1–2 true leaves (Fig. 2). In accordance with the international consortium's protocol, seedlings were transplanted to the demonstration field upon developing 2–4 true leaves (May 2023). Transplantation was scheduled between May 8 and May 12. By this time, the field had been fully prepared, soil samples had been collected, plots were arranged, and the irrigation system had been installed.



Figure 2. Germinated seedlings of sea fennel (*Crithmum maritimum* L.) in vials filled with organic peats. The photos were taken on 25th April 2023.

Soil bed preparation followed conventional tillage practices, including deep ploughing to a depth of 35–40 cm in the fall, followed by soil bed formation using a cultivator prior to transplantation. The experimental layout consisted of rows spaced 45 cm apart, with plants within each row spaced 22.5 cm apart (9.8 plants/m²). Each plot measured 1.8 × 5.0 meters, with a total of 36 plots. The experimental field covered 390 m², of which 324 m² was used for planting.



Transplanting of the sea fennel (*Crithmum maritimum* L.) seedlings to the field between 8-12 May 2023.

The study incorporated two main treatment factors: (1) ecotype (ATL, CAN, and URL) and (2) fertilization (bio-fertilizer application versus control with no fertilization). A split-plot experimental design based on a randomized complete block (RCB) design with three replications was implemented. A biostimulant was applied to the seedlings at the early growth stage in the greenhouse. In the field trial, half of the plots received bio-fertilizer, while the other half remained untreated.

The bio-fertilizer treatment consisted of a commercial organic fertilizer (Organomineral®) containing 12% N, 12% P₂O₅, and 12% K₂O, applied in comparison with a no-fertilization control.

Soil

pH	7.88
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Fe (ppm)	0.18
Cu (ppm)	1.10
Mg (ppm)	367
Zn (ppm)	1.95
Na (ppm)	73
Mn (ppm)	1.17

Table 1. Physicochemical properties of the soil sampled from the experimental site in Izmir. The samples were collected before the sea fennel seedlings transplanted to the demo field trail (2023).

Soil samples (0–30 cm depth) were collected from the experimental site before transplanting. The samples were analyzed for various physicochemical properties, including soil texture, pH, organic matter content, electrical conductivity, total nitrogen (N), available phosphorus (P), exchangeable calcium (Ca), potassium (K), sodium (Na), iodine (I), magnesium (Mg), and major anions (Table 1). Throughout the growing period, irrigation was carried out using a drip irrigation system. Field observations confirmed that sea fennel plants were unable to survive under rainfed conditions at the experimental site. Weed management was performed through mechanical removal, targeting *Sorghum halepense* L., the predominant weed species in the field. Hourly average temperature and total precipitation data for 2023 and 2024 at the experimental site (İzmir-Bornova) are presented in Fig. 4. Mean temperatures in 2024 were recorded as 1.2°C (7.2%) higher than in 2023. Total precipitation in 2023 was 780 mm higher than long-term averages, while precipitation levels in 2024 were projected to be at least 20% lower by the end of the year.

Plant sampling and harvesting were conducted on October 5 (Fig. 5). The aerial parts of sea fennel were separated into two categories: edible portions (sprouts, tender young leaves, and stems) and refuse (older leaves and fibrous stems). Both fractions were stored at +4°C immediately after harvesting for subsequent analysis and utilization.

