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Toxic Impact of Soil Microplastics (PVC) on Two Weeds: Changes in Growth, Phenology and Photosynthesis Efficiency

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Abstract: Experimental evidence on the bio-ecological effects of microplastics on terrestrial plants is still lacking. In this study, we hypothesized that soil polluted with polyvinyl chloride (PVC) microparticles can negatively influence plant traits, photosynthetic efficiency and phenology of two weeds but with different strength in relation to the species' life traits. Therefore, we conducted an experiment in a common garden growing the wild species *Senecio inaequidens* and *Centaurea cyanus* for about 60 days. The possible toxic effects of soil microplastics (1% of PVC in 100 g of soil medium) were investigated, coupling an analyses on plant traits with an evaluation of the microplastic-induced changes in terms of phenology and photosynthetic efficiency. Overall, results showed that plants in control pots were higher and larger than those in treated ones (*C. cyanus* plant width: $p < 0.05$; *S. inaequidens*—plant height: $p < 0.05$; plant width: $p < 0.05$). Moreover, for *C. cyanus*, photosynthetic efficiency (index Fv/Fm) was significantly lower in the treatment than that in control ($p < 0.05$). About phenology, the second leaf of *S. inaequidens* emerged earlier in control than that in treatments (day 12.2 ± 0.25 and 14.3 ± 0.3, respectively; $p < 0.001$). The obtained results highlight that PVC microparticles may have had negative effects on soil–plant system reducing the performance of plants. Since, up to now, research on the interaction between soil microplastics and terrestrial plants has mainly focused on agricultural plants, this work fills a gap of knowledge regarding wild species (weeds), highlighting the possible future impact of microplastics on biodiversity.

Keywords: wild species; polyvinyl chloride; toxicity; biodiversity; soil pollution; soil properties

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1. Introduction

In recent years, scientists all over the world have observed and investigated the microplastic pollution phenomenon in marine ecosystems, and more recently in inland freshwaters, with increasing alarm for its potential negative consequences [1–3]. However, microplastics in terrestrial systems have been still scarcely investigated, although recent works have suggested that plastic wastes are probably more present in soils than in the oceans [4–6]. In industrialized countries, terrestrial emissions of microplastics seem to mainly originate from farmlands because of sewage sludge treatments which are used to improve soil fertility and favor plant growth [7,8]. However, recent studies reported that environmental microplastic concentrations (soil, urban areas, freshwaters, glaciers, and seas) are related to rainfall and atmospheric deposition [2,9,10].

Microplastics can potentially alter the development and life cycle of organisms by direct and indirect pathways, and ultimately, they can be transferred to food chains [11,12]. In terrestrial environments, microplastic pollution negatively influence ecological functions by affecting microbial activity and soil properties (elemental composition and organic matter) [13]. However, changes in soil properties may greatly vary depending on the type of polymer [14].

Experimental evidences on the bio-ecological effects of microplastics on higher plants are increasing. In particular, exposition to such pollutants has been observed to induce change to morpho-functional traits in several crop species. For instance, Qi et al. [15] investigated the effects of microplastic and plastic films on the main traits of *Triticum aestivum* L. highlighting a negative impact on the reproductive fitness and on the above- and below-ground biomass. Indeed, plastic bioaccumulation (LDPE) is expected to decrease biomass, nutrient up-take and nutritional value of crops causing a significant decline in transpiration, nitrogen content, and growth and in some cases also in yield [16,17]. On the same species, plastic residues were observed to induce changes in soil bacterial community structure, soil pH, electrical conductivity, and C:N ratio [18].

Microplastic pollution has been shown to produce contrasting effects on higher plants depending on the involved species and on the type of polymer. For instance, soil polluted with different kind of polymers such as poly-butylene-adipate-co-terephthalate, polyethylene and polylactic acid modified root and shoot traits of *Phaseolus vulgaris* L. Among such polymers, the first compound induced most pronounced biomass reduction [19]. In *Lactuca sativa* L. PVC microparticles influenced both root morphology and development and photosynthesis (chlorophyll and carotenoid content) with contrasting effects [20]. Furthermore, polystyrene nanoplastics negatively affected the total biomass of *Cucumis sativus* L. altering its sugar metabolism, photosynthetic pigments and antioxidant production [21].

