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Article

The assessment and the within-plant variation of the morpho-physiological traits and VOCs profile in endemic and rare Salvia ceratophylloides Ard. (Lamiaceae)

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Abstract: *Salvia ceratophylloides* (Ard.) is an endemic, rare, threatened plant species recently rediscovered in very few individuals in two different sites of South Italy. The study of within-plant variation more than among-plant one is fundamental to understand the plant adaptation to the local conditions, especially in rare species, and consequently to preserve plant biodiversity. Here, we reported the variation of the morpho-ecophysiological and metabolic traits between the sessile and petiolate leaf of *S. ceratophylloides* plants in two different sites for understanding the adaptation strategies for surviving in these habitats. The *S. ceratophylloides* individuals exhibited different net photosynthetic rate, maximum quantum yield, light intensity for the saturation of the photosynthetic machinery, stomatal conductance, transpiration rate, leaf area, fractal dimension and some VOCs between the different leaf types. This within-plant morpho-physiological and metabolic variation was depended on the site. These results provide empirical evidence of sharply within-plant variation of the morpho-physiological traits and VOCs profiles in *S. ceratophylloides* which could be because of adaptation to the local conditions.

Keywords: Gas exchanges, LMA, rare species, *Salvia ceratophylloides* Ard., VOC, within-plant plasticity

1. Introduction

Salvia ceratophylloides Ard. is a perennial herbaceous plant, endemic to Southern Calabria, declared as extinct in 1997 [1] and recently rediscovered in a hundred mature individuals distributed in two sites, 2 Km apart, around the Reggio Calabria hills [2, 3]. Nowadays, the *S. ceratophylloides* populations are threatened with extinction because of the habitat modification and destruction by wild urbanization and agriculture [3]. The spread of alien species, favored by climate change, establishes an additional threat factor [4, 5]. Despite its vulnerability, no quantitative information on the morphological and ecophysiological traits of *S. ceratophylloides* are actually available, although they are very important for understanding the habitat requirements for the conservation of the endemic species.

The viability of endangered and rare species, as *S. ceratophylloides*, depends on their capability to maintain or even increase its fitness under short- and/or long-term continuous and climate change. Because of its rarity, this endemic species usually pointed out specific and narrow habitat requirements suggesting that their responses must occur only

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in the actual habitat determining local adaptation through phenotypic plasticity and/or genetic variation [6].

Although the results appeared sometimes contrasting, the plant phenotypic plasticity assumed a significant role in the viability of rare and endangered species. For example, Noel et al. [7] observed a high degree of phenotypic plasticity that conferred an increased fitness in *Ranunculus nodiflorus* Ten. suggesting the maintenance of the micro-environment heterogeneity as habitat-based strategy for its conservation. By contrary, Westerband et al. [8] observed low phenotypic plasticity in response to drought stress in Schiedea obovate (Sherff) W.L. Wagner & Weller, an endangered, endemic Hawaiian shrub, showing a high risk of extinction in the future climate change scenarios. Based from earlier works of the Winn [9, 10] and the De Kroon's hypothesis [11], which dealt with plant phenotypic variation at sub-individual level (i.e. organ or module), recently the 'within-plant' rather than "among-plant" phenotypic variation could represent the major source of population-level diversity in several functional traits [12, 13]. Recent works pointed out the multiple ecological aspects of the 'within-plant variation' such as the improvement of the exploitation of the heterogeneous-distributed resource [14, 15], the adaptation to biotic and biotic gradients [16], the spreading of the ecological breadth of species and individuals [17, 18], the increase of the functional diversity of populations [17], and the alteration of plant-antagonist interactions [19-22]. In spite, no quantitative characterization of the within-plant variation in endangered and rare plant species has been tested yet.

In this respect, we started a two years field study for the characterization of the *S. ceratophylloides* by leaf-level morpho-ecophysiological and metabolic analysis from its natural habitat. In particular, this species exhibited a leaf morphology characterized by contemporary presence (petiolate leaf) and absence of petiole (sessile leaf), which could suggest a potential within-plant variation. Further, the individuals of this species were in two different sites, distant < 2 km each other, suggesting no genetic variability between the two populations [23] and indicating the phenotypic plasticity as the most driver for the local adaptation. In particular, we focused on the photosynthetic performances as a marker of tolerance and growth of species to predict the optimal habitat conditions for the conservation of rare species [24] but also to provide the capacity of plant adaptation to new conditions associated with climate change and the likely changes in plant communities [25]. Further, we took in account the leaf mass per area (LMA) as a morphological trait strictly correlated with the functional syndrome and consequently to plant growth and development and, ultimately, plant fitness [26]. At the same time, the metabolic profiles of volatile organic compound (VOC) could provide information on the plant status and the plant-, microbial- and arthropode-plant communications [27]. The evaluation of all these functional traits could offer useful information for effectively promoting translocation and mitigation operations to restore *Salvia ceratophylloides* rare plant species.

In this framework, the present study has been addressed to assess the morpho-ecophysiological and metabolic traits of *S. ceratophylloides* from its natural habitat and to investigate about the following questions: 1) does the within-plant variation of the photosynthetic performance, morphological traits and metabolic profiles occur? 2) Is the within-plant morpho-physiological and metabolic variation of *S. ceratophylloides* modified between the two localities?.

2. Results

2.1. Physiological performances and morphological traits of S. ceratophylloides

The net photosynthetic rate (P_N) and the photosynthetic photon flux density (I) curves of *S. ceratophylloides* leaves were well-fitted ($R^2 > 0.95$ and $p < 0.05$) and –described by Ye mathematical model [28] (Figure 1). The parameters estimated by non-linear regression have been reported in Table 1. As observed, the leaf type produced a significant and strong effect on the most photosynthetic parameters. In particular, the sessile leaves had a higher I_{sat} (1578 μmol(photon) m^{-2} s^{-1}), I_{max} (766 μmol(photon) m^{-2} s^{-1}), P_{Nmax} (11.22 μmol(CO_2) m^{-2} s^{-1}) and $\phi(I_0 - I_{comp})$ (0.030 μmol(CO_2) μmol(photon) $^{-1}$) level than petiolate

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ones which showed a lower dark respiration rate (R_D) (0.69 μmol(CO_2) m^{-2} s^{-1}). This photosynthetic pattern has been observed in both sites except for the P_{Nmax}, which has been statistically different between the two leaf types in relation to the site ($p < 0.05$ LT × Sit interaction, Table 1): the petiolate leaves (6.85 μmol(CO_2) m^{-2} s^{-1}) showed lower value of P_{Nmax} respect to the sessile ones (19.70 μmol(CO_2) m^{-2} s^{-1}) at Mo site. Further, the significant LT × Sit interaction observed for the I_{comp} and R_D indicated the parameters difference between the leaf types has been affected by site factor. Indeed, the I_{comp} of the sessile leaf was higher than petiolate one (8 vs 22 μmol(photon) m^{-2} s^{-1}) at Mo site only, and same pattern has been observed for the R_D (0.53 vs 1.40 μmol(CO_2) m^{-2} s^{-1}) (Table 1).

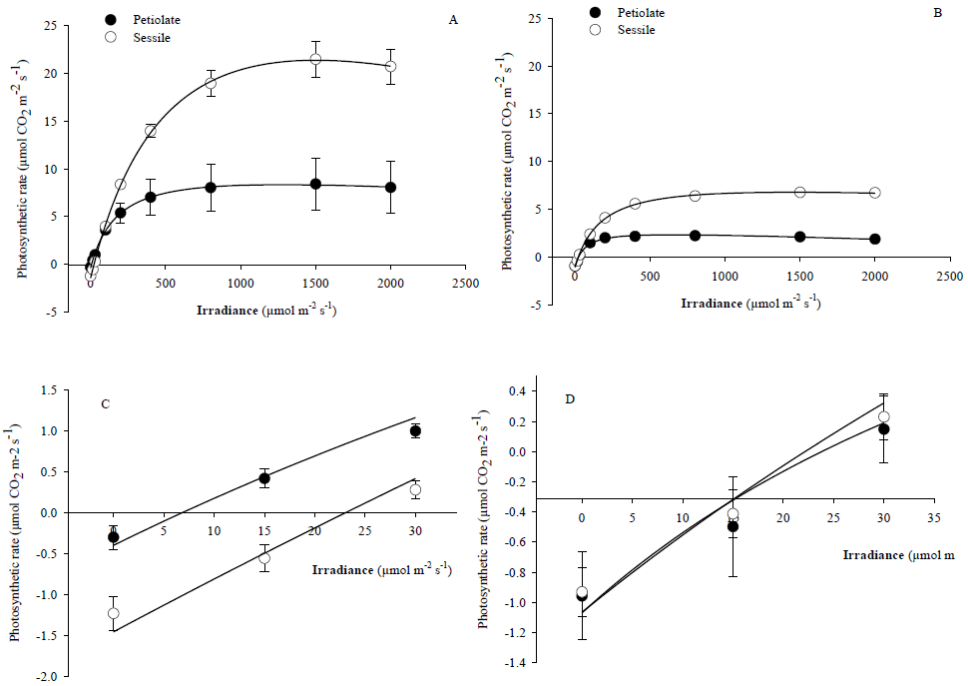

Figure 1. Leaf photosynthetic light-response curves measured on petiolate (●) and sessile leaves (○) of the *Salvia ceratophylloides* at (A and C) Mosorrofa site (Mo) and (B and D) Puzzi site (Pu). The C and D panels showed the curves at the lowest irradiance values. Data points represent means (n = 4-9). Light curves have been fitted by non-linear regression using the Ye et al. model [28].

