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Citizen Science to Investigate the Ecophysiological Responses of Mediterranean Shrubland Vegetation in an Urban Open-Air Laboratory

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Abstract: Urban biodiversity is essential for enhancing liveability for both humans and wildlife by providing a range of ecosystem services. Therefore, it is crucial to raise awareness among citizens, particularly schoolchildren, about the significance of biodiversity in urban environments. To this end, an Open-Air Laboratory was established in an urban park of an Italian Mediterranean city (Livorno), where natural vegetation flourishes. This initiative engaged schoolchildren in experimental campaigns to collect ecophysiological data on local wild woody species. Specifically, the students were tasked with identifying various wild woody species and, under the guidance of researchers, recording specific leaf ecophysiological traits, such as leaf mass per area, leaf pigments, and chlorophyll fluorescence. The results, which highlighted seasonal variations in leaf ecophysiological traits and interspecific differences, were analysed in relation to the environmental conditions documented by the schoolchildren. This analysis revealed distinct plant strategies for coping with winter and summer stressful periods. The methodology employed in this project, which involved schoolchildren in research activities, not only fostered environmental awareness among young participants but also serves as a pilot model for public engagement in scientific research.

Keywords: ecosystem services; informal green spaces; Mediterranean maquis; photosynthesis; plant biodiversity; schoolchildren biodiversity education

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1. Introduction

Plant diversity plays a significant role in Mediterranean urban and suburban landscapes. Nowadays, it is well established that plant communities provide a multitude of ecosystem services that contribute to the overall liveability of urban environments [1,2]. In this context, it has been shown that the more biodiverse these communities are, the greater their contribution to ecosystem services [3,4]. Among these services, woody species can mitigate the urban heat island effect during the summer, improve air quality by removing particulate matter and gaseous pollutants, and enhance the aesthetic value of urban areas,

as well as the restorative potential of green spaces [5,6]. Furthermore, many urban areas, including those recently developed, lack green spaces, resulting in environments that are unsuitable for wild flora and fauna, which contributes to an unhealthy environment for humans [7]. In this regard, urban parks, due to their proximity to city centres and ease of access, can be viewed as Open-Air Laboratories (OALs) where biodiversity and plant ecophysiology can be studied, with the assistance of visitors participating in citizen science activities [8,9]. Urban green spaces that maintain natural or semi-natural surfaces are particularly ideal for conducting such studies [10,11]. These areas are also referred to as "informal green spaces" and are recognised as providers of numerous ecosystem services, including the facilitation of educational activities [12].

Raising public awareness of environmental issues and the importance of biodiversity conservation among citizens is crucial, especially for children, who are tomorrow's adults. Some authors have emphasised the significance of concepts such as the "extinction of experience" and the excess of virtual experiences that focus primarily on exotic species [13,14]. Engaging individuals, particularly schoolchildren, in hands-on, environment-related scientific research can be essential for making these topics more accessible and fostering a desire to learn more [15]. From this perspective, involving them in activities in OALs is an effective way to enhance their learning experience [16,17]. Citizen science is regarded as a powerful means of implementing initiatives aimed at biodiversity conservation [18] and has the potential to contribute to the United Nations' Sustainable Development Goals [19], as well as to help reduce inequality [20]. Furthermore, identifying locations that are both easily accessible and sufficiently safe for schoolchildren, along with providing plant species suitable for an OAL, is a significant milestone in enhancing the general public's environmental awareness and knowledge. Cities, particularly urban natural and semi-natural areas, can serve as laboratories for studying and evaluating the effectiveness of Nature-based Solutions (NbS), which seek to utilise native wild plant species for urban green infrastructures [21].

The Mediterranean urban environment is of particular interest for several reasons. Plant species that grow at low altitudes, especially during the dry season, must be able to tolerate severely stressful conditions, such as high temperatures and water scarcity [22]. The adaptation of the photosynthetic apparatus to Mediterranean environmental conditions results in these ecosystems exhibiting some of the highest net primary productivities worldwide [23]. Consequently, Mediterranean species are particularly intriguing due to their photosynthetic strategies before, during, and after the most stressful periods. Their ability to cope with such stresses makes them a viable choice for cultivation in urban green areas [24]. Thanks to their greater plasticity and variability in functional traits, they can survive in extreme environmental conditions, thereby enhancing overall ecosystem resilience and providing maximum ecosystem services [25]. The primary school curriculum in Italy encompasses the study of living organisms, including their structures, functions, and interactions with the environment. While photosynthesis is typically addressed in lower secondary school, previous research indicates that primary students can grasp this process to a certain extent [26]. Furthermore, the direct involvement of schoolchildren in studying seasonal changes in plants is particularly beneficial, especially when scientific instruments are utilised [27]. However, it is important to note the lack of projects that engage citizens in studying ecosystem functions [28], particularly those that provide young students with direct involvement in measuring photosynthesis.

To enhance public awareness of issues related to urban plant biodiversity and its interaction with the environment, an OAL has been established in the urban park of Villa Corridi, located in the Mediterranean coastal city of Livorno, Tuscany, Italy. This initiative leverages the presence of primary and secondary schools within the park, as

well as the naturally occurring Mediterranean maquis vegetation. The OAL serves as a living laboratory for schoolchildren, aiming to (i) introduce them to the scientific study of plant biodiversity in Mediterranean thermophile woodlands; and (ii) encourage students to explore these environments and measure ecophysiological leaf traits using scientific instruments and methods employed by researchers to generate replicable and reliable scientific data.

2. Materials and Methods

2.1. Site Description

Livorno is a city situated along the coast of Tuscany (northern Central Italy), with a population of approximately 153,000 residents and an area of about 104 km^2. The chemical industry and harbour activities play a key role for the city's economy and contribute significantly to its overall pollution status. The climate of Livorno is, according to the Köppen–Geiger Climate Classification, typically Mediterranean climate, characterised as warm temperate with dry, hot summers (Csa) [29,30] (Figure S1). In terms of bioclimatic classification [31], Livorno exhibits a Mediterranean macrobioclimate, a transitional Oceanic–Mediterranean bioclimate, a Mesomediterranean thermotype, and a subhumid ombrotype. The Villa Corridi park is situated on the outskirts of Livorno (Figure 1a) and spans approximately 114,000 m^2. The park is primarily designed as a formal green space, encompassing around 81,200 m^2 of lawns and trees, mainly Aleppo pines. However, over the past 50 years, a semi-natural vegetation has developed on its south-facing side. This area features cultivated pines and trails that facilitate access for visitors while still showcasing species typical of the Mediterranean maquis, which is well-represented in protected areas of the surrounding hillside.