Ecotoxicity and genotoxicity of nano- and microplastics in plants have also been tested. Jiang et al. [22] highlighted that polystyrene increased oxidative stress (i.e., superoxide dismutase and peroxidase activities) in *Vicia faba* L. tissues. In addition, it has been observed that nanoparticles can accumulate in roots, likely blocking cell connections (or cell wall pores) for nutrient transport [22].

Studies focusing on wild species are still scarce [23,24]. Field experiment investigating plastic (bag macroparticle) deposition on dune systems indicated morphological anomalies, reduced survival and colonization success of dune plants (e.g., *Glaucium flavum* Crantz, *Sporobolus pumilus* (Roth) P.M. Peterson & Saarela and *Thinopyrum junceum* (L.) Á. Löve), especially due to non-biodegradable plastics in combination to other environmental factors [25,26]. In controlled conditions, Lozano and Rillig [27] investigated the effects of microplastic fibers in plant communities showing that shoot and root mass can increase with microfiber addition, an effect likely related to a reduced soil bulk density and an improved aeration, allowing a better penetration of roots in the soil.

In this study, we hypothesized that PVC microparticles can negatively influence plant growth in two weeds but with different strength in relation to the species' life traits. To test our hypothesis, we conducted an experiment in controlled conditions using the Asteraceae *Senecio inaequidens* DC. (South African ragwort), and *Centaurea cyanus* L. (Cornflower) a perennial and annual species, respectively. Several traits were considered to assess the impacts of the PVC microplastics on the growth of the two wild species (i.e., plant height, plant width, photosynthetic efficiency, plant phenology).

2. Materials and Methods

2.1. Plant Material

The two target species *C. cyanus* and *S. inaequidens* are common weeds in agricultural and ruderal habitats that also act as invasive species outside their native range. We collected two invasive weeds, since thanks to their ability to colonize a variety of habitats, they are supposed to be particularly resistant to changes induced in soil characteristics due to microplastic application. In particular:

(a) *C. cyanus* is an annual therophyte native to the Eastern Mediterranean and now subcosmopolite having expanded its range following wheat cultivation (archaeophyte; [28]). *C. cyanus* historically spread worldwide associated with cereal crops as segetal species. In the invasion range (Africa, America, and Asia), the species can be found in several habitats such as grasslands, woodlands, roadsides and disturbed sites. For this experi-

ment, seeds were collected in a cultivated area of the Po valley (Busto Arsizio, N-Italy; 45.599663 N; 8.816384 E).

(b) *S. inaequidens* is a perennial chamaephyte native to South Africa and highly invasive in Europe. *S. inaequidens* has been introduced in Europe by the end of the 19th century with sheep's wool commerce [29]. It is currently invasive in many European countries where it can be mostly found in disturbed sites, such as roadsides, railways embankments and quarries, but also in semi-natural grasslands and vineyards [30]. For this experiment, seeds of *S. inaequidens* were collected in a highly invaded area in the former quarry of Collepedrino (Bergamo, N-Italy; 45.779639 N; 9.523546 E).

2.2. Exposure of Plants to Soil Microplastics

Seeds were sown in pot (6.5 cm diameter × 6 cm depth) containing 100 g of potting soil (Compo-Bio, pH 6.5) mixed with sand (40% and 60%, respectively). Treatments were added with 1 g of Polyvinyl chloride (PVC) microplastics powder (Werth-Metall) having a size lower than 250 µm. The used concentration of 1% (*w*/*w*) has been frequently used in previous experimental works (e.g., [19,20]). In total, 10 control and 10 treatments pots per species were set up (i.e., 40 pots) (Figure 1).