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Table 1 – Leaf-level photosynthetic parameters of different leaf types (P: petiolate; S: sessile) of *Salvia ceratophylloides* individuals of two sites (Mosorrofa, Mo; Puzzi, Pu) estimated by nonlinear regression using the Ye et al. model [28]. Different lowercase letters represent significant differences at $p < 0.05$ among the average within column (Tukey's test). Different capital case letters represent statistically significant differences among the means along the rows ($p < 0.05$, Tukey's test).

		Site (Sit)		
	Leaf type (LT)	**Mo**	**Pu**	**Leaf type average**
Icomp	P	8^{b}	26^{a}	***18*** ^{x}
[μmol(photon) m^{-2} s^{-1}]	S	22^{a}	22^{a}	***22*** ^{x}
	Site average	***16*** ^{A}	***23*** ^{A}	
Imax	P	312^{b}	310^{b}	***311*** ^{y}
[μmol(photon) m^{-2} s^{-1}]	S	839^{a}	725^{a}	***766*** ^{x}
	Site average	***655*** ^{A}	***577*** ^{A}	
I_{sat}	P	1027^{b}	818^{b}	***911*** ^{y}
[μmol(photon) m^{-2} s^{-1}]	S	1559^{a}	1588^{a}	***1578*** ^{x}
	Site average	***1323*** ^{A}	***1313*** ^{A}	
$P_{N(Imax)}$	P	6.85^{b}	2.17^{b}	***4.25*** ^{y}
[μmol(CO_2) m^{-2} s^{-1}]	S	19.70^{a}	6.51^{b}	***11.22*** ^{x}
	Site average	***14.00*** ^{A}	***4.96*** ^{B}	
R_D	P	0.53^{b}	1.07^{ab}	***0.69*** ^{y}
[μmol(CO_2) m^{-2} s^{-1}]	S	1.40^{a}	1.09^{ab}	***1.19*** ^{x}
	Site average	***1.01*** ^{A}	***1.08*** ^{A}	
$\phi_{(I0\text{-}comp)}$	P	0.025^{b}	0.0095^{c}	***0.016*** ^{y}
[μmol(CO_2)	S	0.046^{a}	0.021^{b}	***0.030*** ^{x}
μmol(photon) $^{-1}$]	***Site average***	***0.037*** ^{A}	***0.016*** ^{B}	

#Statistical analysis: two-way ANOVA with 4-9 replications (LT: leaf type; Sit: sites; LTxSit: Leaf type x Sites interaction); *0.05 > $p < 0.01$; **0.01 > $p < 0.001$; ***0.001 > p; NS: not significant.

Figure S3 showed the comparisons of the Isat and the I_{comp} of *S. ceratophylloides* with those of different functional plant groups. The minimum Isat value of *S. ceratophylloides* fell between that of schlerophylls in habitat at a high light intensity and heliophytes, while the maximum one was within the range between heliophytes and C4 plants. The minimum I_{comp} value of *S. ceratophylloides* was between epiphytes and spring geophytes, while the maximum one was between spring geophytes and heliophytes.

Table 2 and 3 reported the stomatal conductance and the transpiration rate of both leaves of *S. ceratophylloides* measured at 200 and 800 μmol(photon) m^{-2} s^{-1} corresponding to the light intensities around to the Imax values of the P and S leaves, respectively. Similar to the photosynthetic pattern, the P leaves pointed out a significant lower stomatal conductance and transpiration rate than S at both light intensities. But this effect was different between the sites ($p < 0.01$ for LT × Sit interaction, Table 2 and 3): the S leaves showed higher levels of stomatal conductance and transpiration rate respect to the P ones only in the Mo site while any difference between the leaf type has been produced in Pu site. The effect of the site was highly significant ($p < 0.001$, Tables 2 and 3) for both ecophysiological parameters, with the Mo site showing the higher values than Pu one.

The leaf morphology of *S. ceratophylloides* has been reported in Table 4. Leaf type affected the leaf fresh weight, leaf area, leaf water content, and fractal dimension, which were higher in petiolate leaves (Table 4). This pattern, however, has been modified in relation to the site for the LFW and LA only with higher values in the petiolate in Pu site

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only ($p < 0.05$ LT × Sit interaction, Table 4). Finally, the site factor affected the LFW, the LDW, and the LWC with higher values in the Mo site (Table 4).

Table 2 – Leaf-level stomatal conductance and transpiration rate of different leaf types (P: petiolate; S: sessile) of *Salvia ceratophylloides* individuals of two sites (Mosorrofa, Mo; Puzzi, Pu) measured at light intensity of 200 μmol m^{-2} s^{-1}. Different lower-case letters represent significant differences at $p < 0.05$ among the average within the column (Tukey's test). Different capital case letters represent statistically significant differences among the means along the rows ($p < 0.05$, Tukey's test).

		Sites (Sit)		
	Leaf type (LT)	**Mo**	**Pu**	**Leaf type average**
Stomatal conductance	P	0.032^{b}	0.016^{b}	***0.023^{y}***
(mol H_2O m^{-2} s^{-1})	S	0.113^{a}	0.029^{b}	***0.059^{x}***
	Site average	***0.077^{A}***	***0.025^{B}***	
Transpiration rate	P	0.87^{b}	0.55^{b}	***0.69^{y}***
(mol H_2O m^{-2} s^{-1})	S	2.59^{a}	0.92^{b}	***1.52^{x}***
	Site average	***1.82^{A}***	***0.79^{B}***	

#Statistical analysis: two-way ANOVA with 4-9 replications (LT: leaf type; Sit: sites; LT × Sit: Leaf type × Sites interaction); *0.05 > p < 0.01; **0.01 > p < 0.001; ***0.001 > p; NS: not significant.

Table 3 – Leaf-level stomatal conductance and transpiration rate of different leaf types (P: petiolate; S: sessile) of *Salvia ceratophylloides* individuals of two sites (Mosorrofa, Mo; Puzzi, Pu) measured at light intensity of 800 μmol m^{-2} s^{-1}. Different lower-case letters represent significant differences at $p < 0.05$ among the average within the column (Tukey's test). Different capital case letters represent statistically significant differences among the means along the rows ($p < 0.05$, Tukey's test).

		Sites (Sit)		
	Leaf type (LT)	**Mo**	**Pu**	**Leaf type average**
Stomatal conductance	P	0.032^{b}	0.016^{b}	***0.023^{y}***
(mol H_2O m^{-2} s^{-1})	S	0.107^{a}	0.032^{b}	***0.059^{x}***
	Site average	***0.074^{A}***	***0.026^{B}***	
Transpiration rate	P	0.83^{b}	0.55^{b}	***0.67^{y}***
(mol H_2O m^{-2} s^{-1})	S	2.44^{a}	1.03^{b}	***1.53^{x}***
	Site average	***1.72^{A}***	***0.86^{B}***	

#Statistical analysis: two-way ANOVA with 4-9 replications (LT: leaf type; Sit: sites; LTxSit: Leaf type × Sites interaction); *0.05 > p < 0.01; **0.01 > p < 0.001; ***0.001 > p; NS: not significant.

Table 4 – Biometric and morphological parameters of different leaf types (P: petiolate; S: sessile) of *Salvia ceratophylloides* individuals of two sites (Mosorrofa, Mo; Puzzi, Pu). Different lower-case letters indicated significant differences at $p < 0.05$ among the

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average within the column (Tukey's test). Different capital case letters indicated statistically significant differences among the means along the rows ($p < 0.05$, Tukey's test).