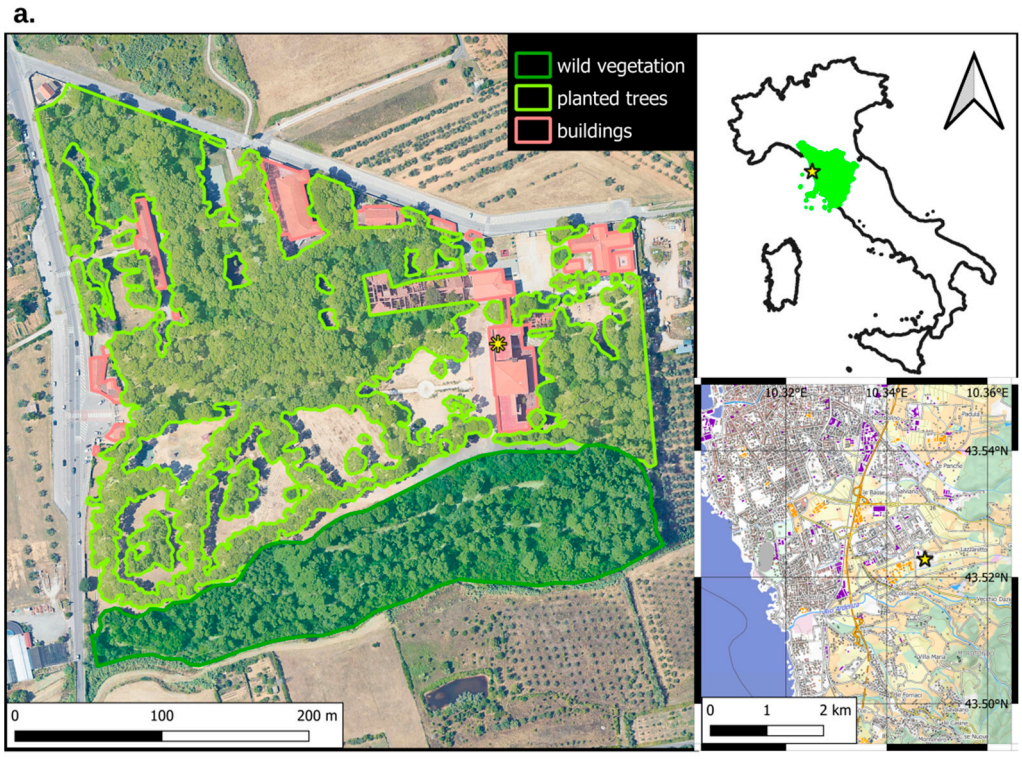


Figure 1. *Cont.*

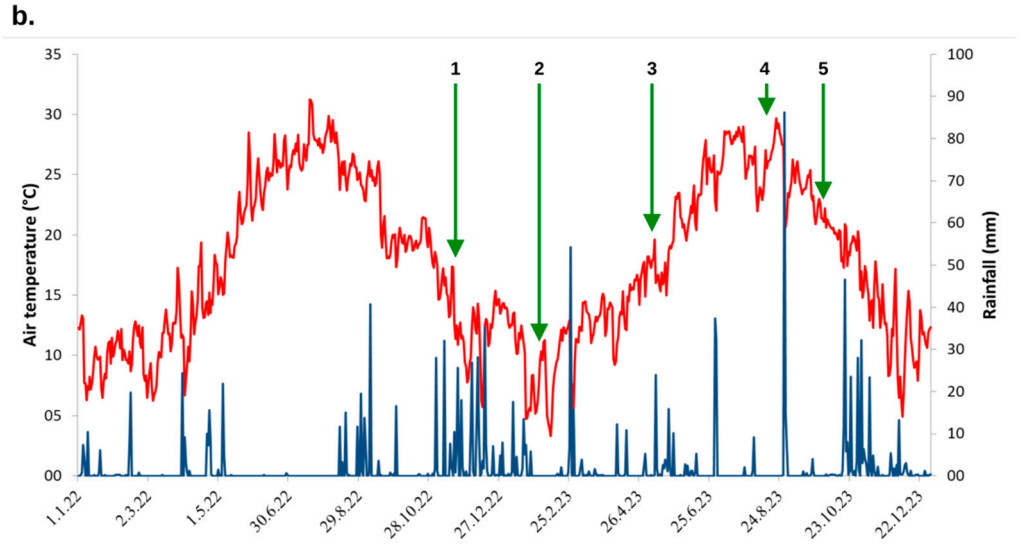


Figure 1. Site vegetation and thermopluviometric chart: (**a**) Left: cartography of the vegetation in the study site (created with QGIS 3.22; 43°31.373′ N 10°20.899′ E). Deep green, wild vegetation (study area); light green: planted trees; pink, buildings; asterisk, position of the meteorological station. Cartographic basis: Google Earth. Bottom right: star, position of the study site in relation to the city of Livorno. Cartographic basis: Regione Toscana. Top right: star, position of the study site in Tuscany. Cartographic basis: Regione Toscana. (**b**) Daily average air temperatures and rainfall measured during the two years of investigation. Green arrows indicate the sampling dates.

2.2. Classroom Activities and the Establishment of an OAL with Students and Teachers

Two classes from the 5th grade of primary school (10 years old) and the 2nd grade of lower secondary school (11–12 years old), along with their teachers, participated in the implementation of an OAL in the park of Villa Corridi during two events held in Autumn 2022 and Spring 2023. A preparatory session was conducted in the classroom, covering topics such as biodiversity on Earth and plant ecophysiology, which were aligned with the objectives of the activities carried out in the park. In this context, a brief introduction to the citizen science application iNaturalist was also provided. Care was taken to employ a "teaching through questioning" approach [32], while avoiding an excessively frontal method. Following the field activities, additional classroom meetings were held to present the results to both the schoolchildren and teachers. These sessions utilised a brainstorming approach, allowing participants to express their ideas spontaneously and fostering creative thinking. Laboratory sessions focused on meteorology and climatology were conducted for schoolchildren and their teachers. These sessions included specific lessons on the operation of the meteorological station, the process of acquiring various meteorological parameters, and the recording of archived data, along with their applications in different fields. To broaden the OAL experience to a wider audience, additional training sessions were organised in the park of Villa Corridi between 2022 and 2023, engaging other schoolchildren and their teachers. These sessions included the same activities conducted in the OAL and focused on plant species identification, the evaluation of their photosynthetic performance, and the collection of meteorological data.