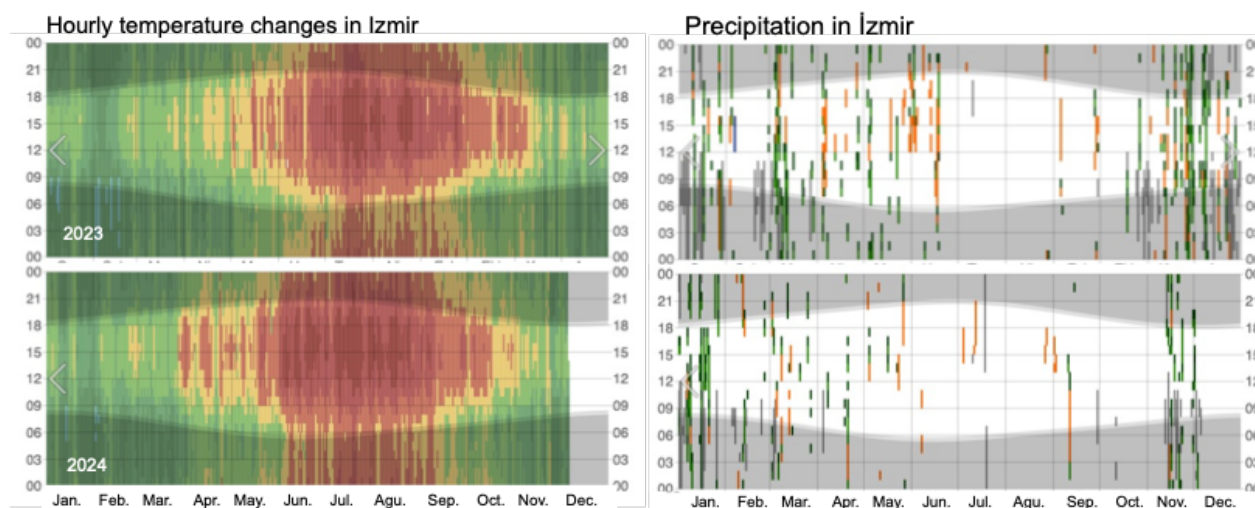


Figure 4. Hourly average temperature and total precipitation during 2023 and 2024 at the experimental site (Izmir-Bornova).

Some plant samples were stored in liquid nitrogen and subsequently transferred to -70°C for further chemical analysis. For dry matter determination, plant material was dried in a forced draft oven at 105°C until a constant weight was achieved. Dry matter content was expressed as g per 100 g fresh weight (FW). Additionally, leaf area (m^2) and color measurements were performed using the CIE Lab* color scale to assess lightness (L^*), redness (a^*), and yellowness (b^*).

Several biochemical properties of sea fennel samples were analyzed using spectrophotometric methods (UV-VIS Cary 50 spectrophotometer), as high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS) were not available in the laboratory.



Figure 5. Sampling of sea fennel (*Crithmum maritimum* L.) plants on October 5th (left photo). Storing plant samples in liquid nitrogen (right-down photo).

Antioxidant activity was determined using the Ferric Reducing Antioxidant Power (FRAP) assay as described by Benzie and Strain (2009). Plant samples (0.5 g) were extracted with 20 mL of 80% (v/v) ethanol and centrifuged for 20 min at 4°C. The reaction was initiated by adding 0.05 mL of the extract to 1.50 mL of FRAP reagent and 0.15 mL of distilled water. Absorbance was measured spectrophotometrically at 593 nm, and results were expressed as μ moles FRAP per gram of dry matter.

Total flavonoid content was measured using the aluminum chloride colorimetric assay (Zhinsel et al., 1999). Extracts were treated with NaNO_2 , AlCl_3 , and NaOH solutions before incubation in darkness for 30 min. Absorbance was measured at 510 nm, with results expressed as mg rutin per gram of dry matter.

Antiradical activity was determined following the method of Brand-Williams et al. (1995). A 0.1 mL extract was mixed with 3.9 mL of DPPH• solution, and absorbance at 515 nm was recorded over 10 min. The efficient concentration (EC_{50}) was calculated as the amount of antioxidant required to reduce the initial DPPH• concentration by 50%.

Carotenoid analysis of sea fennel leaves was performed using a solvent extraction method followed by UV-Vis spectrometric detection, according to Biswas et al. (2011). All analyses were conducted using validated spectrophotometric techniques, ensuring reliable quantification of the biochemical parameters assessed in this study.

4.3.3 Findings

Effect of bio-fertilizer application versus no fertilization on the biomass distribution of sea fennel in 2023, including edible (leaf, stem, and total) plant fractions, as well as the relative proportion of refused plant parts in the total biomass of three populations, are shown in Fig. 6. Considering the average biomass values of the edible parts of all three populations, a 19% increase was observed in the plots with biological fertilizer application, yielding 191 g/plant, compared to the 160 g/plant from the control plots. When considering the refused parts of the plant, the effect of biological fertilization was more pronounced, resulting in a 3.5-fold increase compared to control plots. The biomass of the refused parts was 28 g/plant in the biologically fertilized plots, compared to 8 g/plant in the unfertilized plots.