		Sites (Sit)		
	Leaf type (LT)	**Mo**	**Pu**	**Leaf type average**
Leaf fresh weight	P	1.33^{a}	1.38^{a}	***1.36^{x}***
[g leaf^{-1}]	S	1.40^{a}	0.43^{b}	***0.78^{y}***
	Site average	***1.37^{A}***	***0.77^{B}***	
Leaf dry weight	P	0.23^{a}	0.22^{b}	***0.22^{x}***
[g leaf^{-1}]	S	0.27^{a}	0.12^{b}	***0.17^{x}***
	Site average	***0.25^{A}***	***0.16^{B}***	
Leaf area	P	41.4^{ab}	54.4^{a}	***48.6^{x}***
[cm^{2}]	S	43.6^{ab}	19.9^{b}	***28.3^{y}***
	Site average	***32.2^{A}***	***42.6^{A}***	
Leaf mass x area	P	55.4^{a}	44.1^{a}	***49.1^{x}***
[g m^{-2}]	S	62.3^{a}	61.6^{a}	***61.8^{x}***
	Site average	***59.2^{A}***	***55.3^{A}***	
Leaf dry content	P	0.17^{a}	0.18^{a}	***0.18^{x}***
[g dry weight g^{-1}	S	0.19^{a}	0.28^{a}	***0.25^{x}***
fresh weight]	***Site average***	***0.18^{A}***	***0.24^{A}***	
Leaf water content	P	0.027^{a}	0.020^{a}	***0.023^{x}***
[g H_2O cm^{-2} leaf	S	0.025^{b}	0.016^{b}	***0.019^{y}***
area]	***Site average***	***0.026^{A}***	***0.017^{B}***	
Fractal dimension	P	1.67^{a}	1.73^{a}	***1.70^{x}***
	S	1.51^{b}	1.65^{b}	***1.56^{y}***
	Site average	***1.69^{A}***	***1.59^{A}***	

#Statistical analysis: two-way ANOVA with 4-9 replications (LT: leaf type; Sit: sites; LT × Sit: Leaf type × Sites interaction); *0.05 > p < 0.01; **0.01 > p < 0.001; ***0.001 > p; NS: not significant.

Figure S4 pointed out the value average (± SD) of LMA of *S. ceratophylloides* in comparison with that of the sun- and shade-species herbs, evergreen angiosperm, evergreen species, herbs, and different *Salvia* species. The LMA range of *S. ceratophylloides* fell in that of the herbs, evergreen species, *S. mellifera*, *S. hispanica*, *S. officinalis*, and sun species.

2.2. VOCs analysis of Salvia ceratophylloides in its habitat

Figure 2 showed the PCA of GC-MS spectra of different leaves and sites of *Salvia ceratophylloides*. The two-dimensional PCA score plot revealed a separation in VOCs profile induced by leaf type, but this difference was more clear in Mo site than Pu one. The VOCs profile was also different between the two *Salvia* sites.

In table S1 are summarized the VOCs detected by GC-MS: 39 compounds have been identified of which the most representative chemical class was the monoterpene with 17 constituents followed by sesquiterpene (7 chemicals), monoterpene alcohol (4), aldehyde (4), keton (3), alcohol (2), aliphatic esters (1) and ether (1).

Comparing the amount of the XCMS-extracted peak intensities of each chemical between the S and P leaf types, 13 compounds emitted by both P and S were statistically different (Table 5). In particular, p-Cymene, Sabinene, Terpinolene, β-Pinene, γ-Terpinene, α-Terpineol, α-Cubebene, α-Muurolene, Isovaleraldehyde, 5-Methylheptan-3-one, Pentan-3-one, β-tujone, and Dimethyl Sulfide were higher in S than P leaves. However, the higher emission of β-tujone and α-Terpineol in S leaves has been only observed in Mo site (significant LT × Sit interaction, Table 5) and the same pattern has beee revealed

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for D-germacrene, which was not modified by both leaf type and site as single factors. Only 6 compounds were differently affected by sites: p-Cymene, α-Terpineol, α-Copaene and α-Cubebene have been emitted in Pu more than Mo site which, conversely, produced more β-tujone and Dimethyl Sulfide (Table 5).

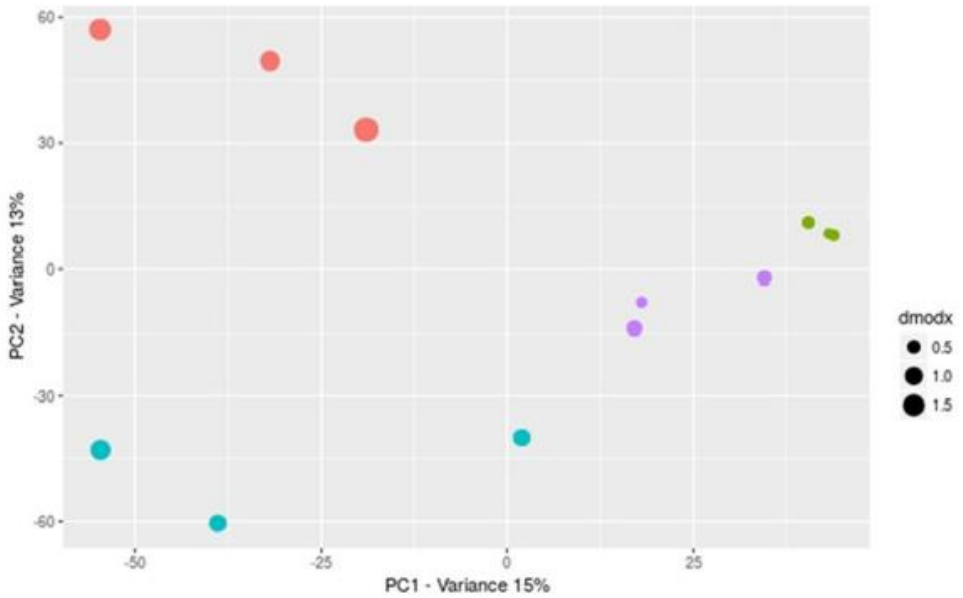

Figure 2 - Principal component analysis of untargeted metabolomics data from different leaves (sessile and petiolate) and sites (Mo and Pu) of *Salvia ceratophylloides* individuals: Mo-sessile (red), Mo-petiolate (green), Pu-sessile (blue) and Pu-petiolate (purple)

Table 5 - Chemical characterization of volatile organic compounds in fresh sessile and petiolate leaves of two sites (Mosorrofa, Mo; Puzzi, Pu) of *Salvia ceratophylloides* plants.

	Compound	**#Statistics**	**Sessile**		**Petiolate**	
			Pu	**Mo**	**Pu**	**Mo**
1	p-Cymene	**LT 7.78*** **Sit 14.16**** LT × Sit 0.21^{NS}	195813	82392	108569	19607
2	Pinocarvone	LT 0.04^{NS} **Sit 6.57*** LT × Sit 0.07^{NS}	2406	987	2444	696
3	Sabinene	**LT 11.80**** Sit 0.34^{NS} LT × Sit 1.70^{NS}	554775	873306	195242	73210
4	Terpinolene	**LT 12.40**** Sit 0.40^{NS} LT × Sit 3.09^{NS}	128554	218320	62198	19784
5	β-Pinene	**LT 7.30***	92968	150052	53391	35502

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		Sit 0.47^{NS} LT × Sit 1.73^{NS}				
6	γ-Terpinene	**LT 5.40*** Sit 0.19^{NS} LT × Sit 0.04^{NS}	16341	13366	4610	3508
7	α-Terpineol	**LT 8.13*** **Sit 12.91**** **LT × Sit 9.04***	10220^{b}	80003^{a}	11854^{b}	18054^{b}
8	D-Germacrene	LT 0.11^{NS} Sit 3.47^{NS} **LT × Sit 4.22***	2554^{a}	169^{b}	1102^{a}	1218^{a}
9	α-Copaene	LT 0.62^{NS} **Sit 11.05*** LT × Sit 0.67^{NS}	3517	601	2385	625
10	α-Cubebene	**LT 8.21*** **Sit 19.35**** LT × Sit 1.02	4460201	1371705	2247264	312465
11	α-Muurolene	**LT 9.49*** Sit 0.56^{NS} LT × Sit 0.99^{NS}	14382	15236	7105	1038
12	Isovaleraldehyde	**LT 6.10*** Sit 0.52^{NS} LT × Sit 0.52^{NS}	81876770	46391341	3426789	3466464
13	5-Methylheptan-3-one	**LT 5.70*** Sit 0.21^{NS} LT × Sit 0.08^{NS}	7776	8204	1578	3291
14	Pentan-3-one	**LT 7.73*** Sit 2.44^{NS} LT × Sit 1.20^{NS}	321989	649080	114170	171753
15	β-tujone	**LT 17.37**** **Sit 6.21*** **LT × Sit 12.54****	65370^{b}	168599^{a}	54660^{b}	36692^{b}
16	(3z)-3-Hexenyl acetate	LT 3.58NS Sit 5.09NS **LT × Sit 5.46***	1253^{a}	0^{b}	99^{ab}	122^{ab}

