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BIOCHEMISTRY & PHYSIOLOGY - ORIGINAL ARTICLE

From the wild to the field: effect of foliar or soil application of inorganic or semi-organic fertilizers on various parameters of four local endemic plant species of Crete (Greece)

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Abstract

Domestication of wild plant species of interest coupled with introduction of pilot cultivation practices and appropriate fertilization schemes could be an efficient alternative for addressing sustainable exploitation needs of threatened and/or declining wild phytogenetic resources. In this field study focusing on four threatened wild-growing plants (*Carlina diae*, *Origanum dictamnus*, *Origanum microphyllum*, and *Sideritis syriaca* subsp. *syriaca*) confined to Crete (local endemics), the effects of three types of fertilization (inorganic, plain semi-organic or co-applied with commercial biostimulants based on edible raw plant materials) by two methods (foliar/soil application) on plants' growth were investigated. Specifically, plant parameters such as aboveground biomass yield, content and uptake of nutrients by plants as well as arbuscular mycorrhizal fungi root colonization were evaluated. Results revealed a distinction in plants' multi-elemental stoichiometry, except in the case of *Origanum* species, in which the variance of their ionomics showed a significant overlap. The elements most closely related to yield were K and Zn, while Pearson tests showed various correlations between studied essential nutrients. Among them, the positive correlations between B and Ca, and Mg (with r value up to 0.9 at $p \leq 0.001$, in all studied species except *O. dictamnus*); the negative correlations between K and Mg (in *O. dictamnus* and *S. syriaca* subsp. *Syriaca*, $r = -0.5$ at $p \leq 0.001$); and the positive correlation patterns between Fe and Mn, or Cu and Zn, respectively (in all studied species) , were observed. Biostimulant application alone or in combination with semi-organic fertilizers increased the yield of *C. diae* (up to 161%) and of the two *Origanum* species studied (up to 70% and 68% for *O. dictamnus* and for *O. microphyllum*, respectively), while inorganic fertilization was beneficial for *S. syriaca* subsp. *syriaca* (up to 170% increase) and *O. microphyllum* (up to 79% increase). However, no solid conclusions could be derived in respect of the preference of the four species for any fertilizers' application method (foliar or by soil).

Keywords Arbuscular mycorrhizal fungi · Biostimulant · Endemic Mediterranean herbs · Marconutrients · Micronutrients · Plant nutrition · Plant yield

1 Introduction

The growing demand for quality, healthier and non-toxic natural products is a well-established consuming trend mainly in developed countries, which in turn qualifies the exploitation of wild-growing plant populations in the agro-alimentary, medicinal-cosmetic, or ornamental-horticultural economic sectors (Cheminal et al. 2020). However, the above practice often raises questions regarding potential ecological risks regarding the depletion or decline of wild phytogenetic resources (Hamilton 2004). Thus, any effort aiming to the domestication of specific wild plant species of interest, as well as the introduction of pilot cultivation

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practices and appropriate fertilization schemes could be an efficient alternative and sustainable perspective.

In this line, inorganic fertilizers are considered efficient in addressing soil nutrients' imbalances occurring from crop removal after the harvest, but they have been severely questioned for raising serious environmental concerns related to soil degradation, nitrate pollution of surface and groundwater as well as they have been associated with the outburst of alarming phenomena such as water bodies' eutrophication (Hou et al. 2020). On the other hand, an increasing interest has been emerged in current literature focusing on the establishment of organic/semi-organic fertilization schemes, with or without the addition of organic promoters such as biostimulants. The combined use of conventional, organic fertilizers and biostimulants represents a strategy that is able to sustain high yields and limit undesirable impact (Selim 2020; Gezahegn 2021). Current research in agriculture has developed distinct groups of materials with innovative properties during the last 20 years to improve farming and increase crop production. It is known that diverse fertilization strategies may differentially affect not only plant growth and yield, but also product quality (Tripathi et al. 2014; Kakar et al 2020). Biostimulants actually involve any material applied to plants in low quantities aiming to nutrients' absorbance increase, abiotic stress tolerance and/or crop quality traits, and most importantly, without using fertilizers or pesticides (du Jardin 2015).

In different areas and habitats of the island of Crete (Greece), many local wild-growing plants thrive; many of these are local endemics in different parts of the island (single-island endemics), while their populations in many cases might be considered threatened due to overexploitation or may still be underutilized due to non-established respective consuming habits. Among them, the Endangered *Carlina diae* (Rech. f.) Meusel & Kästner and *Origanum dictamnus* L. (Dittany of Crete) as well as the Critically Endangered *Origanum microphyllum* (Benth.) Vogel and *Sideritis syriaca* L. subsp. *syriaca* stand out due to their potential utilization in the agro-alimentary, medicinal-cosmetic or ornamental-horticultural economic sectors (Bourgou et al. 2021; Krigas et al. 2021; Libiad et al. 2021). More specifically, *C. diae* or Carline thistle of Dia islet of Crete is a unique rock-dweller growing in inaccessible cliff faces and steep calcareous rocks close to the sea level which is confined exclusively to some islets off the north coast of Crete (Dia, Dragonada and Gianisada islets) and some localities of the north Cretan coastline (e.g., in a gorge over Karoumpes bay); it is a protected species (Bern Convention Appendix I and Greek Presidential Decree 67/1981) and has a considerable ornamental potential with ever-lasting characteristics when dried (Grigoriadou et al. 2020; Krigas et al. 2021). *Origanum dictamnus* commonly known as dittany of Crete is also a protected rock-dwelling Cretan endemic plant which has been traditionally used as an infusion or tincture by decoction for cutaneous use, against gingivitis, cough and cold due to its monograph in European Medicines Agency, as food additive to sauces, salads and vermouth, bitters and liqueurs or as basic constituent in recently developed culinary preparations (Krigas et al. 2015). *Origanum microphyllum* commonly known as Cretan marjoram is locally traded in Crete where it is sourced directly from wild-growing populations and is used in dried form for its calming and antispasmodic properties and pleasant spicy scent in infusions or culinary preparations (Fanourakis et al. 2022). *Sideritis syriaca* subsp. *syriaca* commonly known in Crete as Malotira or Cretan Mountain tea is a wild-harvested plant in Crete with wild-growing population in decline which is traditionally used as infusion with approved indications by the European Medicines Agency's monograph for the relief of cough associated with cold and mild gastrointestinal discomfort (Kloukina et al. 2020). Species-wise and due to their multi-purpose interest raised by conservation concerns and economic value, the current investigation is focused on the above-mentioned threatened local endemic plants of Crete (single-island endemics).

With the exception of fertilization regimes applied in pilot cultivation of the Cretan endemics *Verbascum arcturus* L. (Paschalidis et al. 2021) and *O. microphyllum* (Fanourakis et al. 2022), no other domestication effort through systematic fertilization scheme applications has ever been attempted on local endemic plants of Crete to our knowledge; thus this should be considered a research gap that needs to be addressed. Herein, we hypothesized that different kinds of fertilization schemes on the above-mentioned species and subspecies (taxa) in equivalent quantities to those applied on respective cultivated perennial herbs could derive different responses on their biological parameters such as above-ground biomass yield and content and uptake of nutrients by plants (Paschalidis et al. 2021; Fanourakis et al. 2022), or may benefit soil microbiological properties. In addition, we speculated that if the above hypothesis is valid, we could make a first attempt of grouping species-specific fertilization needs or charting some basic interrelationships between different patterns of nutrients' bioavailability and their relation to yield responses. In this frame, three objectives were investigated: (i) the effects of different conventional and semi-organic fertilization schemes on plant parameters such as above-ground biomass yield, nutrients' absorption and arbuscular mycorrhizal fungi (AMF) root colonization properties, (ii) the effects of application or co-application of plant biostimulants on the above-mentioned parameters and (iii) the possibility of grouping the different species and subspecies according to their fertilization needs and concomitant interrelationships or antagonistic interactions between different essential macro- and micronutrients.

2 Materials and methods

Origin of plant material – Ten authorized botanical expeditions were organized in 2019 to explore different areas for wild-growing *C. diae*, *O. dictamnus*, *O. microphyllum* and *S. syriaca* subsp. *syriaca* populations with natural vigorous growth in rocky wild habitats of Crete. The seed collections were performed using the authorized special permit of the Institute of Plant Breeding and Phytogenetic Resources (IPBPR), Hellenic Agricultural Organization Demeter (Permit 82,336/879 of 18/5/2019 & 26,895/1527 of 21/4/2021), which is issued yearly by the Greek Ministry of Environment and Energy.

The collected herbarium samples and seeds of the four species were taxonomically identified, and consequently, a unique IPEN (International Plant Exchange Network) accession number was given by IPBPR. New plants were initially raised ex situ through pilot seed germination trials or pilot rooting by cuttings based on published information (Grigoriadou et al. 2019; Kloukina et al. 2020; Sarropoulou et al. 2022) and standard working propagation protocols used routinely in the nursery of IPBPR (data not shown). These pilot propagation trials resulted in ex situ raised plants which were then transplanted in 2-L plastic pots and were placed in a non-heated greenhouse of the company GLAVAKIS FRUIT TREES, Piperia, Aridaia, Greece (40°58′ 01.47″ N, 22° 01′ 23.87″ E).