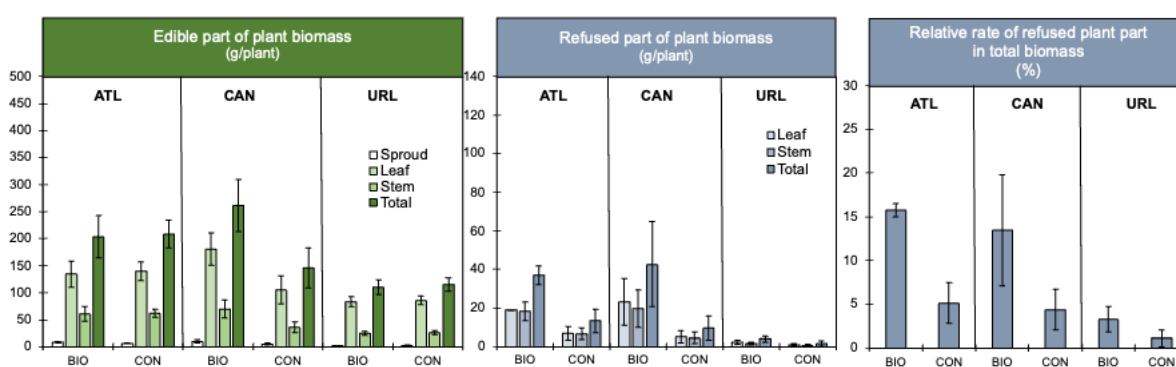


Figure 6. Effect of bio-fertilizer application versus no fertilization on the biomass distribution of sea fennel (*Crithmum maritimum* L.) in 2023, including edible (sprout, leaf, stem, and total) and refused (leaf, stem, and total) plant fractions, as well as the relative proportion of refused plant parts in the total biomass of three populations (ATL: Atlantis, CAN: Çandarlı, and URL: Urla).

The highest biomass values were recorded in the ATL and CAN populations, with 206 g/plant and 204 g/plant, respectively, followed by the URL population with 130 g/plant (Fig. 6). The lowest proportion of refused parts was observed in the URL population (2%). Moreover, biological fertilization increased the ratio of refused plant parts in the total biomass by an average of 7%.

In the second year of the field trial (2024), an average 68% increase in the edible biomass was recorded (Fig. 7). The effect of biological fertilizer application was further enhanced, with 340 g/plant of edible biomass obtained in fertilized plots and 266 g/plant in unfertilized plots, resulting in a 28% increase due to fertilization. On the other hand, the biomass of the refused plant parts increased by 75% in the fertilized plots, reaching 70 g/plant, compared to 40 g/plant in the unfertilized plots.

The highest biomass value in 2024 was recorded in the ATL population, with 410 g/plant, followed by the CAN and URL populations with 265 g/plant and 234 g/plant, respectively (Fig. 7). The lowest proportion of refused parts was observed in the URL population (8%). Additionally, biological fertilization increased the ratio of refused plant parts in total biomass by an average of 3%.

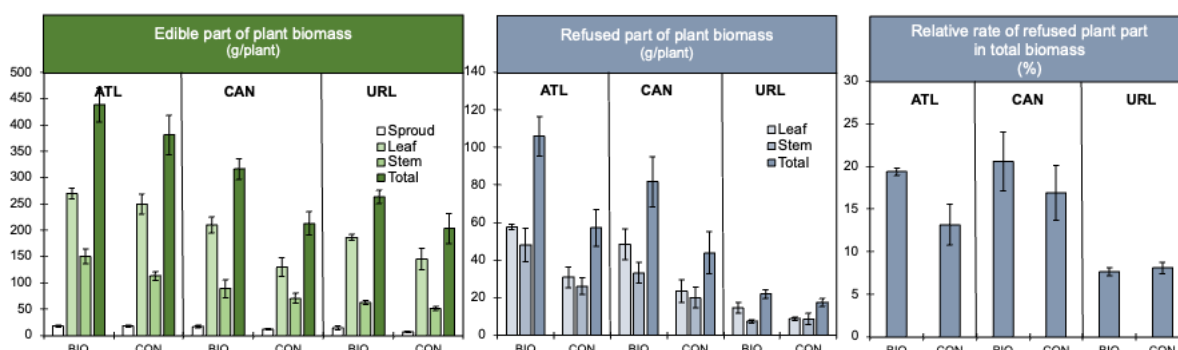


Figure 7. Effect of bio-fertilizer application versus no fertilization on the biomass distribution of sea fennel (*Crithmum maritimum* L.) in 2024, including edible (sprout, leaf, stem, and total) and refused (leaf, stem, and total) plant fractions, as well as the relative proportion of refused plant parts in the total biomass of three populations (ATL: Atlantis, CAN: Çandarlı, and URL: Urla).