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17	Dimethyl Sulfide	**LT 23.77**** **Sit 5.34*** LT × Sit 1.40^{NS}	29866633	54386837	3926181	11857751

3. Discussion

3.1. The assessment of the morpho-physiological traits of the rare Salvia ceratophylloides Ard.

Salvia ceratophylloides Ard., endemic, rare, and critically endangered plant species, has been recently rediscovered on some sites around the Reggio Calabria hills [2, 3]. The knowledge of the morphological and ecophysiological traits are very important for understanding the habitat requirements for the conservation of the endemic species [24] but also for providing their capacity to adapt to new environmental conditions associated with climate change and consequently to the plant communities distribution [25]. The responses of these traits in *Salvia ceratophylloides* are reported here for the first time. In particular, we focused on two functional traits, the photosynthetic light-response curve and the leaf mass per area, which indicate the habitat preferences and responsive to the environmental conditions [24, 29, 30]. The photosynthetic response curves to the PAR photon flux and, in particular, the I_{sat} (818-1588 μmol(photon) m^{-2} s^{-1}) and I_{comp} values (8-26 μmol(photon) m^{-2} s^{-1}) suggested that *S. ceratophylloides* is well adapted to the sunny habitat. Indeed, the minimum and maximum values of the Isat fell within the range defined by sclerophyll of sunny habitat and C4 plants (Figure S3) and, the Icomp values have been included between spring geophytes and heliophytes (Figure S3). The LMA values (44.1-55.4 g m^{-2}) of *S. ceratophylloides* are comprised in the LMA range of the herbs [29] and evergreen species [30] (Figure S4). Among the different *Salvia* species, the LMA of *S. ceratophylloides* was similar to that of *S. mellifera*, *S. hispanica* and *S. officinalis* [31-33] but lower than that of *S. mohavensis* and *S. dorrii var. dorrii* [31] and higher than that of *S. glutinosa* and *S. pratensis* [34, 35] (Figure S4). The different range of the LMA of *S. ceratophylloides* respect to that of some *Salvia* species, have been probably correlated with its functional response to the environmental conditions, such as water and light availability [29, 30]. For example, the higher LMA value in *S. mohavensis* and *S. dorrii var. dorrii* was because of the adaptation to their native desert area (mountain ranges of the Mojave Desert of southern California, south-western Nevada, and northern Baja California Norte, Mexico) [31]. Although the *S. pratensis* has been strictly related to *S. ceratophylloides* (belong to the same sect. Plethiosphace: [33]) as distributed to the similar area (native from Europe: [37]), it showed lower LMA value [34] probably because of the different growing conditions of *S. pratensis* (pot and growth chamber) in Mommer's experiments. Finally, the LMA values (57 g m^{-2}) of *S. ceratophylloides* fell in the range of sun species (Figure S4) confirming its preference to the open sunny habitat as for the most *Salvia* species [32, 38].

3.2. Does the within-plant variation of the photosynthetic performance, morphological traits and metabolic profiles occured?

Recently, the within-plant variation as an expression of intraspecific phenotypic plasticity is strongly considered for its role in plant evolution and ecology at individual, population, and community levels [12, 13]. For example, the within-plant variation in leaf morpho-physiological traits allows the adaptation of each individual to optimize i) its capturing structures to the heterogeneous local environmental conditions such as light, temperature and CO_2 gradients within plant canopy in trees [14] and in perennial herbs [17], and ii) its cost-expensive defenses against herbivory and pathogens [39, 40]. Further, the knowledges of the leaf-level photosynthetic performances within the plant allows to scale at canopy level [41] and to understand the competitive strategies for exploring the within-canopy heterogeneous light, and CO_2 availability. In this respect, the within-plant variation of *S. ceratophylloides* by comparing the morpho-physiological and metabolic

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traits of petiole and sessile leaves have been here tested, for the first time at our knowledge.

The *S. ceratophylloides* individuals exhibited a clear within-plant variation determined by the differences in most functional traits considered between the two leaf types (Table 1, 2 and 3 and Fig. 3). Indeed, comparing the leaf types, the S pointed out a better photosynthetic performance by higher net photosynthetic rate and maximum quantum yield, and higher I_{sat} (1578 vs 911 μmol(photon) m^{-2} s^{-1}) but, conversely, they exhibited a lower leaf area and capacity to fill the space as evidenced by FD, respect than the P ones. Hence, a within-plant efficient subdivision of the functional traits for the resource acquisition and use (light, CO_2) has been observed in *S. ceratophylloides* with the short-term low-expensive physiological traits in S leaves (uppermost) and the long-term high-expensive morphological traits in P leaves (lowest). Hence, the leaves placed at the top of the shoot of *S. ceratophylloides* facing high light intensity, temperature, and vapor pressure deficit, could use higher photon and CO_2 fluxes for increased their carbon gains by high I_{sat} and stomatal conductance. The spatial distribution of more efficient structures or functions within plants to fit the micro-environmental heterogeneous conditions and to maximize the photosynthesis and carbon gain is already known in different plant species [42]. Besides the functional traits linked to the resource acquisition, the two leaf types of *S. ceratophylloides* pointed out difference in the stomatal conductance and transpiration with the S leaf showing higher values (Table 2 and 3). The higher stomatal conductance and transpiration rate limited the leaf overheating, as reported by Lin et al. [43] in dry areas species. The lower aerodynamic resistance caused by the smaller size and shape of the sessile leaves (lower leaf area and fractal dimension) was further beneficial allied for avoiding leaf overheating [43, 44] but also for reducing the water loss by smaller total leaf area. Overall, the leaf position in the *S. ceratophylloides* canopy showed different strategies to cope with the fine-scaled environmental gradients: from higher to lower light intensity and from dry to wet conditions along the uppermost sessile and lowest petiolate leaf gradient.

The within-plant variation of *S. ceratophylloides* has been also observed in the VOCs composition and emission. Indeed, the S and P leaves have been sharply separated by VOC-based metabolic profiles (Figure 3) suggesting different intensity and composition between two leaves. Further, the emission of 14 out of 39 identified VOCs has statistically increased in S leaves respect than P ones (Table 5 and Table S1). The VOCs with higher emission in sessile leaves included monoterpenes (p-Cymene, Sabinene, Terpinolene, β-Pinene, γ-Terpinene, and α-Terpineol), sesquiterpenes (D-Germacrene, α-Cubebene, α-Muurolene) and green leaf volatiles ((3z)-3-Hexenyl acetate) mostly involved in defenses against herbivory and pathogens [45] and in responses to abiotic stress [46]. The within-plant variation of VOCs emission in response to herbivory has been already observed in wild and crop species [47, 48] but no evidence at field level has been reported yet. Why do *S. ceratophylloides* plants defend the S more than P leaves by higher VOCs emission? Probably, the upper, younger, sessile leaves are more protected in views of its high performing photosynthetic machinery and nutritive value (the high leaf dry content, although not statistical support) as suggested by optimal defense hypothesis [39, 40]. Overall, these results pointed out the within-plant functional subdivision at morpho-physiological and metabolic levels of *S. ceratophylloides* mimicking what has been already observed in the wide crown of trees for heat tolerance [49], light acquisition and differential expression of genetic polymorphisms in sun and shade leaves of trees [50] and defense responses to herbivory [51].

The within-plant variation of both morpho-physiological and metabolic traits has been affected by site suggesting that *Salvia* plants have been adapted to local conditions. Indeed, the morpho-physiological patterns of the S and P leaves changed between the two sites for most traits (on 12 that showed the leaf type factor as statistically significant, nine traits pointed out LT × Sit interaction). The S leaves pointed out a higher photosynthetic rate, stomatal conductance, and transpiration rate associated with higher dark respiration and I_{comp} than P ones in the Mo site only. These results could be because of phenotypic plasticity response to the local conditions [52, 53] because preliminary results showed a

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low genetic variability of *S. ceratophylloides* populations [23]. It has been observed that the within-plant phenotypic variation responded to the microhabitat environmental heterogeneity or fine-grained (small-scale) environmental variations [9, 11, 14, 15] rather than macro-geographical or coarse-grained environmental variations [17, 54, 55]. In this respect, we can hypothesize that the *S. ceratophylloides* individuals in the Mo site, showing a statistically significant within-plant variation of the leaf morpho-physiological traits, could face with a higher microhabitat environmental heterogeneity, especially for light intensity and/or temperature gradients (the most important abiotic stresses affecting the leaf growth), than Pu site. Opedal et al. [56] observed that the microhabitat environmental heterogeneity increased with the topographically complex sites changing the intraspecific traits of 16 plant species. The Pu site is characterized by flatter, more open terrains and higher altitude than Mo one, which conversely is placed at a lower altitude at the base of the valley, closed and with rough terrains (Figure 1) likely determining a short duration of light and steeply thermal and light gradients in the latter location.