2.3. Acquisition of Meteorological Data

The environmental conditions of the Villa Corridi park were recorded by schoolchildren and their teachers using a Davis ISS Vantage Pro 2 Plus meteorological station (Davis Instruments, Hayward, CA, USA) equipped with various sensors, including a rain gauge, thermo-hygrometer, cup anemometer, solar radiation sensor, UV radiation sensor, and a Vantage Pro 2 Plus Console unit with a barometer and internal thermo-hygrometer

(Figure 1a). The station has been installed since 2017 on the terrace of Villa Corridi Primary School as part of an agreement ratified between CNR IBE and the school board for educational and research activities outlined in the "ECOPLANET" macro-project, specifically the "Green Action Research" sub-project. This initiative aims to enhance understanding of climate change and implement monitoring activities to support outdoor education in pursuit of the Sustainable Environmental Goals of the 2030 Agenda. From January 2022 to December 2023, students and teachers collected and analysed data, including air temperature and daily mean precipitation (Figure 1b), to evaluate the effects of seasonal environmental conditions on the monitored leaf functional traits.

2.4. Survey of Plant Diversity in the Urban Park

A survey of the wild vegetation was conducted to identify the shrub and tree species most prevalent in the park, which, with the exception of one, are characteristic of the sclerophyllous Mediterranean maquis and woodlands in the surrounding areas. Among the observed species, a list was compiled that includes those taxa selected for ecophysiological analysis: *Arbutus unedo* L., *Cistus creticus* L. subsp. *eriocephalus* (Viv.) Greuter & Burdet, *Laurus nobilis* L., *Ligustrum lucidum* W.T.Aiton, *Myrtus communis* L. subsp. *communis*, *Pinus halepensis* Miller, *Phillyrea angustifolia* L., *P. latifolia* L., *Pistacia lentiscus* L., *Quercus ilex* L., *Rhamnus alaternus* L. subsp. *alaternus*, and *Viburnum tinus* L. subsp. *tinus*. Subsequently, a subset of eight taxa was selected for activities with schoolchildren to align with the school schedule: *C. creticus* subsp. *eriocephalus*, *L. nobilis*, *M. communis*, *P. halepensis*, *P. lentiscus*, *Q. ilex*, *R. alaternus*, and *V. tinus* subsp. *tinus* (Figures S2–S4). These species were chosen to provide a set of diagnostic morphological characters that are (a) easy for beginners to evaluate, (b) representative of the most important characters used in botany, and (c) involve not only the sense of sight but also touch and smell (Table 1). Specifically, information cards were prepared for each species, featuring a colour photograph of the plant sourced from non-copyrighted botanical works, along with a brief description and a simple list of diagnostic characters (the English version in the Supplementary Material, Figures S2 and S3). It is important to note that in these cards, the terms "shrub" and "tree" are used not in a strict botanical sense, but rather to distinguish between plants that are up to two meters tall and those that exceed this height. Subsequently, schoolchildren were divided into groups, with each group receiving two cards at a time. The selection of the two species presented simultaneously was designed to encourage pupils to compare two types of plants that clearly differed in several morphological characters. The students were then encouraged to collaborate in order to identify a small specimen of the correct species. A collaborative approach was promoted to minimise competition between groups, while emphasizing that the project's goals were to enhance their knowledge of wild flora and its ecology, to collaborate with researchers, and to have fun. Additionally, the children's observational skills, along with their ability to connect a simple text and an image to the living plants, were carefully nurtured. Efforts were also made to structure the activity as a game, making the experience more engaging for the pupils and maintaining their attention [33]. Once the specimens were collected, they were verified by experts. In cases of misidentification, the specific errors were explained. No scores were assigned for correct identifications, nor was a ranking established. Teachers were then asked to photograph the identified species and upload the georeferenced images to the citizen science application iNaturalist to map the observed plants.

Table 1. Main morphological diagnostic features of the studied species.

Species	Habitus	Leaves						
		Phyllotaxy	Simple (s) or Compound (c)	Colour	Texture	Indumentum	Margin	Smell
Arbutus unedo	tree	alternate	s	green, slightly discolorous, darker on adaxial side	leathery	glabrous	dentate	grassy
Cistus creticus	shrub	opposite	s	greyish-green, concolorous	rather soft	hairy	entire	grassy
Laurus nobilis	tree	alternate	s	dark green, concolorous	leathery	glabrous	almost entire, usually wavy	pleasant
Myrtus communis	shrub	opposite	s	light green, concolorous	leathery	glabrous	entire	pleasant
Pinus halepensis	tree	fascicled	s	light green	-	glabrous	-	pleasant
Phillyrea angustifolia	shrub	opposite	s	green, slightly discolorous, darker on adaxial side	leathery	glabrous	usually entire, rarely dentate	grassy
Phillyrea latifolia	shrub	opposite	s	green, slightly discolorous, darker on adaxial side	leathery	glabrous	usually dentate, rarely entire	grassy
Pistacia lentiscus	shrub	alternate	c	green, slightly discolorous, darker on adaxial side	leathery	glabrous	entire	pleasant or unpleasant
Quercus ilex	tree	alternate	s	strongly discolorous, dark green on adaxial side, whitish on abaxial side	leathery	glabrous on adaxial side, densely hairy on abaxial side	usually entire, rarely dentate	grassy
Rhamnus alaternus	shrub	alternate	s	green, slightly discolorous, darker on adaxial side	leathery	usually glabrous	dentate, or entire	foetid
Viburnum tinus	shrub	opposite	s	green, slightly discolorous, darker on adaxial side	rather soft	hairy especially on margins	entire	grassy

2.5. Determination of Leaf Ecophysiological Traits in Mediterranean Vegetation

To promote understanding of functional biodiversity, the schoolchildren participated in experimental field campaigns alongside scientists to record leaf ecophysiological traits, such as leaf mass per area (LMA) and photosynthetic performance (Figure S4). Sampling dates and the number of replicates were determined while considering the constraints imposed by the school calendar and timetable. Consequently, to facilitate seasonal comparisons, the same number of replicates per species was maintained during the other sampling dates as well.

For the determination of LMA, leaf disks (1.3 cm^2) and needles (for *P. halepensis*) of a known area were collected, placed in pre-weighed vials, and transported to the laboratory. In the lab, the leaf disks were oven-dried at 50 °C until they reached a constant weight, and then weighed to determine the dry weight (DW). LMA was calculated as the ratio of the DW to the leaf disk area (g m^{-2}).

Photosynthetic activity and light energy dissipation mechanisms were assessed through measurements of leaf pigments, including chlorophylls, flavonoids, and anthocyanins, as well as chlorophyll fluorescence parameters. Measurements were taken on 3–6 different plants for each species on sunny days between 10:00 a.m. and 12:00 a.m. across five seasonal periods: November 2022, January 2023, May 2023, August 2023, and September 2023. The air temperature (Tair) and relative humidity (RH) during the measurement campaigns were obtained by the schoolchildren under the supervision of their teachers using the school meteorological station, while the photosynthetic photon flux density (PPFD) was recorded through the fluorometer sensor (Table 2).