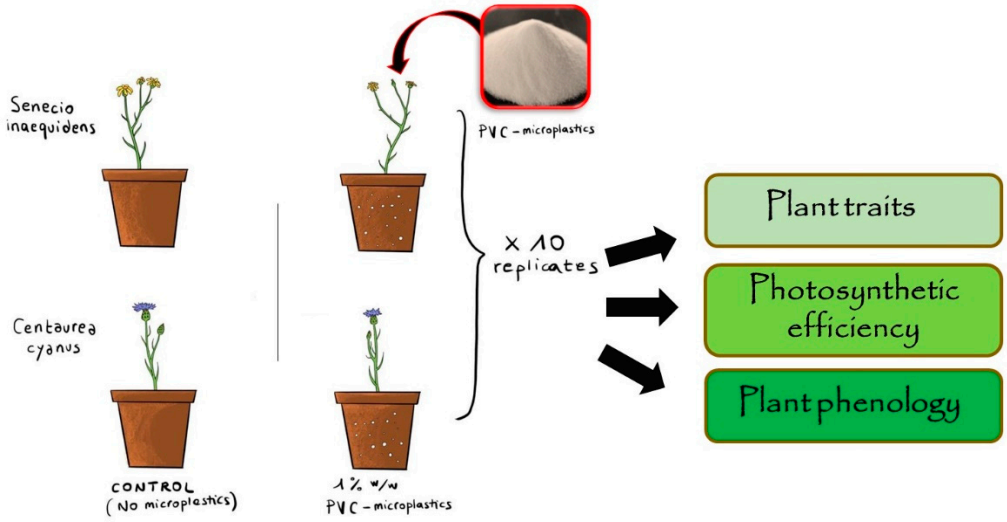

Figure 1. Experimental design: application of soil microplastics (PVC) to *C. cyanus* and *S. inaequidens* and measurements of plant traits, photosynthetic efficiency and plant phenology.

We conducted the experiment from 1 June until 31 July 2020 in a common garden at the Milano-Bicocca University. Mean monthly temperatures were 22.0 °C in June and 25 °C in July; air humidity was 68.4% in June and 65.1 in July. The plants of both species were monitored until second leaf development, then due to the death of several individuals, the experiment was stopped.

2.3. Measurements of Growth Parameters, Phenology and Photosynthetic Efficiency

Plant height (from the collar to the apex; cm) and maximum plant width (cm) were measured once a week after leaf emergency using a steel tape measure. Specific leaf area (SLA; mm^2/mg) was calculated as leaf area divided by leaf dry mass (after the leaf was dried in oven to a constant weight at 70 °C). In particular, leaf area was calculated using the app for mobile phones Easy Leaf Area [31], leaf dry weight (5 biggest leaves) per individual was measured with a precision balance.

In addition, for each species, the dates of the following phenological stages were collected: (a) germination; (b) 1st leaf emission; (c) 2nd leaf emission; (d) flower bud emission; (e) flowering.

The chlorophyll-a fluorescence Fv/Fm (the maximum quantum yield of primary photochemistry of photosystem PS-II), was measured with a Plant Efficiency Analyser

(PEA; Hansatech Instrument Ltd., King's Lynn, UK) with a saturating light intensity of 3500 µmol m^{-2} s^{-1}. This instrument consists of a control unit connected to a sensor head with three LED lights and fluorescence recorder. The fluorescence parameter Fv/Fm was measured in three leaves for each individual plant used in the experiment. Leaves were dark adapted, using a leaf clip, for 20 min before fluorescence measurements. The peak wavelength is 650 nm, which ensures that 95% of the fluorescence comes from Photosystem II (PSII). Details about the instruments can be found in Mathur et al. [32].