Unlike the morpho-physiological traits, the within-plant variation of metabolic profiles was lesser affected by the different sites. Indeed, the PCA pointed out that the metabolic profiles of S and P leaves were separate at both sites, and only 4 single VOCs out 13 exhibited a statistically significant Lt × Sit interaction. Considering that the VOCs emission is more involved to the biotic stress (plant-plant, plant-herbivory, and plant-pathogen interactions) [27], probably the *Salvia* plants in both sites are faced to similar biotic environment heterogeneity or variability (predation, competition, etc.) differently to the abiotic ones (light, temperature, etc.), determining thus the maintaining of the same within-plant VOCs emission in *S. ceratophylloides* at both sites.

Finally, we observed that the metabolite profiling and physiological traits (10 out of 10 parameters, considering the LT × Sit interaction also; Table 1, 2 and 3, Figure 3) varied more than morphological features (4 out 7 parameters; Table 4). Why do *S. ceratophylloides* plants choose to invest in the physiological and metabolic capacity more than morphological-related traits between S and P leaves? The physiological plasticity is a low cost and more rapid response with respect to the cost-expensive changes of morphological traits, allowing the expression of the most adequate plant phenotype in response to the more variable climatic conditions. For example, the plant physiological plasticity is more related to an enhanced ability to colonize gaps and open areas and, hence, exploiting the transient environmental resources at low cost by short-term adjustments, briefly the plant acclimatization [47-60]. Conversely, the plant expensive morphological plasticity is more functional for the plant adaptation at the long-term and probably useful for growing in forest understories [57, 61]. The choice to invest in higher plasticity of the physiological traits than morphological ones has been also reported by Herrera et al. [17], which observed that the within-ramet variation in *Helleborus foetidus* L. was more due to the stomatal features than leaf size- and area-related traits causing an increase of seed number produced by each individual [13].

4. Materials and Methods

4.1. Species and sites

Salvia ceratophylloides Ard. has been studied by Crisafulli et al. [2] and Spampinato et al. [3]. Briefly, it is a perennial herb, scapose hemicryptophyte, with woody and upright stems with a dense pubescence of glandular and simple patent hairs (Figure S1C). The leaves are opposite pinnate-partite with toothed lobes and morphologically distinct in petiolate (basal, 12x4 cm long and less discrete pinnate lobes and presence of the petiole) and sessile (cauline, 3-4 × 1-2 cm long and more incise pinnate lobes, clasp the stem) (Figure S1C). Inflorescences are 20-30 cm in length and made up of 5–6 verticillaster each with 4–6 flowers.

S. ceratophylloides plants have been studied in two different and closer sites (< 2 Km) located around the Reggio Calabria hills, Mosorrofa (Mo) (38° 5′ 42.66″ N latitude, 15°43′ 18.66″E longitude) and Puzzi (Pu) (38° 4′ 52.34″ N latitude, 15° 42′ 29.23″E longitude) (Southern Italy) (Fig. S2). Each site consisted in around 60 and 240 individuals for Mosorrofa and Puzzi, respectively. Further, Mosorrofa site is topographically complex and in a little

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valley while Puzzi pointed out open and flatter terrains (Fig. S1A and B). Both sites are characterized by layers of loose sand alternating with benches of soft calcarenites of Pliocene origin. The soils have a sandy texture with a basic pH and fall into the group Calcaric Cambisols [62].

Species identification is in agreement with Pignatti [63] and specimens are deposited in the Herbarium of Mediterranean University of Reggio Calabria (acronym REGGIO).

The *S. ceratophylloides* location pointed out a Mediterranean climate with average annual temperatures of 18°C and average annual rainfall of 600 mm mostly in autumn-winter, and a dry period in summer. According to Rivas-Martínez [64], the macro-bioclimate is "Mediterranean pluviseasonal oceanic" (upper thermos-Mediterranean thermotype and a lower subhumid ombrotype).

4.2. Measurements and samplings

Measurements and samplings have been conducted during the early summer (May-June) of 2016 and 2017 on two leaf types: 1) upper sessile leaves (S) (two to three nodes from the apex) and 2) lower petiolate leaves (P) (from fifth to sixth nodes from the apex) (Figure 1C). For the morphological and physiological analysis, 4-9 and 5-9 samples for petiolate and sessile leaves, respectively, have been collected from each site; while the metabolic analysis has been carried out on 3 leaves of each type and site.

4.3. Physiological analysis

The net photosynthetic light response curves have been determined by the LI-6400XT portable photosynthesis system (Li-Cor, Inc. Lincoln, Nebraska, USA) at the following irradiance levels: 2000, 1500, 800, 400, 200, 100, 30, 15, and 0 μmol m^{-2} s^{-1}. The net photosynthesis has been measured at 500 cm3 min^{-1} flow rate, 26 °C leaf temperature, CO_2 concentration 400 μmol(CO_2) mol(air)$^{-1}$ (controlled by CO_2 cylinder). Each measurement was made with a minimum and maximum wait time of 120 and 200 s, respectively, and matching the infrared gas analyzers for 50 μmol(CO_2) mol(air)$^{-1}$ difference in the CO_2 concentration between the sample and the reference before every change in irradiance levels. The leaf to-air vapor pressure difference (VPD) has been setted to 1.5 kPa, and continuously monitored around the leaf during measurements and maintained at a constant level by manipulating the humidity of incoming air as needed. The measurements were made on sunny days during 8:30–11:30 am. Finally, stomatal conductance (g_s, mol H_2O m^{-2} s^{-1}) and transpiration rate measurements (T, mmol H_2O m^{-2} s^{-1}) at each light intensity have been evaluated.

4.4. Morphological analysis

After the physiological measurements, the same leaves have been used for the morphological analysis. In particular, leaf fresh weight (LFW, g) and dry weight (LDW, g), determined after oven-drying at 70 °C for 2 days, and leaf area (LA, cm^2) that has been measured by WinRhizo Pro v. 4.0 software package (Instruments Régent Inc., Chemin Sainte-Foy, Québec, Canada) after scanned at a resolution of 300 dpi by WinRhizo STD 1600 (Instruments Régent Inc., Canada). Further, the fractal dimension of the leaf (FD) has been obtained by the "fractal analysis module" through the WinRhizo software, based on the box-counting method with the following settings: maximal pixel size (2.0mm), box sizes ranging from 2 to 32 and filters, and a length/width ratio smaller than 2.00. The FD provides information on the space occupation of the object: the fractal dimension approaches the value of 2 as the leaves become dense to the point of "filling in" a shape. For this reason, the FD has been used to correlate the root architecture to the soil resource acquisition [65], but also as the correction parameter in the LAI-Light interception models [66].

By these parameters, we also calculated the leaf mass per area (LMA, g LDW cm^{-2} LA), strongly related to photosynthetic rate [67], growth rate [68] and decomposition rate [69]; the leaf dry content (LDC, g dry weight/g fresh weight), strongly related with relative growth rate [70], flammability [71], and post-fire regeneration strategy [72]; and leaf water content or leaf succulence (LWC, g H_2O/cm^2 leaf area], which indicates the leaf water content in relation to its leaf area, directly correlated with the plant responses to abiotic stresses [73].

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4.5. VOC analysis: HeadSpace/Solid-Phase Micro-Extraction (HS/SPME) GC-MS analysis

The volatiles (VOCs) produced by petiolate and sessile leaves of *Salvia ceratophylloides* have been chemically characterized using the HS/SPME method. One gram of plant material, per sample and replicate (N=3), has been sealed in a 20 ml vial and allowed to equilibrate for 20 minutes at room temperature. Successively, the SPME gray fiber (StableFlex, divinylbenzene/Carboxen on polydimethylsiloxane coating; 50/30 µm coating; Supleco) has been exposed to plant VOCs for 20 minutes to allow the VOCs adsorption on the fiber.