Table 2. Mean values (± standard error) of photosynthetic photon flux density (PPFD), air temperature (Tair), and relative humidity (RH) recorded every half hour during the chlorophyll fluorescence measurements (~10–12 a.m.).

	Sampling Date	PPFD (μmol m^{-2} s^{-1})	Tair (°C)	RH (%)
1	23 November 2022	1530 ± 6	14.98 ± 0.38	68.60 ± 0.21
2	25 January 2023	1249 ± 22	9.22 ± 0.11	53.83 ± 0.83
3	8 May 2023	1537 ± 37	20.25 ± 0.16	63.50 ± 0.65
4	21 August 2023	1782 ± 15	35.95 ± 0.96	35.75 ± 2.46
5	27 September 2023	1791 ± 9	26.98 ± 0.38	46.50 ± 3.18

Leaf pigment indices were determined on fully expanded and well-exposed leaves of various Mediterranean shrubland species using a portable MPM-100 multi-pigment meter (Opti-Science Inc., Hudson, NH, USA). Measurements of leaf fluorescence (F) ratios were conducted to evaluate indices of anthocyanin (Anth, F660nm/F525nm) and flavonoid (Flav, F660nm/F325nm) content, as well as leaf transmission in the far red and near infrared to assess the chlorophyll content index (Chl, T850nm/T720mm). On the same leaves used for pigment determination, chlorophyll fluorescence was measured utilizing a pulse amplitude modulated fluorometer (Mini-PAM; Heinz Walz GmbH, Effeltrich, Germany). The leaves were pre-darkened for approximately 30 min, and the maximum quantum efficiency of PSII photochemistry (Fv/Fm) was evaluated using the formula $(Fm - F_0)/Fm$, where the difference between the maximum (Fm) and minimum (F_0) fluorescence yield emitted by the leaves in the dark-adapted state represents the variable fluorescence (Fv). The PPFD of the saturating light flash used to determine Fm was approximately 8000 μmol m^{-2} s^{-1}. Measurements of the steady-state effective quantum yield of photosystem II (PSII) photochemistry in the light Y(II) and both the light-regulated Y(NPQ) and non-regulated Y(NO) non-photochemical energy dissipation at PSII were conducted on fully expanded and well-exposed leaves under ambient PPFD, RH, and Tair values (Table 2).

The Y(II) value was calculated using the formula $(Fm' - F')/Fm'$, where Fm' represents the maximum fluorescence yield with all PSII reaction centres in the reduced state, obtained by superimposing a saturating light flash during exposure to actinic light, and F' is the fluorescence at the current state of PSII reaction centres during actinic illumination. The Fv'/Fm' value was determined as $(Fm' - F_0')/Fm'$, where F_0' is the minimum fluorescence yield in the light-adapted state, measured immediately after switching off the actinic light and applying a weak far-red light to preferentially excite PSI and force electrons to drain from PSII. The Y(NPQ) and Y(NO) values were calculated as $(F/Fm')/(F/Fm)$ and F/Fm, respectively. The Y(II) values were utilised to calculate the rate of linear electron transfer (ETR) [34], multiplying Y(II) by the incident PPFD and then correcting for the actual fraction of absorbed light and the relative distribution of absorbed light between the two photosystems, assumed to be 0.5 and 0.84, respectively [35]. The chlorophyll fluorescence decrease ratio (RFd) was calculated as $(Fm - Fs)/Fs$; this parameter is often used as an indicator of the "vitality" of plants and evolution of stress [36].

The schoolchildren were instructed on how to conduct the aforementioned ecophysiological measurements and understand their meaning, while being supervised by an expert throughout the activity. Additionally, the fluorometer was equipped with a screen that enabled the students to verify when the fluorescence signal was stable over time, indicating when a measurement could be taken. Any measurements that were unstable were repeated until valid values were obtained. Finally, a post-processing of the data was performed to discard any values collected under unstable conditions or in inappropriate light exposure compared to external conditions. The schoolchildren were educated about this validation process and the importance of handling scientific data to ensure its reliability. This enabled us to compare the measurements taken throughout the monitoring period and correlate them with the meteorological trends.

2.6. Statistical Analysis

Statistical analyses were performed using the STATISTICA software package 8.0 (StatSoft for Windows, 1998, Tulsa, OK, USA). Data on leaf functional traits were analysed independently using a one-way ANOVA for the factors of species and time. Pairwise multiple comparisons within each factor were conducted using the Fisher LSD method. Linear regressions were employed to test the relationships between individual variables of interest, with determination coefficients (R^2) and significance levels (p-value) reported.

3. Results

An OAL has been established in the urban park of Villa Corridi, involving 19 schoolchildren aged 10 years and 22 schoolchildren aged 11–12 years, along with their teachers, in two separate events. A dedicated project on the iNaturalist website was created, featuring georeferenced photos of selected evergreen shrub and tree species taken by the teachers (https://www.inaturalist.org/projects/la-biodiversita-del-parco-villa-corridi, accessed on 18 October 2024; Figure S5). Additionally, an educational classroom herbarium has been developed and is now available for teaching activities (Figures S6 and S7). The meteorological data recorded by the schoolchildren and their teachers using the school's meteorological station are presented in Figure 1b. These data reveal the typical trends of the Mediterranean climate, characterised by significant seasonal fluctuations in both temperature and precipitation. Average daily temperatures fell below 10 °C almost only during winter, while precipitation was mostly concentrated in autumn, with few occurrences in other seasons. The meteorological trends were used to assess the interaction between climate and ecophysiological leaf traits (i.e., chlorophyll fluorescence parameters and leaf pigment indices) of the studied species. Seasonal variations in light energy dissipation within the