2.4. Data Analysis

To assess the growth trajectory of the main plant traits (height and width; dependent variables X) during time (independent variable, Y), we applied four of the most used equations for modelling plant growth [33]:

(a) Linear model, $Y = b_0, + b_1X$, where b_0 is the value of Y when $X = 0$ while b_1 is the slope;
(b) Exponential, $Y = ae^{kX}$, where a is the value of Y when $X = 0$, while k represents the relative increase or decrease in Y for a unit increase in X;
(c) The three-parameter logistic, $Y = \frac{d}{1+exp(b(X-e))}$, where d is the upper asymptote, e is X value producing a response half-way between d and 0, while b is the slope around the inflection point.
(d) Gompertz, $Y = c + (d)exp\ \{-exp\ [b\ (X - e)]\}$, where the parameters have the same meaning as those in the logistic function.

The parameter estimations of growth models were performed using the "aomisc" package based on R language [34,35]. To assess the curve that best fit the growth trajectory of each morphological trait, we compared the values of the small-sample Akaike's Information Criterion (AIC) of each model (Supplementary Material).

Plant traits (i.e., height and width), photosynthetic efficiency (i.e., Fv/Fm) and phenology were compared between control and treated pots using one-way analysis of variance (ANOVA), followed by Tukey HSD test at the $p < 0.05$ level. Relationship between photosynthetic efficiency and plant traits was detected by means of linear regressions. The assumptions of every model were verified by plotting residuals versus fitted value. All statistical analyses were performed using R version 4.0.2 [36].

3. Results

3.1. Centaurea cyanus

Growth trajectory and plant traits. In total, 20 *C. cyanus* individuals were measured during 7 sessions. Growth trajectory was linear for plant height and logistic for plant width (Figure 2a,b). Growth trajectories of height and width started to diverge (with no superimposition between confidence intervals) before 35 and 30 days (respectively) since sowing (the parameters of the curves are reported in Supplementary Material). Plant width reached the plateau of the curves, while plant height grew regularly. At the end of the experiment, plants in control pots were higher and larger than those in treated ones (plant height, at the edge of significance: $F = 3.58_{(1.15)}$, $p = 0.079$; plant width: $F_{(1,15)} = 6.43$, $p < 0.05$; Figure 3a,b). In particular, it was registered an average decrease of 21.4% and 10.4% in plant height and width, respectively. Specific leaf area (SLA) did not show any difference between treatment and control (Supplementary Material).

Photosynthesis. Photosynthetic efficiency, expressed as the index Fv/Fm, significantly differed between treatment and control plants ($F = 6.12_{(1,18)}$, $p < 0.05$), being higher in control than in treated ones (Figure 4a). Moreover, photosynthetic efficiency increased with plant width (estimate: 0.02 ± 0.008, $t = 2.61_{(1,18)}$, $p < 0.05$; Figure 4b).