According to the results from the field trials, the average fresh yield in 2023 was recorded at 0.58 tons/ha, with a 76% increase in 2024, reaching 1.02 tons/ha (Fig. 8). When considering the average yields over both trial years, the ATL population exhibited the highest yield at 1.05 tons/ha, followed by the CAN and URL populations with yields of 0.80 tons/ha and 0.57 tons/ha, respectively.

In 2023, biological fertilization resulted in a significant yield increase of 96% in the CAN population, whereas no notable effect was observed in the other populations (Fig. 8). In 2024, biological fertilization positively impacted all populations, with an average yield increase of 32%.

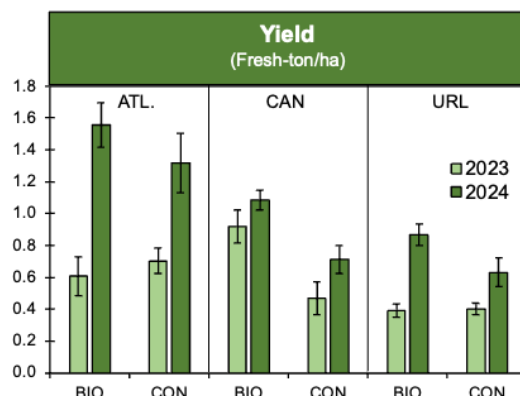


Figure 8. Effect of bio-fertilizer application versus no fertilization on the fresh yield of three sea fennel (*Crithmum maritimum* L.) populations (ATL: Atlantis, CAN: Çandarlı, and URL: Urla) during the 2023 and 2024 growing seasons.

The average relative water content (RWC) of the harvested sea fennel plants in 2023 was 87%, with no statistically significant differences observed between the populations in terms of RWC (Fig. 9). In the same year, the average leaf area did not exhibit significant variation between the ATL and CAN populations, which measured 226 cm² and 232 cm², respectively. However, the leaf area of the URL population was significantly lower than the other two, at 125 cm². Additionally, biofertilization led to an 83% increase in the leaf area of the CAN population. According to the results obtained from the CIELab color analysis, the average L (lightness) value of sea fennel plants was recorded as 47.1. The highest L value was observed in the ATL population treated with biological fertilizer (BIO), at 49.9, while the lowest value was obtained from the ATL population under control conditions (CON), at 41.9.

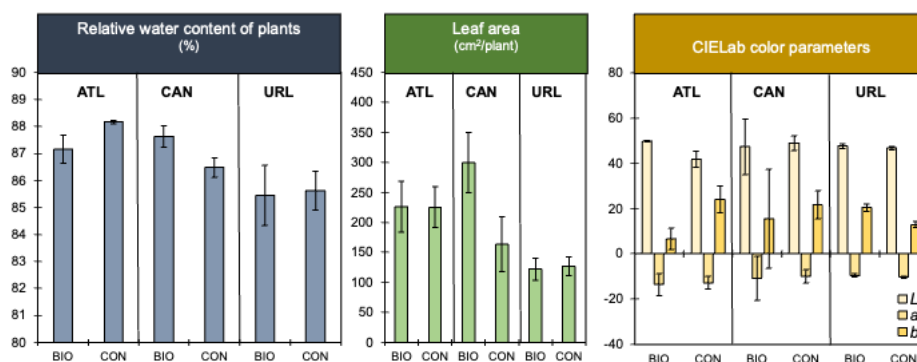


Figure 9. Effect of bio-fertilizer application versus no fertilization on relative water content of plants, leaf area and CIELab color of three sea fennel (*Crithmum maritimum* L.) populations (ATL: Atlantis, CAN: Çandarlı, and URL: Urla) in 2023.

Regarding the a (redness/greenness) values, the ATL population with BIO treatment exhibited the lowest value (-13.5), indicating greater greenness, whereas the URL population with BIO treatment showed the highest value (-9.5) (Fig 9). As for the b (yellowness) values, the highest measurement (24.1) was obtained from the ATL population under control conditions (CON), whereas the lowest measurement (6.7) was recorded for the ATL population under BIO treatment. Overall, biological fertilizer application did not result in consistent or significant changes across the CIELab color parameters in sea fennel populations.