Mediterranean shrubland formation, through both photochemical and non-photochemical processes, are illustrated in Figure 2. The average values for the 11 plant species in this formation revealed a significant decrease in Y(II) during January and August, which was associated with an increase in Y(NO) and Y(NPQ), respectively. This seasonal change in light energy dissipation processes was reflected in the ETR values, which exhibited the lowest seasonal values in January and August, corresponding to the minimum and maximum seasonal daily air temperatures, respectively (Figure 1b). The reduced ETR in January was linked to the lowest seasonal values of both Fv/Fm and Rfd, while the pigment indices (i.e., Chl, Flv, and Anth) reached their maximum seasonal values (Table 3). In contrast, the decreased ETR values in August were associated with only a slight decline in Fv/Fm and a significant reduction in the Chl index (Table 3). Significant interspecific differences in energy dissipation processes (Figure 3a–c), Fv/Fm (Figure 4a), ETR (Figure 4b), and Chl index (Figure 4c) were observed among the 11 Mediterranean species. The significance levels (*p*-values) for the factors of time and species are reported in Tables S1 and S2. All the species tended to increase Y(NPQ) and Y(NO) in summer and winter, respectively (Figure 3b,c). The Fv/Fm values showed only a slight decrease in winter and summer for most species (Figure 4a). This was reflected in a transient decrease in Y(II) (Figure 3a) and ETR (Figure 4b) in all the species, except for *Q. ilex* and *P. angustifolia* in August, and of *Q. ilex*, *P. angustifolia* and *C. creticus* in January. A partial or total recovery of ETR after the summer inhibition was observed in September for all the species, except for *L. lucidum* (Figure 4b). The species that exhibited a reduced ETR in August also showed a decrease in the Chl index (Figure 4c). Consequently, a positive relationship between ETR and Chl index was observed in August (R = 0.83; $p < 0.01$), with *Q. ilex*, *P. angustifolia*, *P. halepensis*, and *P. lentiscus* displaying higher values of both ETR and Chl compared to the other species (Figure 5a). These sclerophyllous species were also characterised by higher LMA values, leading to a positive relationship between ETR and LMA (R = 0.89; $p < 0.01$) (Figure 5b). Conversely, in January, a positive relationship between ETR and Anth index was observed (R = 0.89; $p < 0.05$), with *P. angustifolia, Q. ilex*, *P. halepensis*, and *C. creticus* showing the highest Anth index values (Figure 5c).

Table 3. Seasonal variation of electron transport rate (ETR), maximum photochemical efficiency of PSII (Fv/Fm), chlorophyll fluorescence decrease ratio (Rfd), chlorophyll index (Chl), flavonoid index (Flav), and anthocyanin index (Anth). Values are the average of the 11 plant species of the Mediterranean shrub formation of Villa Corridi urban park. Data were analysed independently by one-way ANOVA. For each parameter, data followed by different letters in the same line are significantly different for the significance level indicated.

	Nov	Jan	May	Aug	Sep	*p*-Value
ETR (μmol m^{-2} s^{-1})	95.3 a	55.2 b	102.5 a	65.6 b	100.2 a	<0.0001
Fv/Fm	0.816 a	0.740 d	0.792 b	0.773 c	0.795 b	<0.0001
Rfd	2.61 c	1.50 d	3.57 b	3.85 b	4.62 a	<0.0001
Chl	1.21 a	1.25 a	1.10 b	0.91 c	0.96 c	<0.0001
Flav	1.07 c	1.33 a	1.20 b	0.98 c	1.19 b	<0.0001
Anth	0.02 c	0.16 a	0.11 b	0.01 c	0.00 c	<0.0001

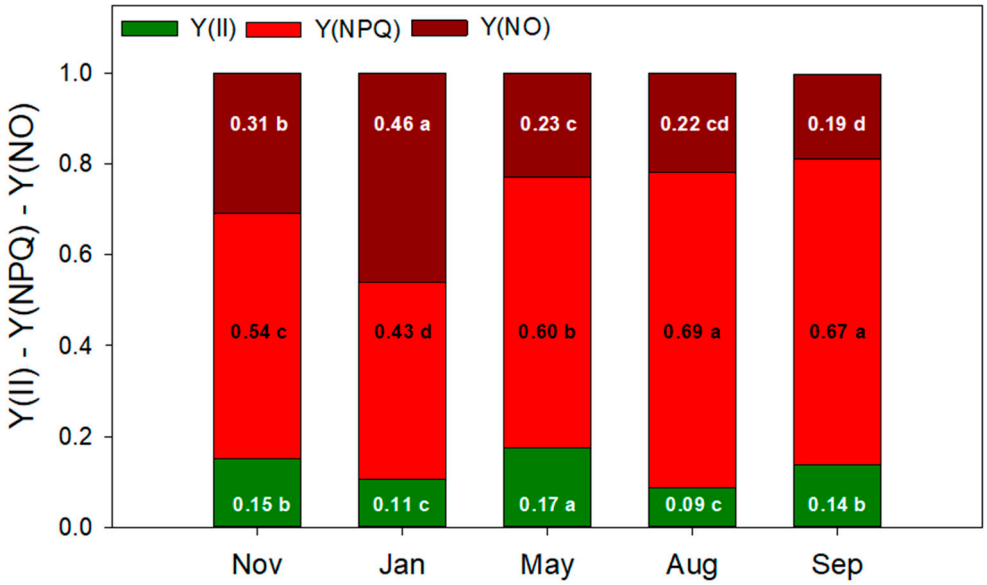


Figure 2. Seasonal variation of steady-state effective quantum yield of photosystem II (PSII) photochemistry in the light Y(II), light-regulated Y(NPQ), and non-regulated Y(NO) non-photochemical energy dissipation at PSII. Values are the average of the 11 plant species of the Mediterranean shrub formation of Villa Corridi urban park. Data were analysed independently by one-way ANOVA. For each parameter, data followed by different letters in the same line are significantly different ($p \leq 0.05$, Fisher's LSD).