An experiment was established in the facilities of the above-mentioned company located in Pella prefecture of northern Greece, in a field of 20 m×25 m. The plants were transplanted in the field on 27th of April 2020. The distances between all plants were 40 cm and between rows were 80 cm, while the rows were 20 m long. A drip-irrigation system was also established for supplying the pilot cultivation with water and fertilizers. The protocol for field experimentation and establishment of plants followed other standard field cultivation protocols for *Origanum* species and medicinal-aromatic plants (Giannoulis et al. 2020; Paschalidis et al. 2021) and was modified to the statistical design chosen herein.

2.1 Establishment of the Field Experiment and Fertilization Treatments

The experimental design consisted of three completely randomized blocks (CRB) per treatment, with10 randomly selected plant individuals of each species or subspecies per block and four blocks per control, which were all randomly located in three different rows per treatment. An automatic irrigation system was installed with 2 L/h adjustable drippers spaced at 0.4 m on the line to supply water to the established plants and it was periodically scheduled to water them three times per week depending on season and local rainfalls. Pest and disease control was not necessary during cultivation, but the removal of weeds was periodically required.

Fertilization treatments were first applied at the end of May (i.e., 24 d following transplanting), and eight application schemes were totally carried out on a biweekly basis till the final harvest. The fertilization treatments involved were the following (see also respective supplementary material file S1):

Th1: semi-organic fertilizers by foliar application; the nutrient solution consisted of THEORUN at 7 mL L^{-1}, THEOFAST at 5 mL L^{-1}, THEOCAL at 1.5 g L^{-1}, 10-47-10 (AGRI.FE.M. LTD Fertilizers, Greece) at 3.2 g L^{-1}, K_2SO_4 (0-0-52, AGRI.FE.M. LTD Fertilizers, Greece) at 2.07 g L^{-1}, micronutrients (Plex Mix, AGRI.FE.M. LTD Fertilizers, Greece) at 1.5 mL L^{-1} and $MgSO_4$ (Mg 25.6%, AGRI.FE.M. LTD Fertilizers, Greece) at 0.6 g L^{-1}.

IF1: conventional inorganic fertilizers by foliar application; the nutrient solution consisted of NH_4NO_3 (34,4-0-0, Neofert®, Neochim PLC, Dimitrovgrad, Bulgaria) at 2.69 g L^{-1}, $Ca(NO_3)_2$ (NITROCAL, Agrohimiki, Greece) at 1.67 g L^{-1}, 10-47-10 at 3.2 g L^{-1}, K_2SO_4 (0-0-52) at 2.27 g L^{-1}, micronutrients Plex Mix at 1.5 mL L^{-1} and $MgSO_4$ (Mg 25.6%) at 0.6 g L^{-1}.

Th2: semi-organic fertilizers by soil application; the nutrient solution consisted of THEORUN at 1.4L/0.1 ha, THEOCAL at 0.3 kg 0.1 ha^{-1}, THEOMASS at 2L 0.1 ha^{-1}, 10-47-10 at 0.64 kg 0.1 ha^{-1}, K_2SO_4 (0-0-52) at 0.64 kg 0.1 ha^{-1}, micronutrients Plex Mix at 0.3 L 0.1 ha^{-1} and $MgSO_4$ (Mg 25.6%) at 0.06 kg 0.1 ha^{-1}.

IF2: conventional inorganic fertilizers by soil application; the nutrient solution consisted of NH_4NO_3 (34.4-0-0) at 0.54 kg 0.1 ha^{-1}, $Ca(NO_3)_2$ (NITROCAL) at 0.34 kg 0.1 ha^{-1}, 10-47-10 at 0.64 kg 0.1 ha^{-1}, K_2SO_4 (0-0-52) at 0.46 kg 0.1 ha^{-1}, micronutrients, Plex Mix at 0.3 L 0.1 ha^{-1} and $MgSO_4$ (Mg 25.6%) at 0.06 kg 0.1 ha^{-1}.

Th3: mixture of plant extracts as biostimulant by soil application; mixture of plant extracts as a biostimulant by soil application (MPE-sa): the nutrient solution consisted of THEOMASS at 2L 0.1 ha^{-1}.

C: Control, with foliar and soil applications only using tap water. More details, regarding composition of the fertilizers used, are provided in the respective supplementary material file (S1).

The biostimulants THEOFAST and THEOMASS were used for foliar and root application, respectively. Both biostimulants consisted of mixtures from edible and powdered plant extracts, and the exact composition is part of the company's "know how," while they are commercially marketed as "Type B" fertilizers for enhancing plant growth.

These biostimulants have been widely used in several crops, and both have been investigated previously with published results for *V. arcturus* (Paschalidis et al. 2021) and *O. microphyllum* (Fanourakis et al. 2022) pilot cultivations established in Crete. More details regarding composition of the fertilizers used are provided in the respective supplementary material file (S1).

Leaf sampling was carried out in the first week of August, during the plants' full-flowering stage, while in the first week of October 2020 harvest of the aboveground biomass was carried out (Fig. 1). Before analysis, all plant samples were oven-dried (70 °C) till constant weight.

Soil and plant analysis – Before the establishment of the field experiment, a composite surface soil sample (0–30 cm in depth) was collected, air-dried, passed through a 2-mm sieve and was analyzed in triplicate for the properties described hereafter (Table 1). Particle size distribution was determined by the hydrometer method (Bouyoucos 1962), organic carbon (C) was determined by the wet oxidation method (Walkley and Black 1934), and $CaCO_3$ was measured using a calcimeter. The pH was determined in a 1:2 (w/v) water suspension, the electrical conductivity was measured in the saturation extract (EC_{se}), and the sodium absorption ratio (SAR) was calculated by the concentrations of water-soluble sodium (Na), calcium (Ca) and magnesium (Mg) (Rhoades 1996). The cation exchange capacity (CEC) was determined by the $[Co(NH_3)_6]Cl_3$ method (ISO 23470).

Soil available phosphorus (P) was extracted using 0.5 M $NaHCO_3$, pH 8.5, and was measured by the molybdenum blue-ascorbic acid method (Kuo 1996). Both NO_3-N and NH_4-N were extracted with 1 M KCl, which were measured using UV–Vis spectrometry and the sodium salicylate—sodium nitroprusside method, respectively (Mulvaney 2018). Exchangeable potassium (K), Ca and Mg were extracted with 1 M CH_3COONH_4, pH 7 (Thomas 2015); K was measured with flame photometry, while Ca and Mg by atomic absorption spectrometry. The micronutrients copper (Cu), zinc (Zn), iron (Fe) and manganese (Mn) were extracted with DTPA (Lindsay 1978) and were measured by atomic absorption spectrometry as well, whereas boron (B) was extracted with hot water and was determined with the azomethine-H method by UV–Vis spectrometry (Keren 1996).

Furthermore, sub-samples of irrigation water, which was used for irrigation and the preparation of fertilizers' solutions applied by foliar, were analyzed for EC and water-soluble B, employing the aforementioned methods of analytical determinations. All analyses were conducted in three replications.

Sub-samples of leaf or above-ground biomass collected at the flowering and harvest stage of each plant species or subspecies, respectively, were ashed at 500 °C for a four-hour minimum (Mills et al. 1996), and then the ash was dissolved in 2 M HCl, following filtration. The filtrate was used for the determination of P, K, Ca, Mg, Cu, Zn, Fe, Mn and B employing the analytical methods described previously for soil analysis. In addition, plant samples were analyzed for total nitrogen (N) by the Kjeldahl method (Bremner 1996). The yield parameter of each species or subspecies was assessed from the dry weight of above-ground biomass at the harvest stage, whereas the uptake parameter was calculated by the product of yield of each species or subspecies times each elements' concentration. Three replicates were assessed per treatment, four samples were pooled for each replicate (collected from different plant individuals), and the assay was performed twice.

Due to their greater commercial value, the harvested plants of *O. dictamnus* and *O. microphyllum* were further analyzed for AMF root colonization. Specifically, the plants were up-rooted; the root samples were cut, washed with tap water on a sieve and placed in centrifuge tubes with 10% KOH at 80 °C for 40 min. Then the alkali was removed with several changes with tap water and the roots were acidified with drops of 5 M HCl before being stained with 0.05% trypan blue solution (Sylvia 1994). The percentage of arbuscular mycorrhizal fungi (AMF) root colonization was counted under a compound microscope at 100X and 400X when needed according to McGonigle et al. (1990).

Statistical analysis – For each plant parameter determined within the same species or subspecies, analysis of variance (ANOVA) was conducted using the statistical package SPSS, version 26, and the protected LSD test was used for mean comparisons, at $p \leq 0.05$. Furthermore, correlation analysis and principal component analysis (PCA) were applied to data using the Statgraphics software (STATGRAPHICS, CENTURION XVI, version 16.1.11, STATPOINT TECHNOLOGIES, Inc). The AMF root colonization data were arcsin transformed prior to the statistical analysis.