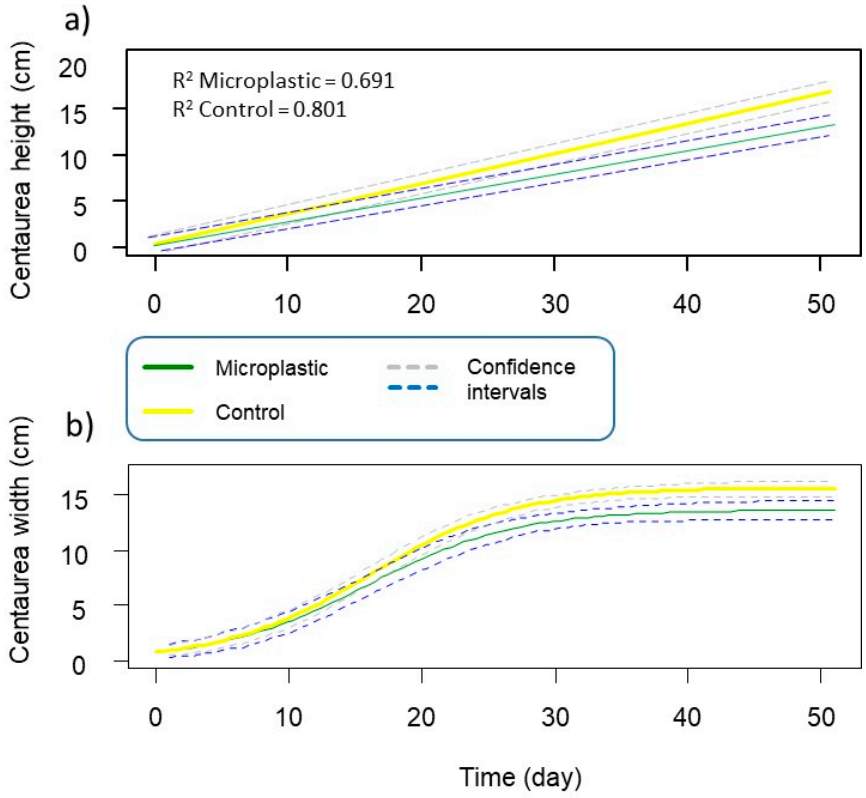

Figure 2. Linear and logistic growth trajectories for plant height (**a**) and width (**b**) of *C. cyanus* during a period of about two months.

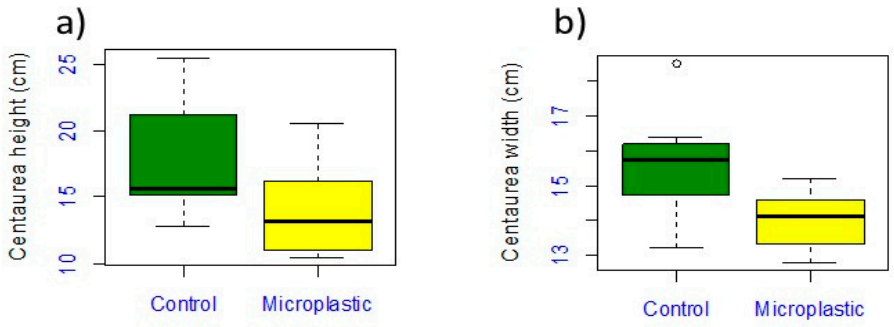

Figure 3. Plant height (**a**) and width (**b**) of *C. cyanus* in control and microplastic treatment.

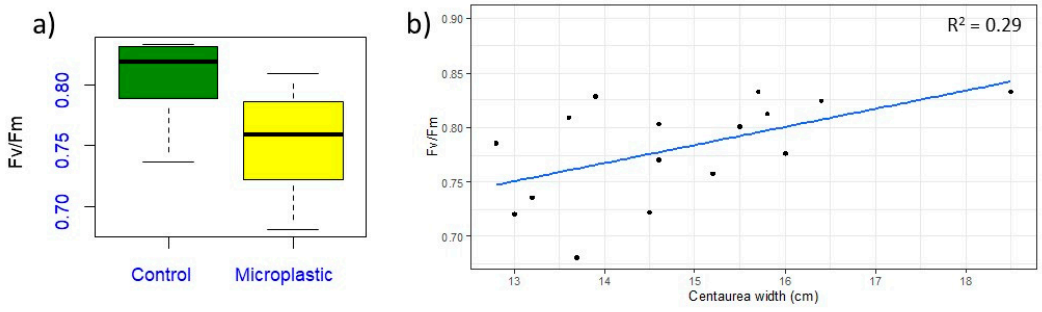

Figure 4. (**a**) Photosynthetic efficiency of *C. cyanus*, expressed as the index Fv/Fm, significantly differed between control and treatment; (**b**) increasing trend of photosynthetic efficiency with plant width.

Phenology. Plant phenology was similar between treatments in the first phases of development. Individuals in control and treated pots germinated at day 3.3 (±0.13) ad 3.2 (±0.15) after seeding, while the first leaf emerged at day 6.5 (±0.19) and 6.8 (±0.22), respectively. However, even if at the edge of significance ($F = 2.91_{(1,18)}$, $p = 0.1$), the second leaf emerged earlier in control plants than in treated ones (at day 14.8 ± 0.56 and 16.1 ± 0.56, respectively).