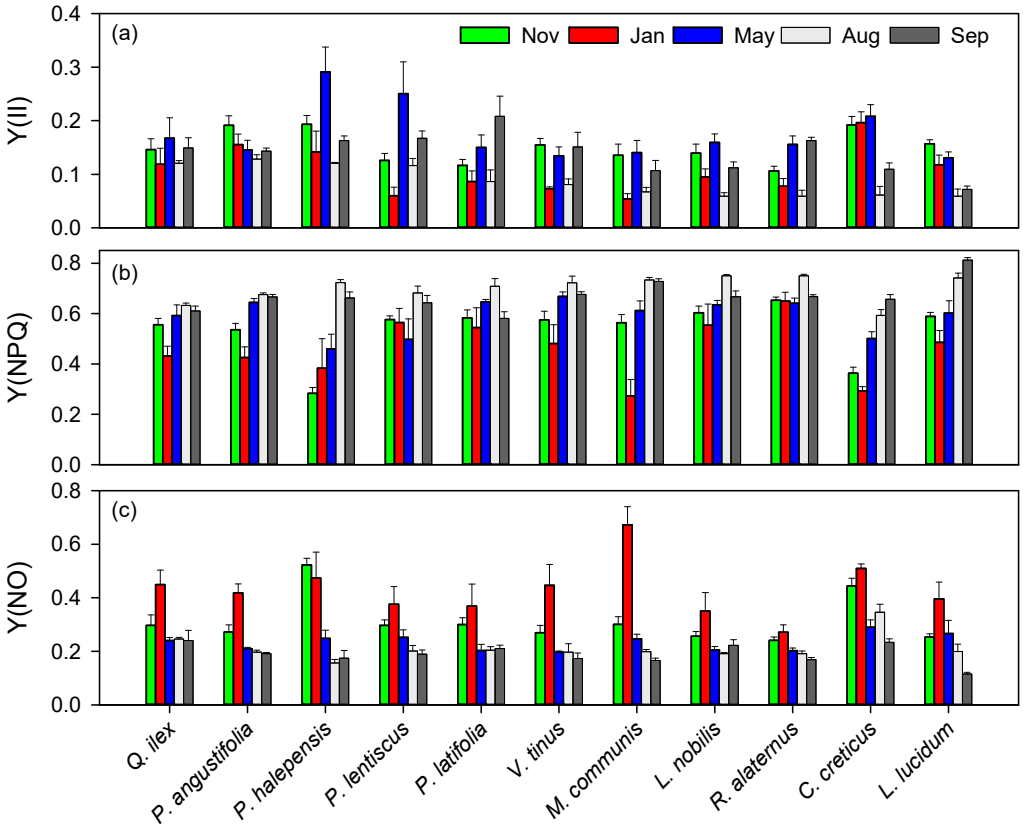


Figure 3. Seasonal variation of (**a**) steady-state effective quantum yield of photosystem II (PSII) photochemistry in the light Y(II), (**b**) light-regulated Y(NPQ), and (**c**) non-regulated Y(NO) non-photochemical energy dissipation at PSII in the 11 plant species of the Mediterranean shrub formation of the park of Villa Corridi. Data were analysed independently by one-way ANOVA for the variable factors of species and time (Table S1).

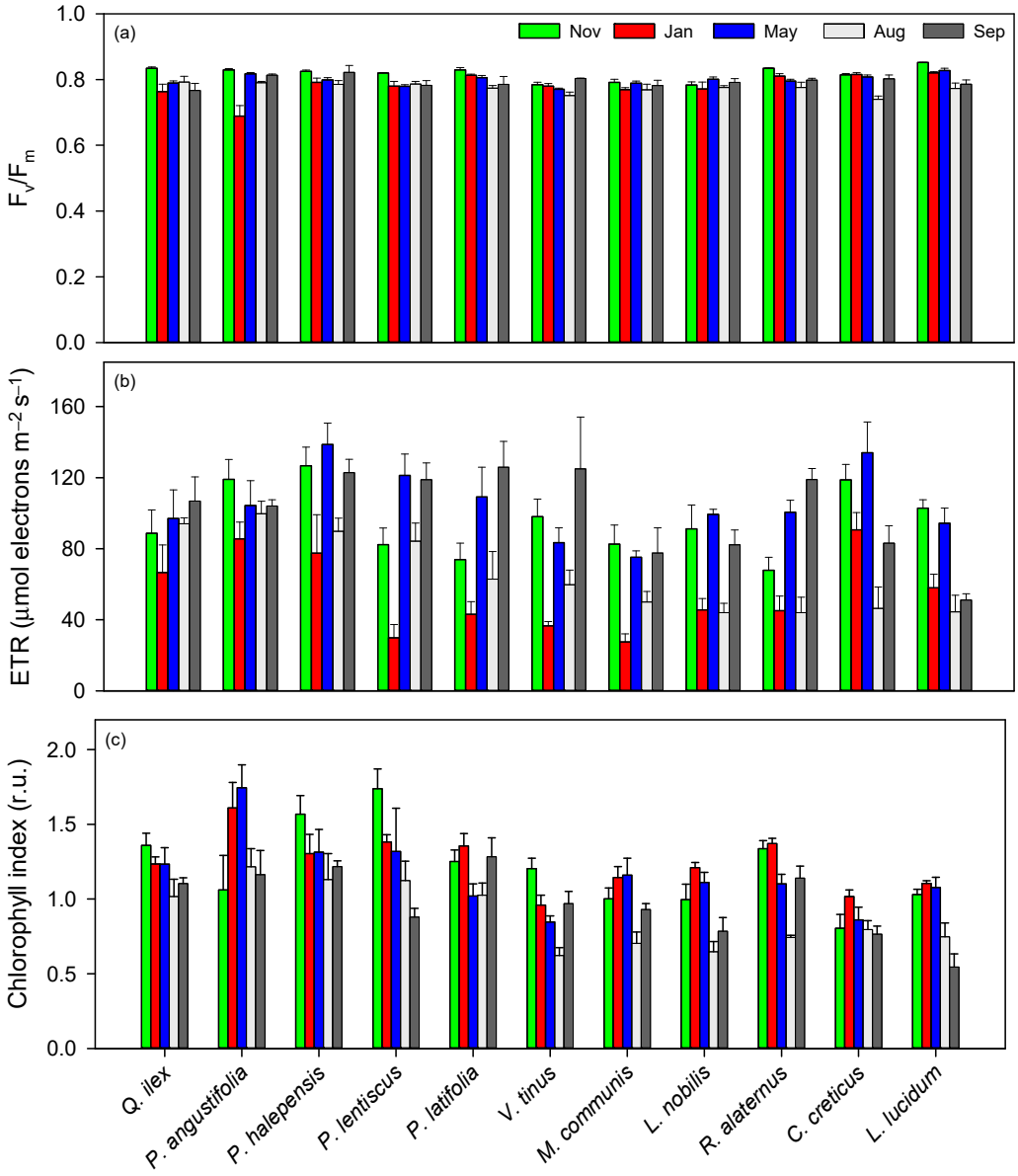


Figure 4. Seasonal variation of (**a**) maximum photochemical efficiency of photosystem II in the dark (Fv/Fm), (**b**) electron transport rate (ETR), and (**c**) chlorophyll index (Chl) in the 11 plant species of the Mediterranean shrub formation of Villa Corridi park. Data were analysed independently by one-way ANOVA for the variable factors of species and time (Table S2).

Approximately 200 schoolchildren (ages 9 to 11 years) and 16 teachers participated in additional training sessions in the OAL, identifying and collecting samples of wild woody species, measuring their photosynthetic performance, and gathering meteorological data.