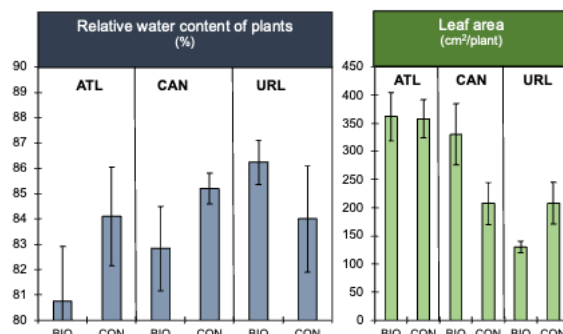


Figure 10. Effect of bio-fertilizer application versus no fertilization on relative water content of plants and leaf area of three sea fennel (*Crithmum maritimum* L.) populations (ATL: Atlantis, CAN: Çandarlı, and URL: Urla) in 2023.

In the second year of the study (2024), the relative water content (RWC) of sea fennel plants decreased by 3.5% compared to the first year, reaching 84% (Fig. 10). However, greater variation was observed between populations and treatments. The highest RWC value was recorded in the URL population treated with biological fertilizer at 86%, while the lowest RWC was observed in the ATL population under the same treatment at 81%. Leaf area measurements showed a similar trend to the first year, with the highest values recorded in the ATL and CAN populations, reaching 360 cm² and 270 cm², respectively. The leaf area of the URL population was lower, measured at 169 cm².

According to the biochemical analyses of sea fennel populations, total antioxidant activity (FRAP) values ranged from 112 to 177 µmol FRAP/g DW (Table 2). The highest antioxidant activity was recorded in the flowers samples of URL population (URL-F) (177 µmol FRAP/g DW), while the lowest was in leaf samples of CAN population (CAN-L) (112 µmol FRAP/g DW). Total flavonoid content varied significantly among samples, with the highest value observed in leaf samples of ATL population (ATL-F) (11.2 mg/g DW) and the lowest in CAN-L (2.71 mg/g DW).

Antiradical activity (DPPH) ranged from 66 to 265 µg/mL (Table 2). The lowest IC₅₀ value, indicating the highest antiradical activity, was observed in leaf samples of URL population (URL-L) (66 µg/mL), while the highest IC₅₀ was recorded in URL-F (265 µg/mL). Total carotenoid content was highest in URL-L (113.1 mg/g DW) and lowest in flower samples of CAN population (CAN-F) (28.6 mg/g DW).

Total antioxidant activity, total flavonoids, antiradical activity, total carotenoids, total phenolic compounds, total tocopherol and vitamin C content of leaves (L) and flowers (F) of three sea fennel (*Crithmum maritimum* L.) populations (ATL: Atlantis, CAN: Çandarlı, and URL: Urla).

Ecotypes	[1] Total antioxidant activity $\mu\text{mol FRAP/g DW}$	[2] Total flavonoids mg/g DW	[3] Antiradical activity DPPH as IC_{50} , $\mu\text{g/ml}$	[4] Total carotenoids mg/g DW	[5] Total phenolic compounds mg/g DW	[6] Total tocopherol mg/kg DW	[7] Vitamin C mg/g DW
ATL-L	172.5 $\pm$ 28.2	2.89 $\pm$ 0.88	136.0 $\pm$ 23.3	86.2 $\pm$ 22.5	6.12 $\pm$ 2.13	369 $\pm$ 19.4	1.72 $\pm$ 0.19
ATL-F	146.5 $\pm$ 32.1	11.2 $\pm$ 1.66	243.8 $\pm$ 40.6	47.7 $\pm$ 13.6	16.2 $\pm$ 3.18	23.9 $\pm$ 8.8	1.80 $\pm$ 0.09
CAN-L	112.3 $\pm$ 24.1	2.71 $\pm$ 1.04	87.7 $\pm$ 18.2	48.3 $\pm$ 17.4	2.10 $\pm$ 0.98	218 $\pm$ 15.7	1.19 $\pm$ 0.24
CAN-F	154.0 $\pm$ 17.0	6.03 $\pm$ 1.12	220.1 $\pm$ 27.0	28.6 $\pm$ 12.7	10.8 $\pm$ 2.44	44.0 $\pm$ 3.45	0.92 $\pm$ 0.16
URL-L	163.8 $\pm$ 18.8	3.44 $\pm$ 1.29	66.3 $\pm$ 12.4	113.1 $\pm$ 33.6	2.84 $\pm$ 0.34	143 $\pm$ 16.6	2.04 $\pm$ 0.37
URL-F	177.2 $\pm$ 27.3	3.82 $\pm$ 0.78	265.1 $\pm$ 31.5	50.4 $\pm$ 12.7	8.79 $\pm$ 2.05	14.9 $\pm$ 3.02	1.86 $\pm$ 0.11