3 Results

Soil properties of the experimental field and quality of the irrigation water Briefly, the soil of the experimental field was alkaline in reaction and calcareous and sandy loam in texture. In addition, it had low content of organic C, EC_{se} and SAR (Table 1). As far as soil's fertility status is concerned, available NO_3-N was high and P and K were marginally sufficient and deficient, respectively (Dahnke 1990; Fixen and Grove 1990; Haby et al. 1990). Moreover, soil

Fig. 1 Representative illustration of the effect of three types of fertilization (inorganic, plain semi-organic or co-applied with biostimulant) applied by two methods (foliar/soil application) on growth of *Carlina diae* individuals (top row), *Origanum dictamnus* individuals (second row), *Origanum microphyllum* individuals (third row) and *Sideritis syriaca* subsp. *syriaca* individuals (bottom row)

Table 1 Certain soil physical–chemical properties and available concentrations of the macro- and micronutrients of the experimental field in Piperia, Aridea, northern Greece

Soil texture				CEC	EC_{se}	SAR
Sand	Silt	Clay	Characterization			
(%)				($cmol_c\ kg^{-1}$)	($dS\ m^{-1}$)	
68.0 ± 2.4	27.7 ± 2.3	4.3 ± 0.3	Sandy loam	7.6 ± 0.4	0.56 ± 0.05	0.16 ± 0.04
pH	$CaCO_3$	OC	NO_3-N	NH_4-N	P	K
(1:2 w/v)	(%)		($mg\ kg^{-1}$)			
7.8 ± 0.1	2.4 ± 0.7	1.5 ± 0.3	21.3 ± 0.7	12.3 ± 1.0	10.0 ± 1.7	37.7 ± 12.1
Ca	Mg	Cu	Zn	Fe	Mn	B
($mg\ kg^{-1}$)						
3280 ± 494	140 ± 17	2.4 ± 0.3	1.5 ± 0.2	14.4 ± 0.3	8.9 ± 1.2	0.75 ± 0.10

Values represent means ± standard deviation ($n = 3$)

CEC Cation exchange capacity, EC_{se} Electrical conductivity of the saturation extract, *SAR* Sodium absorption ratio, *OC* organic carbon

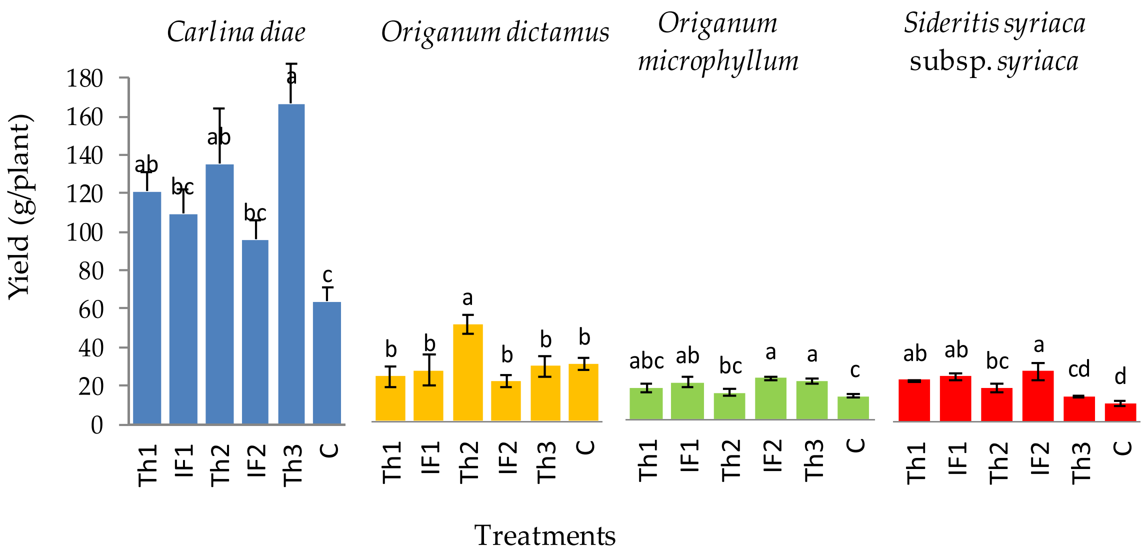

Fig. 2 Above-ground biomass yield of the four threatened Cretan endemic species and subspecies (*Carlina diae*, *Origanum dictamnus*, *Origanum microphyllum*, *Sideritis syriaca* subsp. *syriaca*) established in pilot cultivation with Th1: semi-organic fertilizers by foliar application; IF1: conventional inorganic fertilizers by foliar application; Th2: semi-organic fertilizers by soil application; IF2: conventional inorganic fertilizers by soil application; Th3: mixture of plant extracts as biostimulant by soil application; C: water application (control). Values represent means ± standard errors ($n = 3$). Within each species, different letters indicate significant differences among means, employing the protected LSD test, at $p \leq 0.05$

available B and metallic micronutrients (Table 1) ranged at levels higher or similar to the sufficiency range reported by Sims and Johnson (2018). Irrigation water had pH = 7.5, EC = 0.71 dS m^{-1} and B concentration equal to 0.55 mg L^{-1}. Based on these properties, as far as the quality of irrigation water is concerned, there was no restriction for use for irrigation in respect of salinity problems as well as B phytotoxicity risk (Ayers and Westcot 1985).

Effects of the fertilization schemes on certain parameters of the plant species or sub-species Among the studied four threatened local endemic plants confined exclusively to Crete (*C. diae*, *O. dictamnus*, *O. microphyllum*, *S. syriaca* subsp. *syriaca*) and fertilization treatments applied (Th1, Th2, IF1, IF2, Th3, C), a clear positive effect on dry above-ground biomass yield after harvest was observed in three of them (Fig. 2), while for *O. dictamnus* only soil application of the semi-organic fertilizers (Th2) resulted in a significant increase. More specifically, as regards *C. diae*, significant

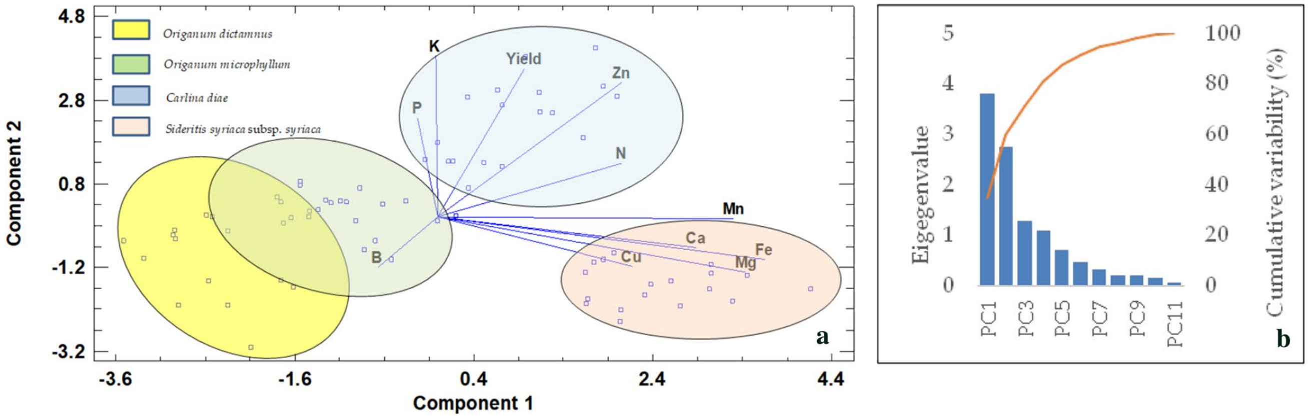

Fig. 3 **a** Biplot of the first two principal components of the PCA of the studied macro- and micronutrients of above-ground biomass (vectors) and the respective distribution of the four threatened Cretan endemic species and subspecies (*Carlina diae, Origanum dictamnus, Origanum microphyllum, Sideritis syriaca* subsp. *syriaca*), **b** eigenvalues of each component, with respective cumulative variability percentages. In Fig. 3a, each colored group represents the distribution pattern of the different species or subspecies, while within each group different spots correspond to the respective treatments or replications

Table 2 The weights of the studied variables for each of the first six components of the PCA

Studied variables	Component 1	Component 2	Component 3	Component 4
Yield	0.137	***0.498***	0.278	0.057
B	−0.088	−0.167	−0.103	***0.867***
Ca	***0.375***	−0.100	0.294	0.227
Cu	0.282	−0.163	***−0.515***	−0.057
Fe	***0.475***	−0.140	−0.045	−0.131
K	−0.003	***0.540***	0.189	0.132
Mg	***0.449***	−0.183	0.022	0.141
Mn	***0.430***	−0.004	0.231	0.124
N	0.268	0.178	−0.293	−0.238
P	−0.030	0.331	***−0.603***	0.244
Zn	0.267	***0.450***	−0.137	0.063

In bold and italic, the variables with the higher weights are indicated

increases were recorded with all semi-organic or biostimulant fertilization schemes under both foliar and soil application methods, whereas in the case of *S. syriaca* subsp. *syriaca* positive treatment effects were observed with both semi-organic and inorganic fertilization, with no significant effect of the biostimulant application (Th3). As far as the *O. microphyllum* is concerned, the inorganic fertilization scheme was found to be more effective when it was applied to soil (IF2), recording an increase nonetheless equal to that of the biostimulant soil application (Th3).

The PCA revealed that the major portion of the total variance (80%) of the studied variables (yield and nutrients' content of above-ground biomass) was grouped between four components, and the two of them explained almost 60% of the variance (Fig. 3a). The distribution of each plant species or subspecies in the two first PCA axes denoted that regardless of the adopted fertilization scheme, they were distributed distinctively with the exception of the two studied *Origanum* species which showed a relative distribution similarity (Fig. 3a). The eigenvalues and proportion of variance explained by each component are presented in Fig. 3b, while Table 2 shows the variables contributing the most to each component. According to these results, the concentrations of Ca, Mg, Fe and Mn in the above-ground biomass were the variables recording the higher weights of the first component, whereas yield parameter with K and Zn concentrations determined the variability of the second.