3.2. *Senecio inaequidens*

Growth trajectory and plant traits. In total, 20 *S. inaequidens* individuals were measured, 10 in control and 10 in treated pots, during 7 sessions, for a total of 140 measures for each vegetation trait. Growth trajectory followed the Gompertz equation for both plant width and plant height (Figure 5a,b). Growth trajectories of both parameters started to diverge (with no superimposition between confidence intervals) after about 35 days since sowing (the parameters of the curves are reported in Supplementary Material). Plant width reached the plateau of the curves, while plant height reached the exponential growth phase of the curve. At the end of the experiment, plants in control pots were higher and larger than those in treated ones (plant height: $F = 5.24_{(1,18)}$, $p < 0.05$; plant width: $F = 7.17_{(1,18)}$, $p < 0.05$; Figure 6a,b). In particular, an average decreases of 28.8% and 22.9% in plant height and width, respectively, were registered. Specific leaf area (SLA) did not show any difference between treatment and control.

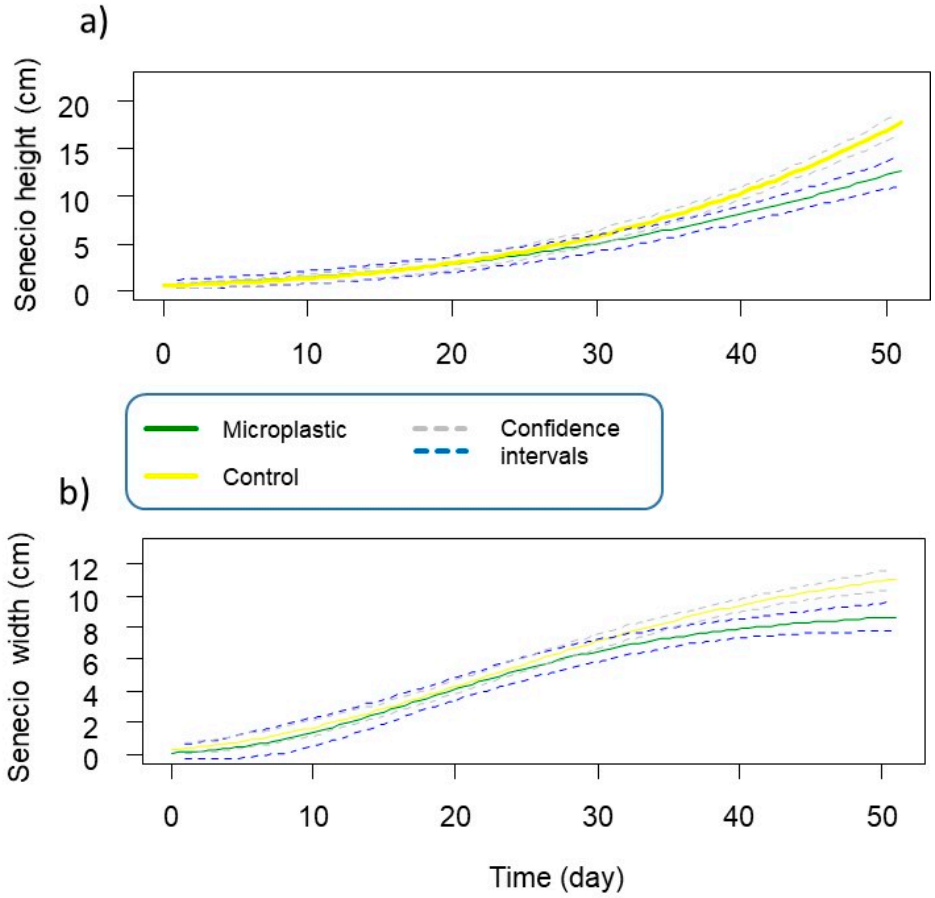

Figure 5. Gompertz growth trajectories for plant height (**a**) and width (**b**) of *S. inaequidens* during a period of about two months.