[1]AO-Benzie and Strain (2009), [2]FLV-Zhishen et al. (1999), [3]AR-Brand-Williams et al. (1995), [4]TC-Arnon and Copper (1949), [5]TPC-Singleton et al.(1999), [6]TTC-Biswas et al (2011), [7]VC-Asghari et al. (2015)

Total phenolic compounds ranged from 2.10 to 16.2 mg/g DW. ATL-F had the highest phenolic content (16.2 mg/g DW), whereas CAN-L had the lowest (2.10 mg/g DW) (Table 2). Tocopherol content varied significantly, with ATL-L showing the highest level (369 mg/kg DW) and URL-F the lowest (14.9 mg/kg DW). Vitamin C content ranged from 0.92 to 2.04 mg/g DW. The highest vitamin C content was detected in URL-L (2.04 mg/g DW), while the lowest was in CAN-F (0.92 mg/g DW).

Overall, biochemical profiles varied notably across ecotypes and plant parts, indicating both genetic and treatment-related differences in the accumulation of bioactive compounds.

4.3.4 Recommendations and guidelines

Based on the outcomes of the demo field trials and the comprehensive laboratory analyses, the following recommendations and guidelines are proposed to support the sustainable production of organic sea fennel crops. These guidelines are intended to serve as a practical reference for growers, agronomists, and research teams striving to optimize production under organic conditions.

Seedling propagation

For optimal seedling development, it is advisable to sow sea fennel seeds in late February or early March. This timing allows seedlings to reach an optimal growth stage by mid-April, which has been identified as the best period for transplanting into the field. Transplanting at this time ensures that the young plants are vigorous enough to establish well in the field environment, while still avoiding the high temperature ranges that occur later in the season. Sowing after April (to seedbeds) is considered risky because the region experiences significantly higher temperatures during that period, a risk that is further compounded by ongoing climate change. In cases where sowing is delayed, it is crucial to initiate irrigation early to mitigate the adverse effects of heat stress on the seedlings.

Sustainable Sea Fennel Production Overview

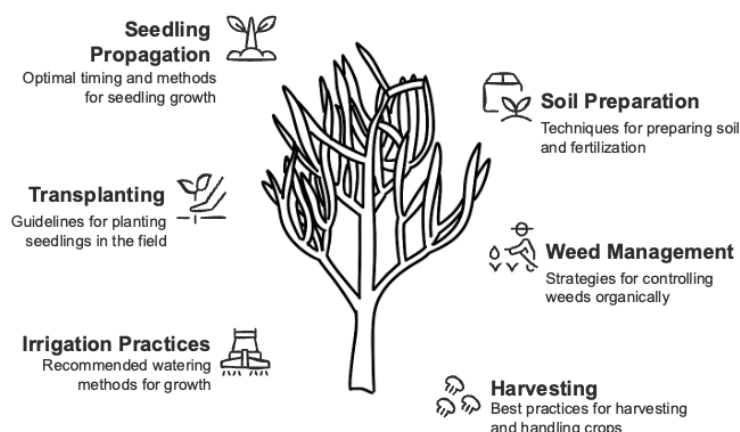


Figure 11. Sustainable sea fennel production overview scheme

An alternative approach that has shown promising results in preliminary trials is direct sowing in the field. Direct sowing could be particularly effective if implemented at the end of January or the beginning of February, as these dates may offer a more favorable microclimate for seed germination and early seedling establishment before the onset of warmer conditions. Both propagation methods, nursery-based propagation with subsequent transplantation and direct field sowing, have their advantages. Nursery propagation offers greater control over the early growth environment, while direct sowing may reduce labor and infrastructure costs and simplify operations.