Effect of fertilization treatments on Carlina diae. Different fertilization treatments resulted in a significant increase in B leaf concentration as regards the flowering stage of *C. diae*, although not directly correlated with the reported increase in yield, whereas respective changes were reflected in both alkali and alkaline earth cations studied (K, Ca, Mg) as well

Table 3 Macro- and micronutrients' concentrations in leaves and aboveground biomass, at the flowering and harvest stage, respectively, of *Carlina diae* plants established in pilot cultivation

Treatment	Flowering	Harvest	Flowering	Harvest	Flowering	Harvest	Flowering	Harvest	Flowering	Harvest
	N		P		K		Ca		Mg	
	(g kg^{-1})									
Th1	10.7a	12.3a	2.3a	2.7a	33.4a	33.9ab	3.6a	20.0ab	3.0a	4.5a
IF1	10.0a	10.2a	2.6a	2.9a	38.0a	26.3bcd	4.8a	16.6c	3.4a	4.4ab
Th2	12.1a	9.4a	2.7a	2.5ab	28.9a	23.6 cd	2.8a	17.5bc	2.7a	4.1abc
IF2	11.3a	8.8a	2.2a	1.8b	32.7a	42.3a	2.6a	21.4a	2.3a	4.5a
Th3	11.7a	8.7a	2.2a	1.9b	34.1a	32.1bc	2.1a	22.5a	2.2a	3.6c
C	10.7a	12.2a	3.0a	2.3ab	31.7a	22.5d	2.6a	17.5bc	2.8a	3.9bc
p F test	NS	NS	NS	0.029	NS	0.004	NS	0.007	NS	0.022
	Cu		Zn		Fe		Mn		B	
	(mg kg^{-1})									
Th1	10.6a	16.8a	18.4a	37.2a	351c	1292b	49.5a	70.8c	35.4b	34.1a
IF1	11.1a	16.9a	24.2a	33.9ab	424bc	1702a	71.0a	111.6a	46.6a	28.9ab
Th2	10.6a	13.7bc	18.6a	24.7d	671ab	1174bc	66.1a	93.2abc	31.8c	26.6bc
IF2	11.6a	14.3b	19.6a	26.2cd	258c	1124bc	64.3a	95.7ab	29.3c	28.7ab
Th3	12.7a	12.4c	20.5a	31.6abc	226c	926c	55.2a	98.4ab	34.8b	28.6b
C	10.9a	13.1bc	19.5a	28.1bcd	945a	1165bc	69.8a	77.5bc	26.2d	21.4c
p F test	NS	<0.001	NS	0.005	0.003	0.012	NS	0.035	<0.001	0.008

Th1, Semi-organic fertilizers by foliar application; IF1, Conventional inorganic fertilizers by foliar application; Th2, Semi-organic fertilizers by soil application; IF2, Conventional inorganic fertilizers by soil application; Th3, Mixture of plant extracts as biostimulant by soil application; C, Water application (control)

NS non-significant. Within each growth stage and element, different letters indicate significant differences among means, employing the protected LSD test, at $p \leq 0.05$

as in all micronutrients (Cu, Zn, Fe, Mn, B) at the harvest stage (Table 3).

The correlation matrix between plant parameters at both growth stages revealed significant and positive correlations between yield and leaf Cu concentration ($r = 0.5$, $p \leq 0.05$) at the flowering stage and a positive correlation trend with Ca concentration of biomass at the harvest stage ($r = 0.44$, $p = 0.07$). Furthermore, at both growth stages, P concentration was significantly and positively correlated with Fe, Cu and Zn, while the same occurred in the case of Mn concentration only at the flowering stage. Moreover, and as expected, on a case-by-case basis for each growth stage, Ca was positively and strongly correlated with Mg and K though negatively with Fe (harvest stage), whereas remarkably it showed positive correlations with Mn at the flowering stage. Respective positive correlations were observed between cation micronutrients, whereas the leaf N:P stoichiometry ratio of the flowering stage showed significant and negative correlations with Fe and Zn ($r = -0.5$, $p \leq 0.05$).

The plant uptake of each macro- and micronutrient followed similar variation trends among different fertilization schemes, which were accompanied by respective significant changes; among them a noteworthy positive effect of the Th3 treatment (biostimulant application) was detected for most of the studied nutrients (Fig. 4).

Effect of fertilization treatments on Origanum dictamnus. In contrast to *C. diae*, the different fertilization schemes caused a significant increase in leaf concentration of all macro- and micronutrients studied in *O. dictamnus*, except for B concentration which practically showed no variations at the flowering stage. Regarding the harvest stage, different sources of fertilization or application methods had generally no remarkable effects; the exceptions were K concentration in above-ground biomass in the case of foliar application of semi-organic fertilizers (Th1) or Fe and Mn concentrations in the case of foliar application of inorganic scheme (IF1), which showed a significant increase (Table 4). In addition, there were no treatment effects on AMF root colonization, with total (hyphae, arbuscules and vesicles) being on average 64% (± 10%), while arbuscular colonization alone was 3% (± 2%).

As expected from the negligible effects of the different fertilizer sources or application methods on the yield of the *O. dictamnus*, no particular correlations occurred

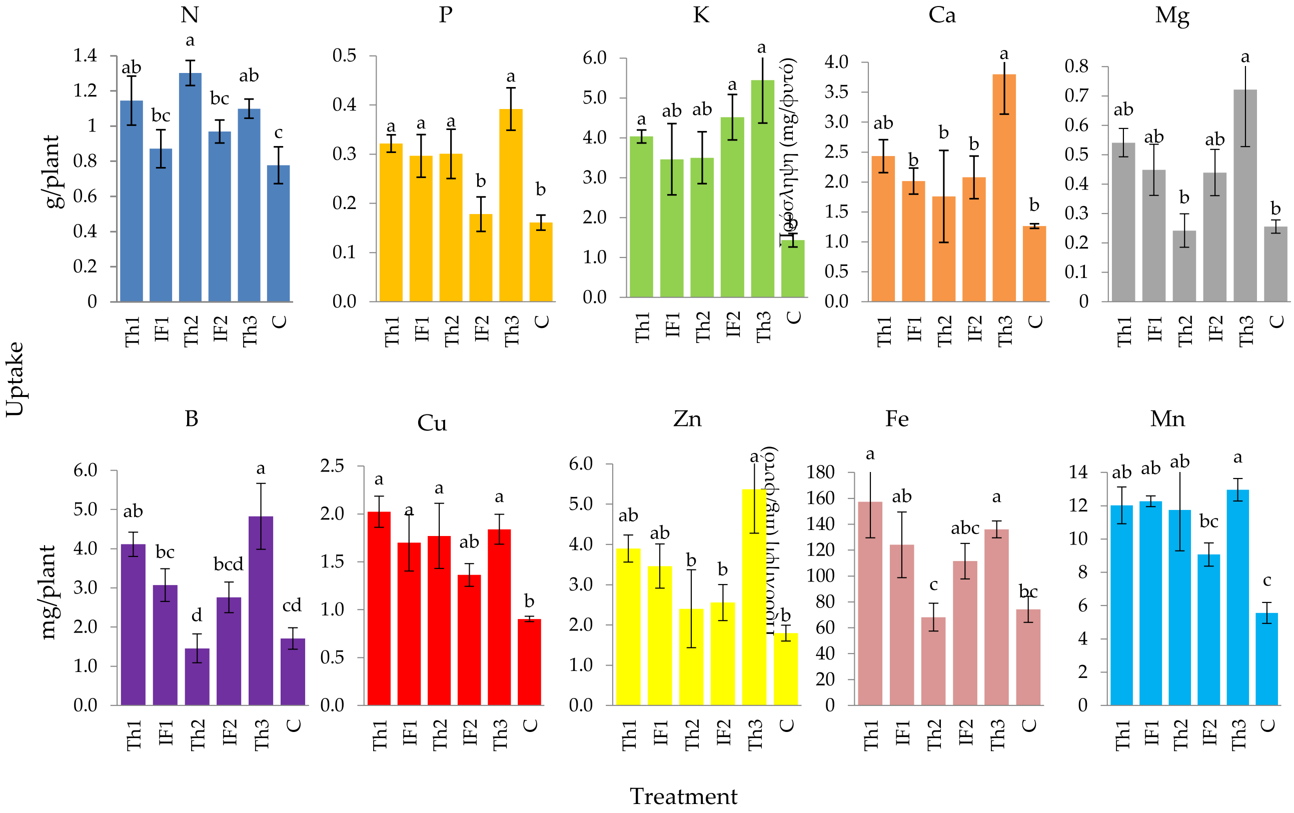

Fig. 4 Macro- and micronutrients' uptake by *Carlina diae* plants established in pilot cultivation with Th1: semi-organic fertilizers by foliar application; IF1: conventional inorganic fertilizers by foliar application; Th2: semi-organic fertilizers by soil application; IF2: conventional inorganic fertilizers by soil application; Th3: mixture of plant extracts as biostimulant by soil application; C: water application (control). Values represent means ± standard errors ($n = 3$). Within each element, different letters indicate significant differences among means, employing the protected LSD test, at $p \leq 0.05$

between above-ground biomass and nutrients' concentrations regardless the growth stage; the only exceptions were a negative effect of leaf Cu concentration at the flowering stage on yield, and also a negative correlation with leaf Mg concentration, which however both appeared as marginally significant ($r = -0.45$, $p = 0.06$).