VOCs have been identified using a Thermo Fisher gas chromatograph apparatus (Trace 1310) coupled with a single quadrupole mass spectrometer (ISQ LT). The capillary column was a TG-5MS 30 m × 0.25 mm × 0.25µm. Helium has been used as carrier gas with a flow of 1 ml/min. Samples have been injected in a split mode with a split ratio of 60. Injector and source have been settled at the temperature of 200 °C and 260 °C, respectively. The temperature ramp has been settled as follow: 7 min at 45 °C, from 45 °C to 80 °C with a rate of 10°C×min, from 80 °C to 200 °C with a rate of 20 °C × min then isocratic for 3 minutes 200 °C. Mass spectra have been recorded in electronic impact (EI) mode at 70 eV, scanning the 45–500 m/z range.

Native raw chromatograms (RAW), previously converted in mzXML using the tool MSconvert of proteowizard [74], have been normalized for TIC intensity, aligned, deconvoluted and peak intensities extracted using the open source software XCMS [75]. For peak analysis the GC/Single Quad (matchedFilter) pre-settled method has been used.

After chromatograms processing and peak picking, features and normalized peak areas have been imported to Excel for further statistical analysis. Compounds identification has been carried out comparing the relative retention time and mass spectra of molecules with those of commercial libraries (NIST Mass Spectral Reference Library) and open source EI spectral libraries (Mass Bank of North America, Golm Metabolome Database) [76, 77].

4.6. Statistical analysis

Light curves have been fitted by nonlinear regression using the Ye [28] model equation:

$$PN = \emptyset_{(I_0 - I_{comp})} \times \frac{1-\beta \times I}{1+\gamma \times I} \times (I - I_{comp}) \quad (1)$$

where: P_N – the net photosynthetic rate [µmol(CO_2) m^{-2} s^{-1}]; I – the photosynthetic photon flux density [µmol(photon) m^{-2} s^{-1}]; I_{comp} – the light compensation point [µmol(photon) m^{-2} s^{-1}]; β – the adjusting factor (dimensionless); γ – the adjusting factor (dimensionless); ϕ (I_0 – I_{comp}) – the quantum yield obtained at the range between I_0 and I_{comp} [µmol(CO_2) µmol(photon)$^{-1}$]. The following leaf-level photosynthetic parameters have been calculated by these equations [28]:

$$P_{gmax} = \emptyset_{(I_0 - I_{comp})} \times \frac{1-\beta \times I}{1+\gamma \times I} \times \left(I - I_{comp}\right) + R_D \quad (2)$$

$$R_D = \emptyset_{(I_0 - I_{comp})} \times I_{comp} \quad (3)$$

$$I_{sat} = \frac{\sqrt{\frac{(\beta+\gamma)\times(1+\gamma \times I_{comp})}{\beta-1}}}{\gamma} \quad (4)$$

I_{sat} – the light saturation point [µmol(photon) m^{-2} s^{-1}]; P_{gmax} – the asymptotic estimate of the maximum gross photosynthetic rate [µmol(CO_2) m^{-2} s^{-1}]; R_D – the dark respiration rate [µmol(CO_2) m^{-2} s^{-1}]. Finally, the ϕ (I_{comp} – I_{200}) has been calculated as the slope of the linear regression of P_N for values of I between Icomp and 200 µmol (photon) m^{-2} s^{-1} representing the "maximum quantum yield".

Finally, according to Lobo et al. [78], we reported the Imax (µmol(photon) m^{-2} s^{-1}) (light saturation point beyond which there is no significant change in P_N) and the P_N (I_{max}) (µmol(CO_2) m^{-2} s^{-1}) (maximum value of P_N obtained at I = I_{max}) instead of I_{sat} and P_{gmax} as more realistically adequate. We used a simple routine to minimize the error sum of squares

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(SSE) for fitting the models, allowing the determination of equation parameters using the Microsoft Excel spreadsheet and Solver function (Microsoft Excel 2010). Non-linear regressions were repeated several times in order to minimize the sum of square of deviation between predicted and experimental values to less than 0.01% between two consecutive fits [79].

In order to evaluate the effect of the Years (Y) (2016 and 2017), we used the one-way ANOVA on the gas exchange parameters, which quickly respond to the environmental conditions. Since the Years factor was not significant ($p > 0.05$) for almost all the gas exchange traits (Table S1), all the morpho-physiological parameters have been analyzed by two-way analysis of variance with the Leaf Type (LT) (sessile and petiolate) and Site (Sit) (Mosorrofa and Puzzi) as main factors and their interaction Lt × Sit. Then, Tukey's test has been used to compare the means of all the parameters of each LT and Sit. All data have been tested for normality (Kolmogorov–Smirnoff test) and homogeneity of variance (Levene median test) and, where required, the data have been transformed.

For the comparison of the LMA and P_N of *S. ceratophylloides* with that of different plant functional groups and *Salvia* spp., we used the Isat and Icomp data obtained from [80, 81] for evergreen angiosperm, [29] for evergreen shrub, [30] for evergreen species, [82] for *S. officinalis*, [34] for *S. pratensis*, [35] for *S. glutinosa*, [33] for *S. hispanica*, [31] for *S. mohavensis*, *S. leucophylla*, *S. dorrii var. dorrii* and *S. mellifera*.

The TIC intensity normalized dataset obtained from metabolomic data analysis have been classified through unsupervised Principal Component Analysis (PCA) where the output consisted of score plots to visualize the contrast among different samples. PCA analysis has been carried out on all the features detected by the analysis. Successively, identified and annotated compounds have been statistically analyzed through univariate two-way analysis of variance with the LT (sessile and petiolate) and Sit (Mosorrofa and Puzzi) as major factors. Then, the Tukey's test has been used to compare the compound means of each leaf type and site ($p<0.05$).

5. Conclusions

The eco-physiological adaptation of *S. ceratphylloides*, a rare and endangered plant species, to its habitat by functional traits has been studied. The higher light saturation and compensation point and leaf mass per area indicated a sunny habitat preference of *S. ceratophylloides*. These results suggested that for its *in situ* conservation, lower competition (low density and diversity) especially with woody species (trees and shrubs), should be favored. However, the *S. ceratophylloides* habitat has been destroyed and continuously fragmented because of anthropogenic disturbance and environmental deterioration and, consequently, further and deepening study needs to identify the main stressful factors that threaten its growth, development, and fitness. For the artificial propagation, *ex situ* conservation, we recommend growing the seedlings at least half sunlight (1200 μmol (photons) m^{-2} s^{-1}).

Further, for the first time, the "continuous within-plant variation" of the morpho-physiological traits and metabolic profiles of endangered and rare plant species has been assessed in the field. The results indicated that the physiologic and metabolic traits explained most of this within-plant plasticity, which has been also affected by the location. Indeed, the sessile and petiolate leaves of *S. ceratophylloides* showed different photosynthetic performances and metabolic profiles, but the sub-individual variation of the photosynthetic-related parameters, differently to the volatilome, has been exhibited in one site only. These within-plant patterns, probably related to the micro-environmental heterogeneity, could optimize the growth and defenses machinery for the fitness's improvement to specific habitats. Overall, the magnitude of the within-plant variation should be taken into consideration when designing sampling schemes for the ecological studies of *S. ceratophylloides*.

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Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Figure S1: title, Table S1: title, Video S1: title.

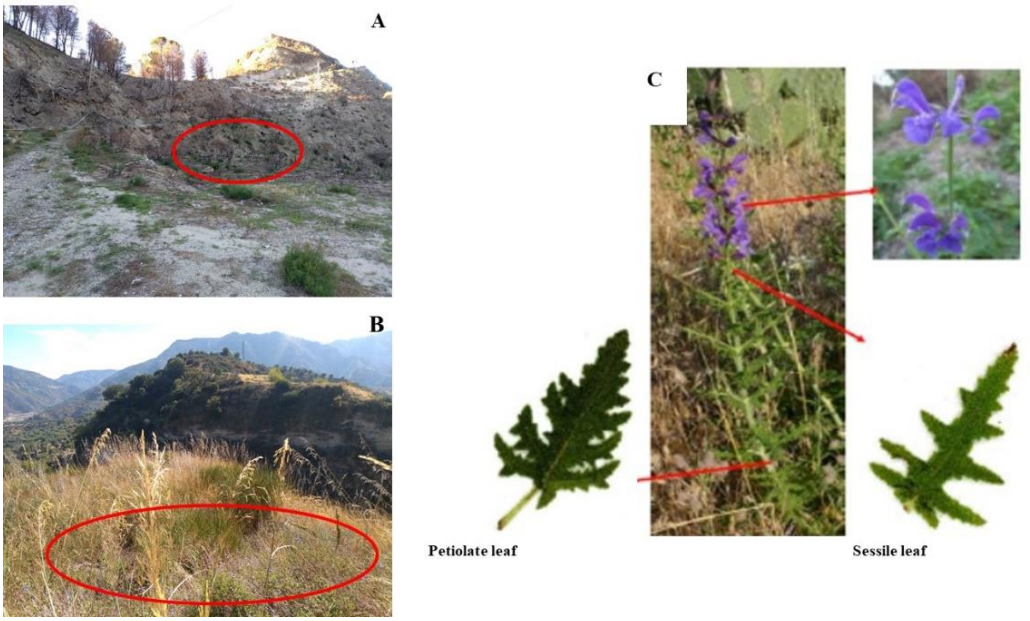

Figure S1. - Plants of *Salvia ceratophylloides* in the two sites, Mosorrofa (A) [Mo] and Puzzi (B) [Pu]. The red circles indicate the places where the *Salvia ceratophylloides* plants have been discovered. (C) Individual plant of *Salvia ceratophylloides* and different leaves: petiolate (P) and sessile leaf (S)

Figure S1. - Position of the sampled populations of *Salvia ceratophylloides*. A and B: Mosorrofa; C: Puzzi.