Thus, growers are encouraged to consider the local climatic conditions and resource availability when selecting the most appropriate propagation method. Detailed field trials and further research will be necessary to refine these recommendations and optimize sea fennel seedling propagation strategies under varying environmental conditions.

Soil preparation and fertilization

Given the vigorous root system of sea fennel, extensive soil preparation is not necessary. Instead, a shallow ploughing to a depth of around 30 cm is sufficient to loosen the soil adequately for transplanting. This limited soil disturbance not only facilitates proper seedling establishment but also supports in water conservation, which is crucial for sustainable production. Following the ploughing, thorough soil bed formation using a cultivator is recommended to create an optimal seedbed that minimizes transplant shock and supports early root development.

In terms of fertilization, a variety of organic fertilizer options are available. Organic manures, in particular, serve as an excellent alternative to synthetic fertilizers. Based on field trials and current agronomic practices, it is recommended to apply 40–60 kg/ha of nitrogen, 30–40 kg/ha phosphorus and potassium to meet the nutritional requirements of sea fennel. This rate is sufficient to promote vigorous growth without risking nutrient leaching or soil degradation. The combination of minimal soil disturbance with targeted organic fertilization not only enhances crop performance but also contributes to the long-term sustainability and health of the soil, ensuring that water is conserved and the ecosystem is maintained.

Transplanting and field establishment

Timely transplantation is critical to ensuring vigorous plant development and successful establishment in the field. Seedlings should ideally be transplanted once they have developed 2-4 true leaves, as research has identified this developmental stage as optimal for minimizing transplant shock and promoting rapid root establishment. The standard recommended field layout involves spacing rows 45 cm apart and positioning individual plants 22.5 cm apart within each row, yielding approximately 9.8 plants/m². This configuration is designed to minimize intra-specific competition while optimizing resource use, such as water and nutrients.

However, adjustments to these distances may be acceptable based on seed or seedling availability and specific site conditions. In cases where a higher seedling density is available, or where improved land cover is desired to enhance weed suppression, reduced spacing may be beneficial. Field trials suggest that spacing of 35-40 cm between rows combined with 15-20 cm within rows can offer advantages in terms of better ground cover and improved weed control, especially after the initial establishment period. These alternative spacings should be tested under local conditions to determine their efficacy in balancing crop competition with resource optimization and weed management.

In summary, while the conventional layout provides a reliable framework for sea fennel establishment, growers are encouraged to consider adaptive spacing strategies that reflect local seedling vigor, weed pressure, and overall management objectives, thereby ensuring the most effective establishment and sustained productivity of the crop.

Weed management

Effective weed control is crucial for reducing competition and promoting healthy crop growth. Mechanical removal is recommended as the primary weed management strategy, specifically targeting problematic species such as *Sorghum halepense* L. Integrating mulching and other non-chemical methods can further support organic production standards.

Irrigation practices

Sea fennel is inherently a halophyte, well adapted to saline and water-limited environments, which enables it to succeed in naturally harsh conditions. However, in locations such as the western coastal regions of Türkiye, supplemental irrigation is necessary to optimize growth and yield, as natural precipitation may not suffice. It is important to strike a balance, as excessive irrigation can reduce the concentration of aromatic compounds that are key to the plant's value.

Based on field observations and agronomic trials, a total of 100 mm of supplemental irrigation, applied in two equal doses of 50 mm during the critical growth months of June and July, is recommended to support optimal yield without compromising the plant's aromatic quality. This irrigation schedule ensures that sea fennel receives adequate moisture during its peak growth phase, while minimizing the risk of over-irrigation, which could otherwise inhibit the synthesis of essential aromatic and bioactive compounds. By carefully managing water input in this manner, growers can achieve a sustainable balance between maximizing biomass production and maintaining the desirable qualitative attributes of the crop.