Considering the leaf nutrients' concentrations at the flowering stage, Cu and Mg (which were negatively correlated with yield) showed positive correlations with each other, as well as with Mn and Zn, respectively. In addition, the leaf N:P stoichiometry ratio at the flowering stage showed significant and negative correlations with Mg, Mn and Zn ($r = -0.6$, $p \leq 0.01$), while concerning nutrients' uptake, Th2 treatment resulted in a significant increase only in the case of K. On the contrary, it is noteworthy that for most macro- and micronutrients, some treatments (mainly Th1 and IF2) induced a significant decrease in their uptake by *O. dictamnus* (Fig. 5).

Effect of fertilization treatments on Origanum microphyllum. Although *O. microphyllum* showed similarities with *O. dictamnus* regarding the general magnitude of nutrients' uptake and leaf or biomass concentrations, the same was not true with regard to the respective responses to the different fertilization schemes applied. Thus, in addition to the significant increases of nutrients' uptake by plants at the harvest stage upon application of both conventional fertilization schemes (IF1 and/or IF2 increased the uptake of P, K, Mg, Cu, Zn, Fe and Mn) and biostimulant (Th3 increased the uptake all studied macro- and micronutrients) (Fig. 6), all nutrients' concentrations (except P) also showed a case-by-case positive response in different treatments as regards the flowering stage. The above trend was also repeated at the harvest stage. However, it was related mainly to N, K and Zn concentrations, the values of which showed the most significant positive variations among different fertilization treatments (Table 5). Regarding the AMF colonization, total colonization was not affected, being on the average 48% (± 16%); however, arbuscular colonization was decreased compared to the control for all treatments but Th2 (Fig. 7).

The correlation matrix between plant parameters at both growth stages revealed a strong and positive correlation of yield with leaf Cu concentration ($r = 0.7$, $p \leq 0.001$) at the flowering stage, whereas positive correlations at the harvest stage were recorded between yield and N, K and Zn

Table 4 Macro- and micronutrients' concentrations in leaves and aboveground biomass, at the flowering and harvest stage, respectively, of *Origanum dictamnus* plants established in pilot cultivation

Treatment	Flowering	Harvest	Flowering	Harvest	Flowering	Harvest	Flowering	Harvest	Flowering	Harvest
	N		P		K		Ca		Mg	
	(g kg^{-1})									
Th1	15.3abc	9.4a	3.3ab	2.6a	15.5b	21.3a	7.7a	15.3a	4.8a	3.4a
IF1	12.7cd	7.9b	3.7a	2.4ab	17.2ab	17.6b	5.9bc	16.2a	4.4ab	3.9a
Th2	14.8bc	6.8b	2.7b	2.4ab	16.9ab	16.9b	7.2a	17.2a	4.0b	3.6a
IF2	16.1ab	7.1b	3.9a	2.0c	18.5a	18.8ab	7.5a	16.4a	4.7a	3.6a
Th3	18.1a	9.8a	3.3ab	2.1bc	18.0a	19.6ab	6.2b	15.6a	4.0b	2.5a
C	11.7d	10.4a	1.7c	2.6a	15.9b	17.2b	5.1c	14.1a	3.9b	3.7a
p F test	0.008	<0.001	0.004	0.011	0.045	0.039	<0.001	NS	0.019	NS
	Cu		Zn		Fe		Mn		B	
	(mg kg^{-1})									
Th1	13.3a	15.1a	22.4ab	23.0ab	1255ab	905ab	104.9a	40.9c	59.2a	36.4a
IF1	11.6c	15.9a	20.9abc	25.5a	976c	1155a	92.6abc	82.0a	64.1a	33.2a
Th2	11.4c	13.2a	20.0abc	17.5c	1407a	702bc	82.3c	58.5b	66.5a	34.0a
IF2	13.7a	13.8a	25.5a	21.9b	1160abc	887abc	101.7ab	60.9b	69.9a	32.6a
Th3	12.7abc	13.1a	17.6bc	20.3bc	1062bc	577c	83.7bc	50.2bc	62.5a	31.4a
C	12.0bc	17.8a	15.6c	22.8ab	997bc	663bc	74.2c	54.4b	63.4a	32.7a
p F test	0.043	NS	0.033	0.003	0.036	0.024	0.023	<0.001	NS	NS

Th1, Semi-organic fertilizers by foliar application; IF1, Conventional inorganic fertilizers by foliar application; Th2, Semi-organic fertilizers by soil application; IF2, Conventional inorganic fertilizers by soil application; Th3, Mixture of plant extracts as biostimulant by soil application; C, Water application (control)

NS non-significant. Within each growth stage and element, different letters indicate significant differences among means, employing the protected LSD test, at $p \leq 0.05$

concentrations of biomass ($r = 0.6$, $p \leq 0.01$). However, it is noteworthy that at the latter growth stage, the yield parameter was negatively correlated with Mg, also showing a negative correlation trend with B ($r = -0.4$, $p = 0.07$) and a negative correlation with AMF colonization, either total or arbuscular ($r = -0.5$, $p \leq 0.05$ and $r = -0.6$, $p \leq 0.05$, respectively). In addition, the arbuscular colonization was negatively correlated with N ($r = -0.58$, $p \leq 0.01$) and Zn ($r = -0.57$, $p \leq 0.01$).

Effect of fertilization treatments on Sideritis syriaca subsp. syriaca. The application of different fertilization schemes resulted in a significant increase of all leaf micronutrients' content as regards the flowering stage, accompanying the reported yield responses presented in Fig. 2. On the other hand, respective changes were not reflected in major macronutrients (N, P, K), whereas Ca and Mg responded positively mainly to the Th2 fertilization scheme (Table 6). In the same line, the above findings were also observed at the harvest stage, showing however some differences in the P content pattern, in which a positive response was recorded with the semi-organic or inorganic foliar applications (Th1, IF1). Nevertheless, a common feature of both growth stages was the significant role of the IF2 fertilization scheme which resulted in increased metallic micronutrients' content, a fact which was also observed for the same treatment as far as the yield parameter is concerned (Fig. 2).

Pearson correlation tests between yield and leaf concentrations of the nutrients at the flowering stage revealed that the P content was a basic constraint factor, showing correspondingly a negative correlation ($r = -0.5$, $p \leq 0.05$). In addition, P was also negatively correlated with Fe ($r = -0.5$, $p \leq 0.05$), K with Mg ($r = -0.5$, $p \leq 0.05$), while as expected, Ca was positively and strongly correlated with Mg ($r = 0.8$, $p \leq 0.001$) and B ($r = 0.7$, $p \leq 0.01$). As far as the metallic micronutrients are concerned, the correlation matrix showed also respective significant and positive connections with each other. At the harvest growth, the effects of the above-mentioned micronutrients' concentrations on yield were more pronounced giving respective positive correlations with Fe, Cu and Zn content.

The overview of plant uptake of nutrients at the harvest stage is presented in Fig. 8. In contrast to similar patterns concerning the above-ground content of each element at this stage, the results revealed different uptake patterns at the harvest stage. Thus, compared to the control, noteworthy significant increases in N, P and K uptake were observed