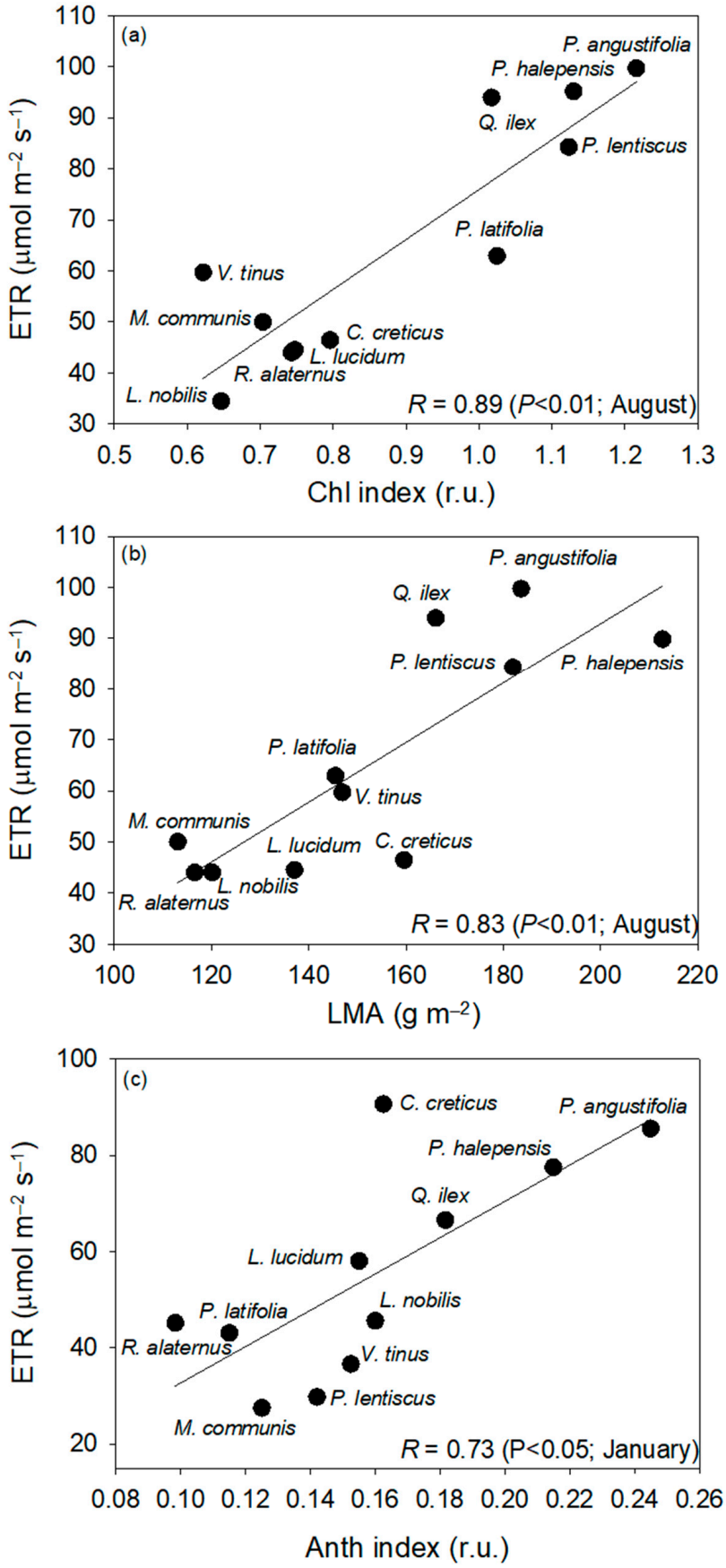


Figure 5. Linear relationships between (**a**) electron transport rate (ETR) and chlorophyll index (Chl) in August; (**b**) ETR and leaf mass per area (LMA) in August; and (**c**) ETR and anthocyanin index (Anth) in January in the 11 plant species of the Mediterranean shrub formation of the park of Villa Corridi.

4. Discussion

The significance of involving children in citizen science activities related to the environment has been extensively discussed and promoted [17]. Active participation in ecological research is particularly valuable for enhancing children's understanding of this scientific field [37]. These activities can also serve as a source of reliable data, provided that quality is ensured through meticulous verification [38,39]. Schoolchildren participating in the experimental campaigns in the park of Villa Corridi searched for selected species and recorded the morphophysiological leaf traits. This location has proven to be an effective living lab for studying wild plant biodiversity and its ecophysiological responses to seasonal environmental changes. This approach, combined with classroom lessons, enabled the participants to (1) enhance their understanding of their surrounding environment and foster a sense of belonging; (2) acquire foundational knowledge of botanical terminology used to identify eight common Mediterranean species and key ecological processes; (3) analyse the connections between biotic and abiotic components of the ecosystem; (4) improve skills such as communication, classification, measurement, inference, and prediction; (5) increase their awareness of the key role of urban green infrastructure, particularly Mediterranean evergreen species, in promoting human well-being and supporting ecosystems; and (6) practice group-collaboration while completing a task. The involvement of schoolchildren and their teachers in citizen science activities related to wild plant species and the environment is particularly significant. During the activities, the students demonstrated their ability to connect a picture and a simple list of diagnostic characters to a living plant. This can be viewed as a preliminary acquisition of basic plant identification knowledge, including habitus (tree vs. small or large shrub), phyllotaxy (opposite vs. alternate), leaf shape (simple vs. compound), leaf colour (concolorous vs. discolorous), leaf margins (entire vs. dentate), leaf scent (scented vs. grassy), and leaf indumentum (hairy vs. glabrous). Previous research has indicated that the general public may not fully understand what biodiversity is and how it relates to ecology [40]. Moreover, children may primarily be attracted to cultivated plants, while wild and less showy species may only capture the interest of researchers [41]. It appears that some children experience a form of "plant blindness" toward native species [14]. The fruitful engagement of children in these research activities through gamification and their direct involvement in specimen collection and analysis has demonstrated that this approach can effectively foster a connection between young students and science. The creation of a photographic atlas on the iNaturalist app, featuring georeferenced observations of the studied species, can also be useful for classroom activities [42]. Each project on iNaturalist includes a map displaying the georeferenced observations from the explored area, allowing for a review of observed biodiversity and familiarization with a technology that simplifies and makes the process of learning the names and identification of wild organisms enjoyable. Additionally, creating a classroom herbarium is an excellent method for teaching botany in schools [43]. Classroom discussions encouraged students to connect the observed plant species with recorded meteorological data and measurements related to plant ecophysiology obtained during activities in the park. This approach, which deviates from the typical classroom routine, exemplifies a multidisciplinary science teaching module [44]. Finally, it is important to emphasise that the informal green space within the park of Villa Corridi has facilitated the establishment of the OAL due to the abundant presence of native wild species. The significance of informal green spaces at the urban level, in terms of the ecosystem services they provide, has been extensively documented [12], particularly in comparison to formal green areas [45].