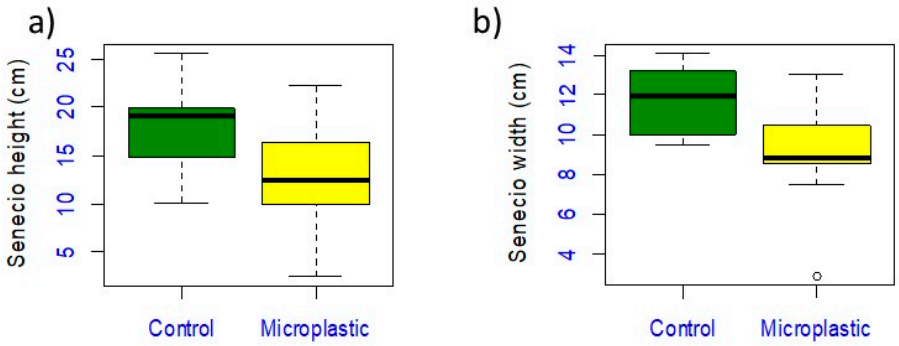

Figure 6. Plant height (**a**) and width (**b**) of *S. inaequidens* in the control and microplastic treatment.

Photosynthesis. Photosynthetic ability, expressed as the index Fv/Fm, did not differ significantly between treatments (Supplementary Material).

Phenology. Plant phenology was similar between treatments in the first phases of development. Individuals in control and treated pots germinated at day 4 (±0.0) ad 3.7 (±0.15) after seeding, while the first leaf emerged at day 8.3 (±0.21) and 8.7 (±0.26), respectively. However, the second leaf emerged earlier in control plants than in treated ones (at day 12.2 ± 0.25 and 14.3 ± 0.3, respectively; $F = 28.97_{(1,18)}$, $p < 0.001$).

4. Discussion

In this study, we found that PVC soil microparticles can negatively influence plant performances in two wild and widespread ruderal species (weeds), *Senecio inaequidens* and *Centaurea cyanus*. As regards the main vegetative traits (plant height and width), PVC microplastics induced lower plant size in both species. On the other hand, results showed a difference in the effects on the two species. While photosynthetic efficiency was only reduced in *C. cyanus*, alteration of leaf phenology, with a delayed emission of leaves, was only observed in *S. inaequidens*. Finally, no effect of microplastics on SLA was recorded for both species. The growth trajectories of the control and treatment of the two species started to diverge between 30 and 40 days after the sowing, but in *C. cyanus*, the effect of microplastics seemed to occur earlier than in *S. inaequidens* (i.e., in the graphs the divergence of the curves of control and treatment is anticipated in *C. cyanus* in comparison to *S. inaequidens*).

Overall, plant traits are controlled genetically and physiologically but they are also influenced by environmental factors and by the presence of pollutants [37]. We deduce that PVC microparticles may have had negative effects on the soil–plant system, reducing the vegetative vigor, the photosynthetic efficiency and the foliar phenology of the studied plants.

Indeed, previous studies indicated some possible physical or biological action mechanisms of microplastics on plants. First of all, microplastics present in soil systems can generate smaller pieces of micro and nanoparticle through fragmentation [38]. On one hand, experiments using fluorescent nanoplastics indicate the presence of particles on the surfaces of the plant tissues (especially of seeds and roots) during all growth stages, physically obstructing the pores of seeds or blocking the roots [39]. Therefore, the particles might reduce water and nutrient uptake and delay germination and plant growth [39,40]. On the other hand, internalization and accumulation in plant tissues and, in some cases, intracellular uptake of nano and microplastics (nanobeads from 20 nm to 5 µm) has been observed in several terrestrial or aquatic species [23]. Both root and shoot has been observed to exhibit different patterns of localization of oxidative stress (i.e., production of ROS) also inducing antioxidant responses [41]. All these mechanisms are involved in the reduction in plant performance (growth).

Another problem derives from the changes in element concentrations in shoot induced by microplastics. For instance, a potassium concentration below the normal threshold

detected in plants was recorded in stems and leaves of plants treated with PVC. Potassium is known to be involved in cell expansion and organ growth [42].