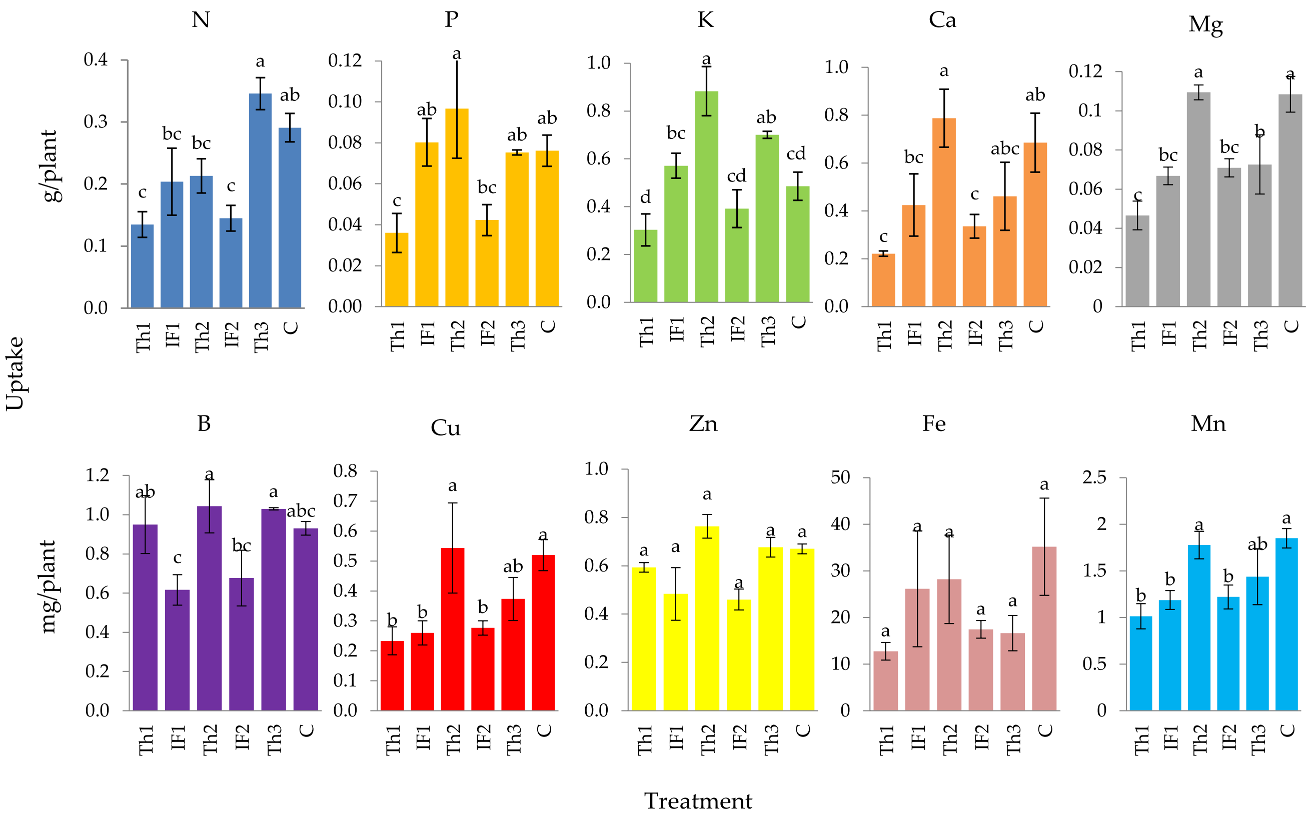

Fig. 5 Macro- and micronutrients' uptake by *Origanum dictamnus* plants established in pilot cultivation with Th1: semi-organic fertilizers by foliar application; IF1: conventional inorganic fertilizers by foliar application; Th2: semi-organic fertilizers by soil application; IF2: conventional inorganic fertilizers by soil application; Th3: mixture of plant extracts as biostimulant by soil application; C: water application (control). Values represent means ± standard errors ($n = 3$). Within each element, different letters indicate significant differences among means, employing the protected LSD test, at $p \leq 0.05$

in foliar fertilization treatments (Th1 and IF1), and with regard to N the same was also recorded in the treatment with soil application of inorganic fertilization (IF2). As regards P uptake, a significant increase was also observed in Th2 treatment, while Ca uptake increased significantly in all semi-organic fertilization or biostimulant treatments, and Mg increased in all fertilization schemes, except for the biostimulant application (Th3). Regarding the micronutrients' uptake by *S. syriaca* subsp. *syriaca*, the two foliar fertilization schemes resulted in significant increases compared to the control in the case of Cu, Zn and Fe, while additionally for Cu the same also occurred with the Th2 treatment (Fig. 8). The sole biostimulant treatment (Th3) differed from the control only in K and Ca, which were higher.

4 Discussion

This study represents the first step to bring into cultivation four local endemic Cretan plants in a field located in North Greece to alleviate the over-collection pressure on their wild-growing populations triggered by their actual or potential economic value (Bourgou et al. 2021; Krigas et al. 2021; Libiad et al. 2021). Moreover, this pilot cultivation took place in a completely different climatic zone compared to their origin (Crete), thus showing their potential for acclimatization. The results of this investigation showed that for all studied plants there was at least one fertilization treatment that increased their yield. The same also applies for similar results recorded on a case-by-case basis regarding the parameters of plant uptake and concentration of investigated macro- and micronutrients. However, the results showed that application of different fertilization schemes caused also different patterns between the three studied biological parameters, e.g., yield, nutrients' uptake and their respective concentrations in leaves at the flowering stage or the above-ground biomass of the harvest. We assume that these different combinations may reflect different mechanisms as well, stemming from changes in the soil environment that appeared due to the application of corresponding treatments as also suggested by Jarrell and Beverly (1981).

In this line, the general picture obtained by the PCA revealed a distinction in plants' multi-elemental stoichiometry, except for the case of the two *Origanum* species studied

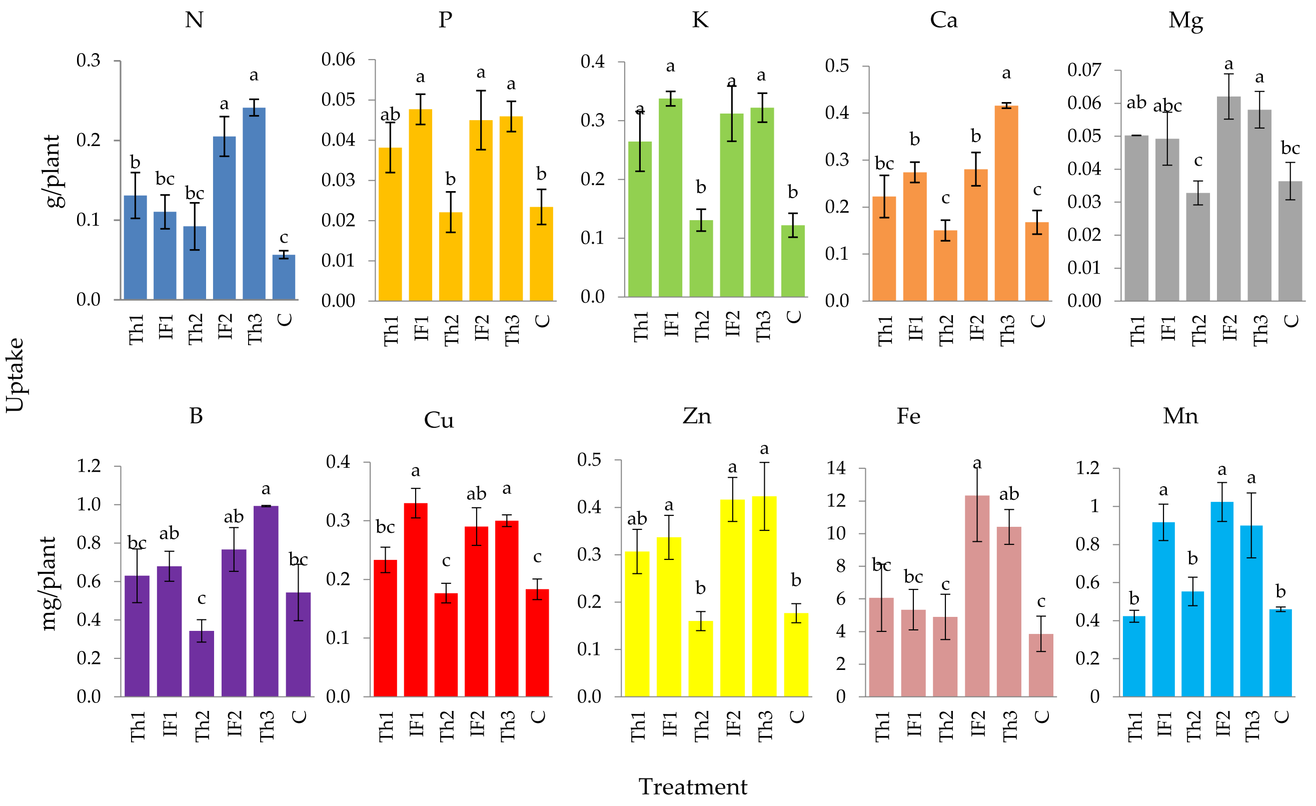

Fig. 6 Macro- and micronutrients' uptake by *Origanum microphyllum* plants established in pilot cultivation with Th1: semi-organic fertilizers by foliar application; IF1: conventional inorganic fertilizers by foliar application; Th2: semi-organic fertilizers by soil application; IF2: conventional inorganic fertilizers by soil application; Th3: mixture of plant extracts as biostimulant by soil application; C: water application (control). Values represent means ± standard errors ($n = 3$). Within each element, different letters indicate significant differences among means, employing the protected LSD test, at $p \leq 0.05$

herein, in which the variance of their ionomics showed a significant overlap. The above findings thereby enclose important information for selecting the optimum fertilization scheme. Under cultivation conditions in this fashion, the degree to which each wild-growing plant species tends to maintain a constant elemental composition in response to the availability of environmental resources is able to determine the respective elements' critical sufficiency limits that need to be detected for optimum cultivation results (Elser et al. 2010; Zhang et al. 2020).

The elements most closely related to the yield parameter of the studied Cretan taxa were K and Zn, as shown by the respective weights of the second component which together with yield contributed substantially to the detected variability. Additionally, the above finding can be further supported by the results of the soil analysis of the field on which the experiment was established. Indeed, exchangeable K was found below the critical sufficiency levels as reported in the literature for soils of northern Greece (Bilias and Barbayiannis 2017). Regarding available Zn in the soil, although its concentration could not be directly considered as limited, this may actually refer to the case of soils with alkaline reaction in which a P-induced Zn deficiency effect could occur (Ipsilantis et al. 2022). With this respect, cases in which Zn concentration on leaves or above-ground biomass of the flowering or harvest stage, respectively, are below 20 mg kg^{-1}—a threshold reported in the literature as a critical sufficiency limit for common field crops (Schulte 2004)—are considered as an indicator of a possible Zn-limiting parameter. In this experiment, Zn was below 20 mg kg^{-1} only for *O. microphyllum*. The above was also taken into account in conjunction with possible coexisting limiting factors like adequate or excessive P concentration above 0.2% (also compared with respective threshold values recorded in the literature for common field crops), low N:P ratios, or excessive B concentration which may also inhibit Zn accumulation on plant tissues (Sarafi et al. 2018).