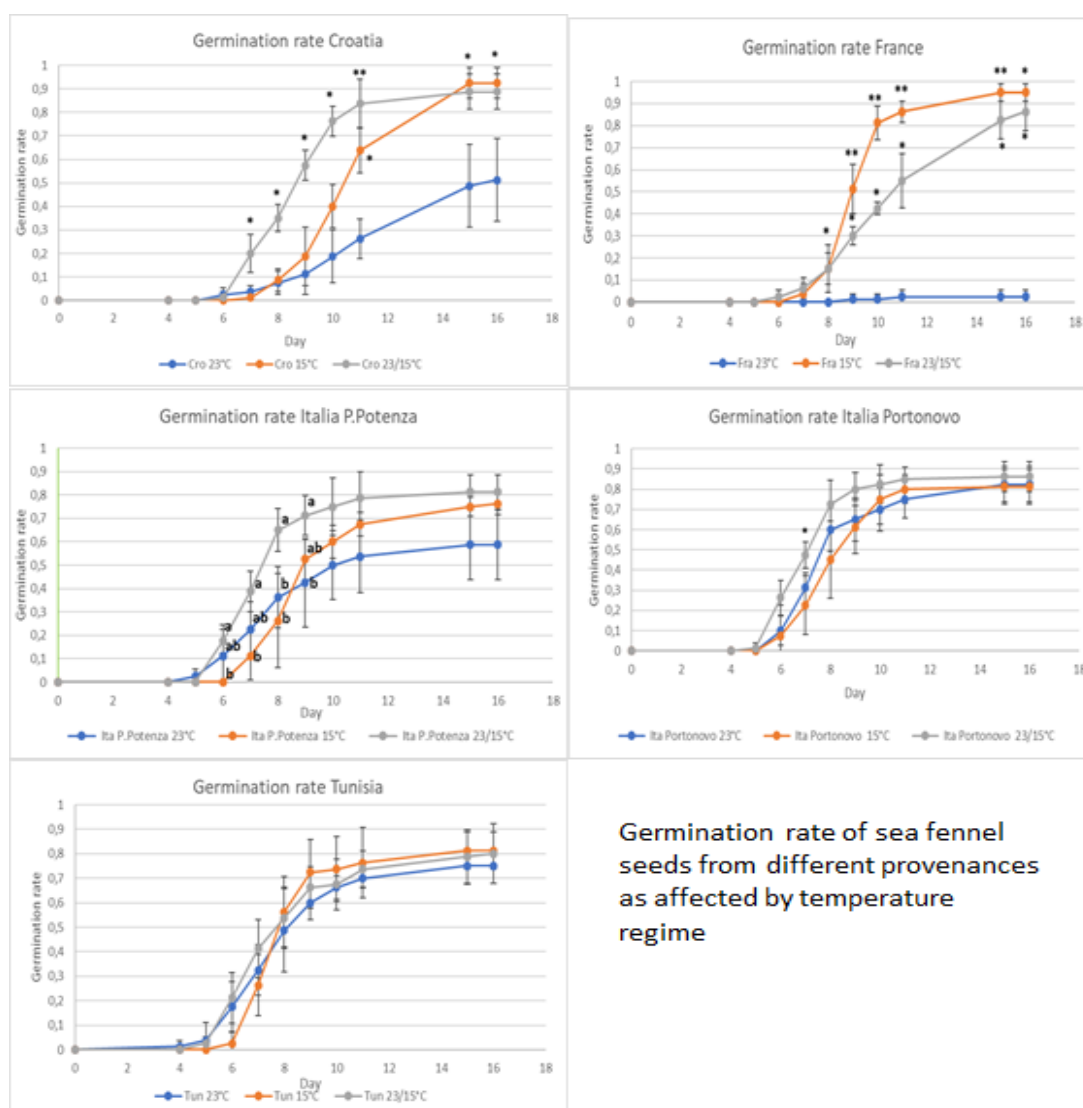
Harvesting and post-harvest handling

Harvesting should be conducted at peak biomass to maximize both yield and quality of the edible and refuse portions. Immediate post-harvest handling is crucial; harvested materials should be cooled promptly (stored at +4°C) to preserve nutritional and bioactive compounds. These practices are essential to ensure that the quality of the raw material is maintained for further processing or value-added applications.

In summary, the above recommendations integrate experimental findings and best agronomic practices to provide a comprehensive framework for the sustainable production of organic sea fennel crops.

4.4 Preliminary germination tests carried out by PARTY6

Sets of 50 seeds provided by each partner (P1, P2, P5, P6, P7) were sown in Spring 2023 on moistened filter paper in petri dish and placed at different temperatures or thermoperiods (at growth chamber conditions). Germination process was then followed for three weeks. This preliminary trial was aimed at setting out differences in germination capacity between sea fennel provenances, and optimizing the germination process for each provenance, which would be very useful for further cultivation of sea fennel in each country.



Germination rate of sea fennel seeds from different provenances as affected by temperature regimes.