The direct engagement of schoolchildren with scientific instruments commonly used by researchers to measure photosynthesis and other leaf traits represents a novel approach

to educational activities. Under expert supervision, they utilised the chlorophyll fluorescence technique as a powerful tool to assess the photosynthetic performance and health status of Mediterranean shrubland species [46]. This experience enabled the participants to (1) apply theoretical knowledge from school modules related to photosynthesis through hands-on field experience; (2) understand the instrumentation and techniques that scientists employ to generate replicable and reliable scientific data; and (3) evaluate how the photosynthetic performance of individual Mediterranean species is influenced by the environmental conditions in which they thrive (e.g., solar radiation, precipitation, air temperature, and relative humidity), as recorded by the school's meteorological station. The results indicated a reduction in the PSII photochemical efficiency and electron transport rate of the Mediterranean shrub vegetation in both August and January. This finding highlights a diminished capacity to dissipate light energy through photochemistry during the hot, dry summer and the cold winter periods. In August, daily air temperatures reached their maximum seasonal values, coinciding with a lack of significant precipitation until the end of the month. Conversely, in January, minimum temperatures dropped to near 0 °C. The decreased Y(II) was linked to an increase in the quantum yield of light-dependent (August) and light-independent (January) non-photochemical fluorescence quenching. These processes represent the energy dissipated as heat through regulated and non-regulated energy dissipation pathways, respectively [47]. These results clearly indicate that the fate of absorbed radiation energy at the PSII level varies between winter and summer, alternating the direction of excessive absorbed energy between regulated and non-regulated energy dissipation pathways. Specifically, during the hot and dry summer months, the Mediterranean maquis formation effectively protected PSII by dissipating excessive light energy through regulated non-photochemical processes. In contrast, during winter, the plants increased the proportion of energy lost through harmful non-regulated dissipative pathways [48]. It has been hypothesised that high Y(NO) values are associated with a longer lifetime of energy excitation, which can lead to the formation of reactive oxygen species [49]. This could lead to photoinhibition and photodamage, as indicated by the reduced Fv/Fm and vitality index observed in winter. Furthermore, the increase in the Flav and Anth indices in January suggests a crucial role for antioxidant defences in mitigating oxidative stress and reducing the risk of photoinhibition [50,51]. Conversely, during the hot and dry summer period, the reduction in photochemical efficiency was associated with an increase in Y(NPQ), highlighting the ability of Mediterranean shrub species to safely dissipate excessive absorbed energy as heat without exhibiting chronic photoinhibition signals. Nevertheless, the temporary decrease in Y(II) and ETR during summer confirms that high air temperatures and excessive solar radiation can lead to a reduction in photosynthetic capacity in Mediterranean vegetation [52,53]. Seasonal variations in photosynthetic performance and energy dissipation processes were examined in relation to changes in leaf pigment composition. The reduced ETR in August was linked to a decrease in the Chl index, indicating a rearrangement of the photosynthetic apparatus in response to multi-stress conditions typical of summer. Previous studies have shown that a reduction in chlorophyll content, which is associated with increased leaf reflectance, may help Mediterranean vegetation mitigate excessive light energy by reducing intercepted solar radiation [54,55]. These results highlight the capacity of evergreen Mediterranean maquis to safely dissipate excess energy as heat (i.e., thermal energy dissipation) during the hot and dry summer months. This mechanism helps prevent chronic photodamage to the photosynthetic apparatus, enabling the recovery of photosynthetic performance in September when environmental conditions become milder. Conversely, cold winter periods can negatively impact Mediterranean vegetation by inducing chronic photoinhibition processes and activating the antioxidant system, as indicated by the increase in the

Anth index. Research has shown that Mediterranean species experience two main stress periods: winter and summer [56]. Low winter temperatures result in chronic photoinhibition, while the hot and dry summer months lead to dynamic photoinhibition and a reduction in pigment content. This suggests that summer photoprotection may arise from a combination of increased non-radiative energy dissipation, alternative electron sinks, and a higher carotenoid-to-chlorophyll ratio [56].

The other question addressed with the schoolchildren was whether there were interspecific differences in how plants respond to environmental changes. In fact, species-specific adaptive responses to seasonal environmental changes were observed among the studied Mediterranean species, depending on their peculiar morphophysiological characteristics. Specifically, sclerophyllous species exhibiting the highest LMA values, such as *Q. ilex*, *P. angustifolia*, *P. lentiscus*, and *P. pinea*, were able to maintain a higher ETR and Chl index during the summer compared to the other species investigated. This suggests a strong tolerance of their photosynthetic apparatus to photoinhibition under hot and dry environmental conditions [57–59]. Notably, the Mediterranean sclerophyllous species can achieve a high capacity for CO_2 uptake despite their large LMA, resulting in high productivity [23]. Moreover, some of these species, such as *P. angustifolia*, are considered well suited for the multi-stress conditions typical of urban environments [60] and have been proposed as promising ornamental species for gardening and landscaping in Mediterranean areas [61]. An exception was represented by *L. lucidum*, which, despite exhibiting high LMA values, showed a limited ability to withstand summer stress conditions and did not recover its photochemical efficiency in September. This observation may be attributed to the optimal conditions for this species, which are found in the central and southern regions of China, where a humid subtropical climate prevails [62]. *Quercus Ilex*, *P. angustifolia*, and *P. pinea* maintained higher ETR values during the winter months compared to the other studied species, highlighting their capacity to mitigate cold stress. This ability is also associated with the synthesis of anthocyanins, which function as an antioxidant system to prevent chronic photoinhibition [50,51]. These findings confirm the significant growth plasticity and adaptability of these species to both summer drought and winter frost events in Mediterranean regions [63]. Conversely, the decline in ETR observed in *P. lentiscus* during the winter suggests a sensitivity of this species to cold stress [64]. A distinctive seasonal behaviour was observed in *C. creticus*, a semi-deciduous species of the Mediterranean maquis. *Cistus* species can shed a substantial portion of their leaves during the summer, showing a combination of drought-tolerance and drought-avoidance strategies [65]. According to our data, these species generally exhibited a downregulation of PSII associated with a reduction in leaf chlorophyll concentrations and a rearrangement in the structure of the light harvesting complex to minimise potential photoinhibition during the summer [66]. This was followed by a recovery of physiological performance during the autumn–winter period [67], thereby confirming the resilience of this semi-deciduous species to the Mediterranean climate [68].

Overall, these data support the plasticity and adaptability of shrubland species to both summer and winter stress periods, making them excellent candidates for NbS in urban areas located in coastal Mediterranean regions.

5. Conclusions

This study represents a successful pilot citizen science initiative aimed at engaging young students in biodiversity monitoring and raising their awareness of environmental issues. The results demonstrated the feasibility of an Open-Air Laboratory, where students explored their everyday environment from a new perspective and observed aspects they had previously overlooked, effectively gamifying science. This approach possibly not only heightened their awareness of the importance of biodiversity but also fostered a greater

sense of belonging. Furthermore, the direct involvement of schoolchildren in collecting ecophysiological data on the mechanisms of plant resilience to environmental constraints was successfully achieved. Ultimately, the methodology employed in this study can serve as a pilot model for similar future initiatives.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/land14020423/s1: Figure S1: Climate chart for the city of Livorno; Figures S2 and S3: Cards with species descriptions; Figure S4: Schoolchildren involvement in project activities; Figure S5: The main page of the project created in the iNaturalist website; Figures S6 and S7: Classroom herbarium specimens; and Tables S1 and S2: Statistical analyses.

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