Pearson tests revealed specific correlations between the nutrients under investigation, while similar patterns have also been reported by other researchers. Among them, it is worth noting the observed positive correlations between B and Ca, and Mg (as recorded in all of the studied taxa except for the *O. dictamnus*), the negative correlations between K and Mg (as recorded for *O. dictamnus* and *S. syriaca* subsp.

Table 5 Macro- and micronutrients' concentrations in leaves and aboveground biomass, at the flowering and harvest stage, respectively, of *Origanum microphyllum* plants established in pilot cultivation

Treatment	Flowering	Harvest	Flowering	Harvest	Flowering	Harvest	Flowering	Harvest	Flowering	Harvest
	N		P		K		Ca		Mg	
	(g kg^{-1})									
Th1	19.1a	8.0bc	3.3a	2.3a	19.0a	16.1a	3.3d	13.4b	3.9c	2.6a
IF1	13.9bc	5.8cd	3.1a	2.1a	18.4a	16.8a	3.6d	14.9b	3.9c	2.7a
Th2	19.7a	6.4cd	3.3a	1.6a	18.2a	9.5c	6.7a	14.6b	4.8ab	2.5a
IF2	17.2ab	9.6ab	3.0a	2.1a	18.2a	14.6ab	5.2bc	13.3b	4.9a	2.9a
Th3	17.5ab	12.2a	3.2a	2.4a	17.8a	18.5a	5.5ab	22.6a	4.5abc	2.5a
C	13.3c	4.8d	3.7a	2.1a	16.5b	11.5bc	3.9cd	16.5b	4.1bc	3.7a
p F test	0.014	< 0.001	NS	NS	0.018	0.006	0.002	0.047	0.045	NS
	Cu		Zn		Fe		Mn		B	
	(mg kg^{-1})									
Th1	11.8a	14.5b	21.3ab	18.6b	675c	355a	59.4c	40.0a	92.1bc	37.8a
IF1	11.6a	19.4a	18.2bc	17.6b	699c	301a	75.7ab	50.0a	88.2bc	37.6a
Th2	8.7b	13.1b	15.7c	11.5d	738bc	355a	82.3a	41.7a	123.9a	27.0a
IF2	13.1a	13.6b	20.9ab	19.3b	976a	467a	78.0ab	48.3a	82.4c	36.6a
Th3	13.7a	15.2b	22.8a	26.9a	902ab	455a	71.5abc	46.4a	100.9b	41.4a
C	8.8b	13.5b	17.9bc	14.7c	732c	348a	68.3bc	56.6a	80.9c	57.6a
p F test	0.006	0.007	0.039	< 0.001	0.010	NS	0.039	NS	< 0.001	NS

Th1, Semi-organic fertilizers by foliar application; IF1, Conventional inorganic fertilizers by foliar application; Th2, Semi-organic fertilizers by soil application; IF2, Conventional inorganic fertilizers by soil application; Th3, Mixture of plant extracts as biostimulant by soil application; C, Water application (control)

NS Non-significant. Within each growth stage and element, different letters indicate significant differences among means, employing the protected LSD test, at $p \leq 0.05$

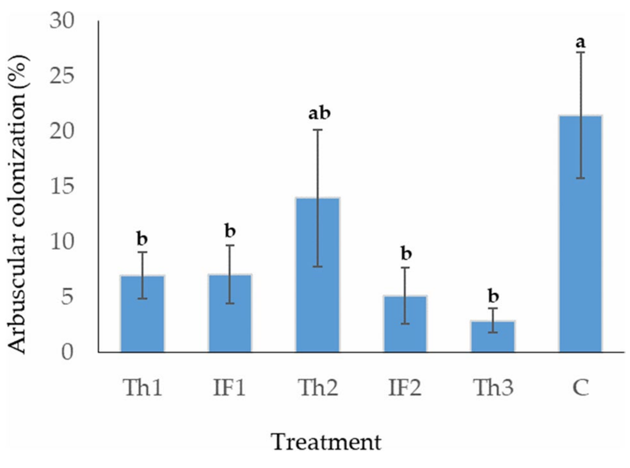

Fig. 7 Arbuscular mycorrhizal fungi root colonization percentage of *Origanum microphyllum* plants established in pilot cultivation with Th1: semi-organic fertilizers by foliar application; IF1: conventional inorganic fertilizers by foliar application; Th2: semi-organic fertilizers by soil application; IF2: conventional inorganic fertilizers by soil application; Th3: mixture of plant extracts as biostimulant by soil application; C: water application (control). Values represent means ± standard errors ($n = 3$). Different letters indicate significant differences among means, employing the protected LSD test, at $p \leq 0.05$

syriaca), as well as the positive correlation patterns between Fe and Mn, or Cu and Zn, respectively (recorded in all of the studied taxa). The above-mentioned results are in a general agreement with other studies reported in the literature, whereas they could be attributed to factors such as the common source for the element pairs in the case of positive connections (Ibourki et al. 2022), or the tendency of elements with similar physicochemical properties to share or compete for pathways or transport systems accumulating them in leaves (Watanabe et al. 2015; Zhang et al. 2021). More specifically, it is known that under conditions of reduced K availability, antagonistic interactions between K and Mg might occur in plant tissues (Xie et al. 2021), whereas the positive correlations between B and Ca can be attributed to the fact that B tends to keep Ca in a soluble form within the plant (Tariq and Mott 2006).

On the other hand, we assume that biostimulant application alone (Th3) or in combination with semi-organic fertilizers (Th1, Th2) could have offered an excess of humic substances in direct contact with the plants' above-ground parts or indirectly in the soil rhizosphere as mentioned also in other studies (Franzoni et al. 2022). Many studies have reported that treatments with humic substances can

Table 6 Macro- and micronutrients' concentrations in leaves and aboveground biomass, at the flowering and harvest stage, respectively, of *Sideritis syriaca* subsp. *syriaca* plants established in pilot cultivation

Treatment	Flowering	Harvest	Flowering	Harvest	Flowering	Harvest	Flowering	Harvest	Flowering	Harvest
	N		P		K		Ca		Mg	
	$(\mathrm{g\ kg^{-1}})$									
Th1	8.8c	11.3a	2.0a	2.1ab	13.2c	12.7a	3.4b	20.3b	4.6ab	6.1a
IF1	10.5bc	11.3a	2.1a	2.2a	17.4ab	10.4abc	1.3c	21.6b	3.4c	5.9a
Th2	12.7a	7.2b	2.4a	1.8bc	14.0c	8.2c	5.4a	23.4b	5.2a	6.2a
IF2	11.5ab	10.6a	2.1a	1.8bc	16.9ab	8.9bc	2.6bc	25.1ab	4.8ab	6.3a
Th3	10.1bc	10.1a	2.0a	1.7c	15.5bc	10.8ab	3.6b	30.6a	4.6ab	5.9a
C	11.8ab	12.1a	2.6a	1.7c	18.1a	10.1bc	2.8bc	21.7b	4.0bc	6.2a
p F test	0.007	0.031	NS	0.021	0.007	0.029	0.003	0.040	0.016	NS
	Cu		Zn		Fe		Mn		B	
	$(\mathrm{mg\ kg^{-1}})$									
Th1	12.7b	19.5b	16.1b	24.1b	1019ab	2190cd	71.6bc	71.7d	48.5abc	28.7a
IF1	12.2b	28.3a	18.1b	27.4a	725bc	2552ab	81.4ab	90.5cd	46.6bc	30.6a
Th2	10.3bc	17.1bc	15.3b	19.9c	708bc	2295bc	69.8c	125.5ab	58.0a	34.9a
IF2	18.1a	20.5b	22.6a	23.9b	1222a	2656a	83.3a	132.1a	41.1c	31.3a
Th3	14.4ab	17.5bc	25.0a	20.9bc	1138ab	1862d	86.3a	92.9cd	57.1ab	31.6a
C	7.8c	14.5c	14.7b	20.1c	469c	2121cd	68.4c	105.8bc	44.1c	26.2a
p F test	0.004	<0.001	<0.001	0.002	0.038	0.002	0.007	<0.001	0.024	NS

Th1, Semi-organic fertilizers by foliar application; IF1, Conventional inorganic fertilizers by foliar application; Th2, Semi-organic fertilizers by soil application; IF2, Conventional inorganic fertilizers by soil application; Th3, Mixture of plant extracts as biostimulant by soil application; C, Water application (control)

NS non-significant. Within each growth stage and element, different letters indicate significant differences among means, employing the protected LSD test, at $p \leq 0.05$

stimulate the growth and development of plant roots (Shah et al. 2018; García et al. 2019; Nardi et al. 2021), while such effects are usually attributable to the improvement in the absorption of nutrients and water as well as to their effect on plant metabolism (Canellas et al. 2015). In soil alkaline conditions in which the high pH values might inhibit the absorption of micronutrients, the beneficial role of biostimulants could be also attributed to their capacity in reducing pH of the rhizosphere, thus facilitating the uptake pathways by the root system. The above could also stand in the case of K considering that a solubilizing activity by biostimulant application could have promoted the release of non-exchangeable K by primary minerals such as K-feldspars or micas (Pramanik et al. 2019). Moreover, the addition of humic substances in soils can enhance plant uptake of metallic nutrients by promoting their solubility due to (i) the chelating capacity of the containing soluble phenols which act as leaching promoters and thus blocking metals' sorption in the solid phase (Madrid and Díaz-Barrientos 1998) or (ii) their ability to influence redox processes in soils resulting in the release of reduced elements which are highly soluble (Madrid and Díaz-Barrientos 1994; de la Fuente et al. 2011; Pardo et al. 2017).