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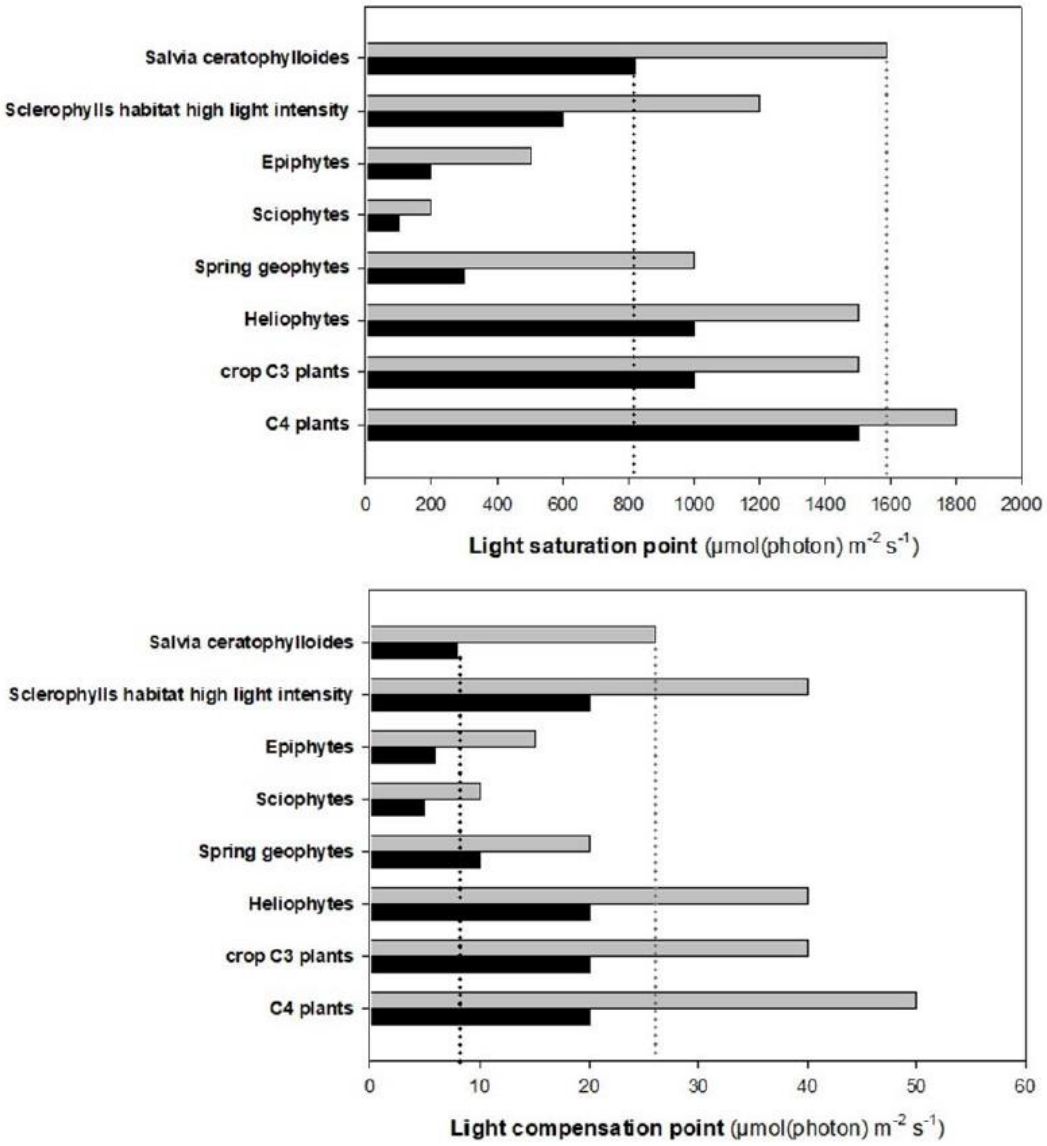

Figure S3. Light saturation point (µmol(photons) m^{-2} s^{-1}) (upper panel) and light compensation point (µmol(photons) m^{-2} s^{-1}) (bottom panel) of different plant functional groups. The data [minimum (■) and maximum value (■)] are derived from Larcher [80]. The dotted lines are drawn for a better comparison with the minimum and maximum value of *Salvia ceratophylloides*.

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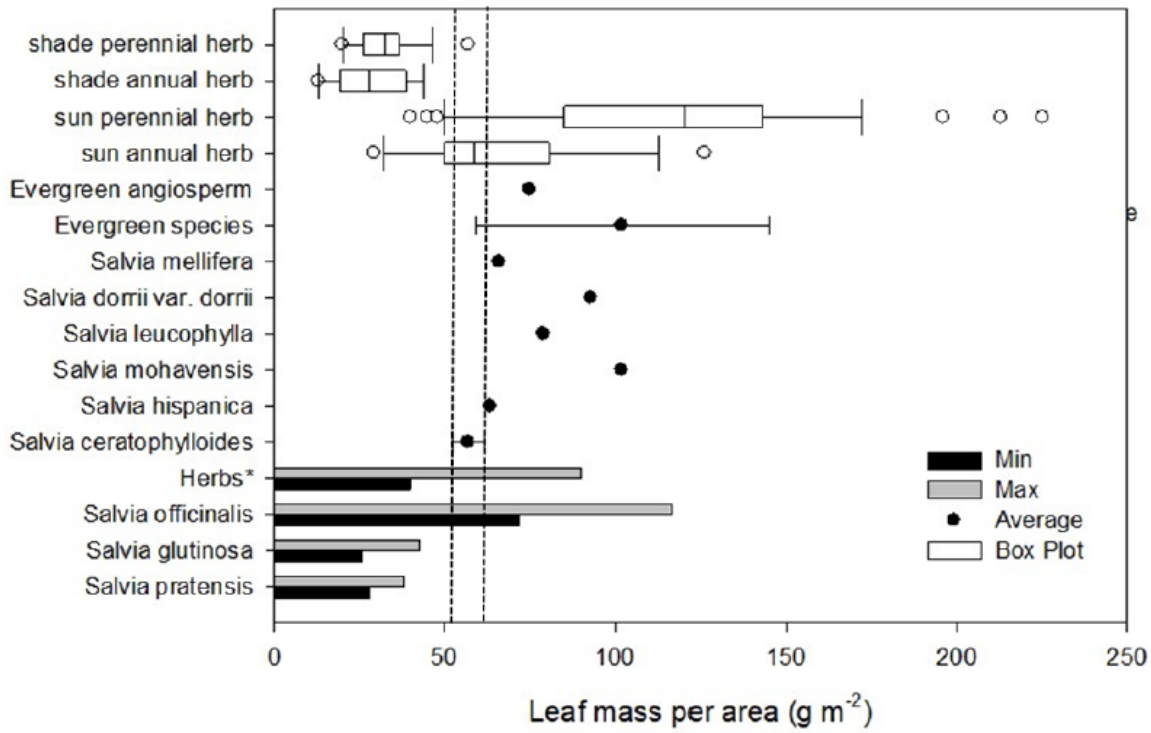

Figure S4. Leaf mass per area (g m-2) of sun- and shade-species herbs, evergreen angiosperm and species, herbs and different *Salvia* species. The data of LMA of Salvia species, herbs, evergreen angiosperm and species are indicated by minimum (■) and maximum value (■) or by the average (black plot point and the standard deviation where reported)] and have been derived from Martins et al. [82] for *S. officinalis*, Mommer et al. [34] for S. pratensis, Paz´-Dyderska et al. [35] for *S. glutinosa*, Goergen et al. [33] for *S. hispanica*, Knight and Ackerley [31] for *S. mohavensis*, *S. leucophylla*, *S. dorrii* var. dorrii and *S. mellifera*, Poorter et al. [29] for herbs, Duursma et al. [81] for evergreen angiosperm and de la Riva et al. [30] for evergreen species. Box plots point out the distribution of LMA values as observed for a wide range of sun- and shade-species herbs both annual and perennial, with the bottom and top part of the box indicating the 25th and 75th percentile, respectively, the two whiskers the 10th and the 90th percentile, respectively, and the horizontal line within the box the median value. The data for the box plot are derived by scientific literature as indicated in Table S1. The dotted lines have been drawn for better comparisons and pointed out the range of LMA values of *Salvia ceratophylloides*.