We speculate that some of these mechanisms alone or in combination have probably acted synergistically offering enhanced bioavailability pathways in the studied Cretan plants, while the above could explain the detected increase in yield that the Th3 application caused to *C. diae* or *O. microphyllum*.

Nevertheless, how humic substances affect plant physiology is still questionable due to the molecular complexity of these substances or the variability of plant responses driven by their application. Thus, the above-mentioned speculations should be further investigated in the future.

Concerning the potential preference of the studied taxa in terms of foliar or soil application method, no clear conclusions could be derived from this study, since each species or subspecies responded uniquely to the applied fertilization methods. On the other hand, more evident patterns of species-specific selectivity in inorganic or semi-organic fertilization schemes were observed, whereas the respective mechanisms responsible for these results can also be speculated. In the case of *O. microphyllum* for example, N, K and Zn inputs resulted in positive yield effects under the inorganic fertilization schemes, while in a noteworthy way the application of biostimulant (Th3) also produced equally

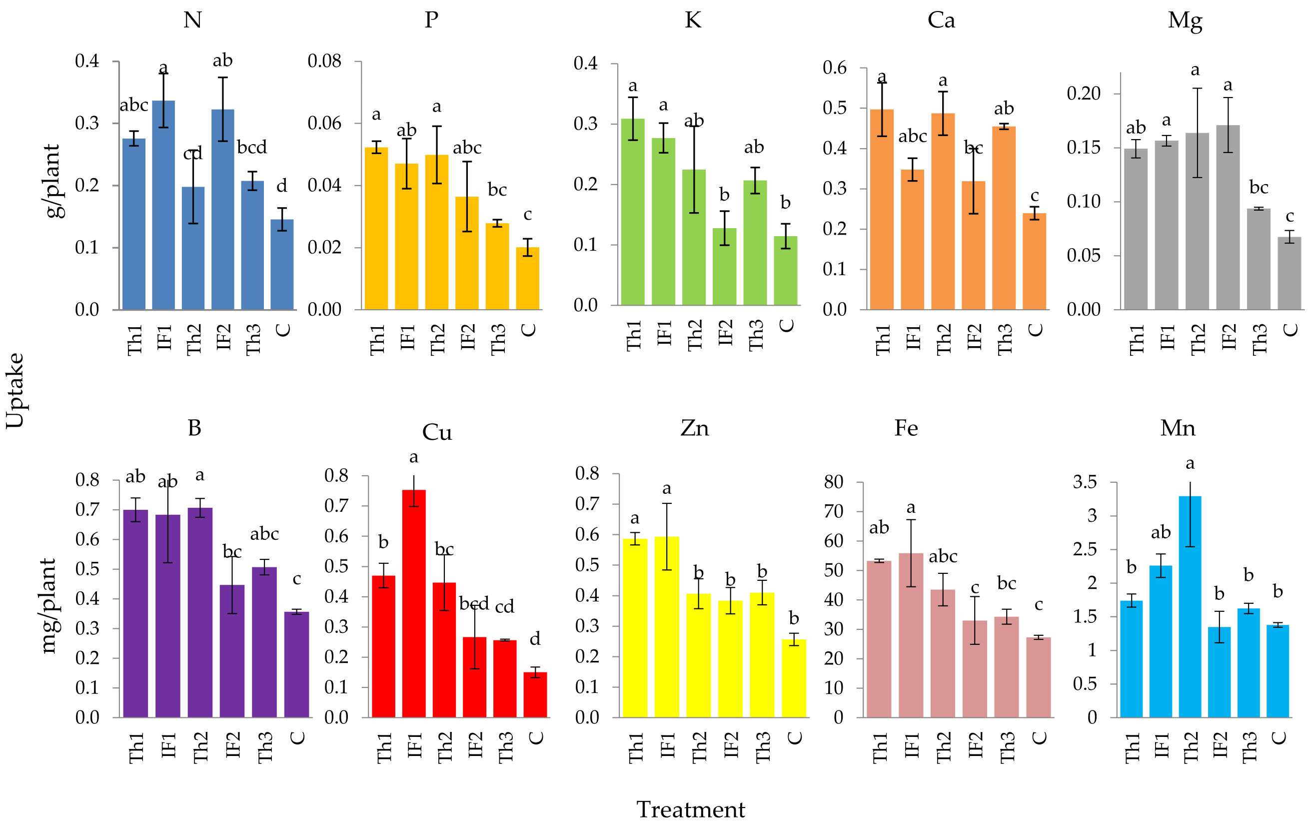

Fig. 8 Macro- and micronutrients' uptake by *Sideritis syriaca* subsp. *syriaca* plants established in pilot cultivation with Th1: semi-organic fertilizers by foliar application; IF1: conventional inorganic fertilizers by foliar application; Th2: semi-organic fertilizers by soil application; IF2: conventional inorganic fertilizers by soil application; Th3: mixture of plant extracts as biostimulant by soil application; C: water application (control). Values represent means ± standard errors ($n=3$). Within each element, different letters indicate significant differences among means, employing the protected LSD test, at $p \leq 0.05$

positive results. On the contrary, Th2 treatment did not manage to trigger corresponding results. These data are in agreement with the reduction in AMF colonization in the treatments with positive yield effects and elevated N, K or Zn inputs. Although it is elevated P that is known to reduce AMF colonization on roots, elevated N has also been shown to reduce AMF root colonization at high N:P ratios (Treseder 2004; Blanke et al. 2005). This also agrees with the negative correlation between N and AMF colonization as detected herein.

We assume that the co-application of biostimulant with the semi-organic fertilization treatment by soil (Th2) caused an increase in the already high levels of B concentration at the flowering stage (123.9 mg kg^{-1}), which in turn acted as an inhibitory factor on Zn accumulation (Sarafi et al. 2018). The above was substantially reflected at the final harvest stage, in which Zn concentration was recorded at remarkably low and probably deficient levels (11.5 mg kg^{-1}). Inversely, inorganic fertilization schemes as well as the biostimulant application alone managed to increase both N and Zn uptake (especially in the case of IF2, and Th3 treatments) and additionally managed to promote K.

However, recent studies have reported conflicting results while referring to the pilot cultivation of Cretan endemic species (*Verbascum arcturus, O. microphyllum*) using the same fertilizer/biostimulant products on comparable fertilization schemes. Paschalids et al. (2021) observed that while *V. Arcturus* was generally unaffected by fertilization treatments, both foliar-applied fertilization types improved the levels of Zn and B in leaves, and soil-applied biostimulant increased leaf Ca. On the other hand, in the case of *O. microphyllum*, Fanourakis et al. (2022) found that plants subjected to foliar fertilization in an integrated nutrient management system (similar to Th1 treatment in the present study) had significantly higher leaf Mn content, while biostimulant application resulted in significantly higher leaf B content.

The dissimilarities between the present study's findings and the aforementioned cases can be attributed to the distinct initial properties of the soil utilized as a medium, particularly in terms of soil fertility. The initial soil employed for cultivating *V. Arcturus* and *O. microphyllum* (Paschalidis et al. 2021; Fanourakis et al. 2022) had high concentrations of soil-available macro- and micronutrients, exceeding their sufficiency levels. Conversely, the soil utilized in the current study lacked sufficient soil-available potassium, falling below the critical sufficiency levels, while soil P was marginally sufficient.

The results herein showed that the four Cretan endemic species selected to be studied as alternative crops responded positively to the fertilization regimes tested. There was at least one fertilization treatment that increased the yield of the studied plants causing, however, different patterns between yield and nutrients' uptake or concentrations in leaves at the flowering stage or in the harvested above-ground biomass. In addition, our findings revealed a distinction in plants' multi-elemental stoichiometry, except for the two *Origanum* species studied herein; in these cases, the variance of their ionomics showed a significant overlap. The elements most closely related to the yield parameter were K and Zn, while species-specific Pearson tests showed distinct correlations between the nutrients under investigation. Either biostimulant application alone (Th3) or in combination with semi-organic fertilizers (Th1, Th2) was found to play a substantial role in increasing the yield of *C. diae* and the two studied *Origanum* species. On the other hand, inorganic fertilization schemes (IF1 and IF2) seemed that operated in a beneficial way in the case of *S. syriaca* subsp. *syriaca* and *O. microphyllum*. Nonetheless, no solid conclusions could be derived yet in respect of the preference of the four species for any fertilizers' application method (foliar or by soil), since they responded uniquely to the applied fertilization schemes.

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Author contributions GT, NK and KG conceived the presented idea. GT, EG and KG supervised the field work. FB, II, ES and TM conducted soil, plant analysis and AMF colonization determination. FB, ES, II and TM worked on the data collection and analysis. FB wrote the first draft of the manuscript. FB, TM, II, ES, NK, GT, KG and EG revised this manuscript. All authors provided critical feedback on the manuscript.

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Data availability All data are included in this article (and its supplementary information files).

Code availability Not applicable.

Declarations

Conflict of interests The authors declare that they have no conflict of interest.

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