Table S1 – Two-way ANOVA results and chemical characterization (average and error standard within brackets) of volatile organic compounds in fresh sessile and petiolate leaves of *Salvia ceratophylloides* harvested in two different sites (Mosorrofa, Mo; Puzzi, Pu). Different lower-case letters indicated significant differences at $p < 0.05$ among the average along the rows (Tukey' test) and they have been only reported when the LT × Sit interaction was significant. The bold identify the statistically significant factors and/or their interaction.

	Compound	**Chemical classes**	**#Statistics**	**Sessile**		**Petiolate**	
				Pu	**Mo**	**Pu**	**Mo**
1	Camphene	Monoterpene	LT 0.75^{NS} Sit 0.64^{NS} LT × Sit 1.25^{NS}	468	141	130	184
2	Camphor		LT 2.78^{NS} Sit 4.82^{NS} LT × Sit 0.86^{NS}	1776	368	606	34

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3	Limonene		LT 5.30^{NS} Sit 1.33^{NS} LT × Sit 0.74^{NS}	77187	72261	53128	19515
4	p-Cymene		**LT 7.78*** **Sit 14.16**** LT × Sit 0.21^{NS}	195813	82392	108569	19607
5	Pinocarvone		LT 0.04^{NS} **Sit 6.57*** LT × Sit 0.07^{NS}	2406	987	2444	696
6	Sabinene		**LT 11.80**** Sit 0.34^{NS} LT × Sit 1.70^{NS}	554775	873306	195242	73210
7	Terpinolene		**LT 12.40**** Sit 0.40^{NS} LT × Sit 3.09^{NS}	128554	218320	62198	19784
8	trans-Sabinene hydrate		LT 0.00^{NS} Sit 0.28^{NS} LT × Sit 3.73^{NS}	3086	4773	5342	2381
9	trans-α-Ocimene		LT 0.56^{NS} Sit 3.02^{NS} LT × Sit 0.04^{NS}	4784232	2005449	3790259	292998
10	α-Pinene		LT 1.04^{NS} Sit 0.68^{NS} LT × Sit 0.60^{NS}	548	104	50	36
11	α-Terpinene		LT 0.01^{NS} Sit 5.21^{NS} LT × Sit 6.02^{NS}	1116	1202	2418	22
12	α-Thujene		LT 3.83^{NS} Sit 4.44^{NS} LT × Sit 3.74^{NS}	1186	114	153	108
13	β-Myrcene		LT 2.54^{NS} Sit 0.15^{NS} LT × Sit 0.01^{NS}	10189	8949	4114	2208
14	β-Ocimene		LT 0.54^{NS} Sit 3.61^{NS} LT × Sit 0.38^{NS}	3309	978	2059	868
15	β-Phelladrene		LT 0.80^{NS} Sit 3.86^{NS} LT × Sit 0.60^{NS}	1327	167	620	117
16	β-Pinene		**LT 7.30*** Sit 0.47^{NS} LT × Sit 1.73^{NS}	92968	150052	53391	35502
17	γ-Terpinene		**LT 5.40***	16341	13366	4610	3508

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			Sit 0.19^{NS} LT × Sit 0.04^{NS}				
18	cis-Pinen-3-ol	monoterpene alcohol	LT 1.40^{NS} Sit 1.13^{NS} LT × Sit 1.46^{NS}	882	51	7	60
19	Eucalyptol		LT 0.42^{NS} Sit 0.00^{NS} LT × Sit 1.52^{NS}	124492	278959	194614	53771
20	Isoborneol		LT 1.48^{NS} Sit 4.42^{NS} LT × Sit 1.48^{NS}	7041	156368	7160	46949
21	α-Terpineol		**LT 8.13*** **Sit 12.91**** **LT × Sit 9.04***	10220^{b}	80003^{a}	11854^{b}	18054^{b}
22	D-Germacrene	sesquiterpene	LT 0.11^{NS} Sit 3.47^{NS} **LT × Sit 4.22***	2554^{a}	169^{b}	1102^{a}	1218^{a}
23	α-Caryophyllene		LT 0.62^{NS} Sit 3.43^{NS} LT × Sit 0.00^{NS}	43235^{a}	19311^{a}	33115^{a}	8972^{a}
24	α-Copaene		LT 0.62^{NS} **Sit 11.05*** LT × Sit 0.67^{NS}	3517	601	2385	625
25	α-Cubebene		**LT 8.21*** **Sit 19.35**** LT × Sit 1.02	4460201	1371705	2247264	312465
26	α-Muurolene		**LT 9.49*** Sit 0.56^{NS} LT × Sit 0.99^{NS}	14382	15236	7105	1038
27	β-Caryophyllene		LT 1.56^{NS} Sit 2.04^{NS} LT × Sit 0.17^{NS}	30708	18290	19500	12669
28	β-Copaene		LT 2.46^{NS} Sit 2.74^{NS} LT × Sit 2.19^{NS}	1001	100	126	74
29	(z)-Hex-3-en-1-ol	alcohol	LT 0.06^{NS} Sit 4.29^{NS} LT × Sit 0.06^{NS}	2721741	7008	2159260	8617
30	1-Octen-3-ol		LT 0.05^{NS} Sit 3.20^{NS} LT × Sit 0.13^{NS}	22710	12017	23730	7653
31	2-Propenal	aldehyde	LT 0.89^{NS} Sit 1.20^{NS}	900	94	153	88

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			LT × Sit 0.87^{NS}				
32	Isovaleraldehyde		**LT 6.10*** Sit 0.52^{NS} LTxSit 0.52^{NS}	81876770	46391341	3426789	3466464
33	Octenal		LT 2.94^{NS} Sit 1.40^{NS} LT × Sit 0.16^{NS}	10441	7566	6602	5171
34	α-Methyl-n-Butanal		LT 5.19^{NS} Sit 0.00^{NS} LT × Sit 0.02^{NS}	32740199	30174050	3454460	4492251
35	5-Methylheptan-3-one	keton	**LT 5.70*** Sit 0.21^{NS} LT × Sit 0.08^{NS}	7776	8204	1578	3291
36	Pentan-3-one		**LT 7.73*** Sit 2.44^{NS} LT × Sit 1.20^{NS}	321989	649080	114170	171753
37	β-tujone		**LT 17.37**** **Sit 6.21*** **LT × Sit 12.54****	65370^{b}	168599^{a}	54660^{b}	36692^{b}
38	(3z)-3-Hexenyl acetate	Aliphatic esters	LT 3.58NS Sit 5.09NS **LT × Sit 5.46***	1253^{a}	0^{b}	99^{ab}	122^{ab}
39	Dimethyl Sulfide	ether	**LT 23.77**** **Sit 5.34*** LT × Sit 1.40^{NS}	29866633	54386837	3926181	11857751

#Statistical analysis: two-way ANOVA with 4-9 replications (LT: leaf type; Sit: sites; LT × Sit: Leaf type × Sites interaction); *0.05 > p < 0.01; **0.01 > p < 0.001; ***0.001 > p; NS: not significant.

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Table S2 – *F* statistic and *p* values (within brackets) of one-way ANOVA of the leaf-level photosynthetic parameters of *Salvia ceratophylloides* individuals estimated by nonlinear regression using the model of Ye et al. (2007) and measured in 2016 and 2017.

Parameters	Statistics
I_{comp} [μmol(photon) m^{-2} s^{-1}]	0.406 (0.530)
I_{max} [μmol(photon) m^{-2} s^{-1}]	1.076 (0.311)
I_{sat} [μmol(photon) m^{-2} s^{-1}]	**5.263 (0.032)**
$P_{N(Imax)}$ [μmol(CO_2) m^{-2} s^{-1}]	3.115 (0.091)
R_D [μmol(CO_2) m^{-2} s^{-1}]	3.808 (0.064)
$\phi_{(Icomp-200)}$ [μmol(CO_2) μmol(photon) $^{-1}$]	**4.561 (0.044)**

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