As a general rule, our results are in accordance with some published research on agricultural and aquatic plants about the great negative effect of PVC on plant traits and physiology [42,43]. For instance, similarly to what we observed for the photosynthetic efficiency of *C. cyanus* that was reduced due to microplastic application, other authors observed that PVC microplastics reduced the value of the photosynthetic performance (i.e., chlorophyll content and/or fluorescence) in plants such as *Utricularia aurea* Lour and *Cucurbita pepo* L. [42,43]. On the other hand, different results were also observed for *S. inaequidens*, in our work, and *Lactuca sativa* L. in the work of Li et al. [20]. Indeed, these last species did not show any kind of alteration of the photosynthetic efficiency due to the PVC application between the treatment and the control. Surprisingly, Pignatelli et al. [44] found significantly higher levels of photosynthetic pigments (Chl-a) in *Lepidium sativum* L. plants treated with PVC microplastics (and other plastic types) than in the control.

In our experiment, we observed a great reduction in plant size (plant height and width) in both species. Similarly, Colzi et al. [42] recorded a decreased size of some traits (shoot fresh weight and leaf lamina) in *C. pepo* when treated with PVC microplastics. In the same work, an alteration of root morphology and biomass in plants exposed to PVC microplastics in the soil was also observed. Consistently with such results, toxicity induced by different polymers of soil microplastic application was often observed to reduce size and biomass of plants [13]. Even though the exact mechanisms are still uncertain, this tendency can be due to a combination of several factors highlighted in previous studies:

(a) The stress caused by toxic compounds released by plastic micro particles; in particular, phthalates in plants are observed to affect germination, growth and metabolism [45,46];
(b) The stress caused by toxic chemicals adsorbed on microplastic surfaces which act as a vector for transport (e.g., heavy metals and hydrophobic organic compounds [47,48];
(c) The change caused in chemical status in soil; for instance, PVC has been observed to cause a significant change in soil available P [49], while other polymers were associated with a modification in soil pH [50,51];
(d) The alteration of the soil's physical properties, with potential modifications in moisture retention and root penetration dynamics [50];
(e) The alteration of microbial community composition and enzymatic activities in soil and plant–microbe interactions on plant shoot [43,52].

As a whole, it is possible that due to biophysical and chemical changes occurring in soil, the absorption of macro and micronutrients (e.g., potassium) from plants is altered, with subsequent effects on their photosynthetic ability and finally on their growth [13,50]. However, several discrepancies in the effect of microplastics on plants can depend on the considered species and exposition time applied to treatments, as well as on the concentration, size, shape and chemical composition of plastic particles [23,53].

Finally, it should be noted that to assess significant effects caused by microplastics to plants, it is generally necessary to apply relatively higher concentrations of particles in the soil than those occurring in terrestrial environment, as already highlighted by van Weert et al. [54]. Although this suggests no immediate consequences for ecological risk [54], recent research highlighted that some plant roots (of wheat and lettuce) are able to uptake microplastics with a size of 2 μm [21]. Considering that bigger plastic particles can degrade into smaller particles [55], further efforts in the understanding of the ecological risk assessment of microplastics for plants are needed.

5. Conclusions

In conclusion, our work highlighted how PVC microparticles in soil can negatively influence plant growth as well as photosynthetic efficiency and plant phenology in the two weeds *C. cyanus* and *S. inaequidens*, but with different strength. First of all, our work fills a gap of knowledge regarding wild species (one native and one invasive alien species, in our area) since, up to now, researches on the interaction between soil microplastics and

terrestrial plants has mainly focused on agricultural plant species. Since a large number of studies emphasize the increasing presence of microplastics in natural habitats, future research will have also to focus on their effect on the growth and fitness of wild species (i.e., threat to biodiversity), especially those subject to extinction risk in fragile habitats.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12051219/s1, Supplementary Material.

Author Contributions: R.G., L.Q., S.C. (Sarah Caronniand) and S.C. (Sandra Citterio). conceived the idea and the sampling design. R.G., L.Q. and E.C. led the writing of the manuscript and of the statistical analyses. R.G., E.C., C.M. and L.Q. participated in the field data collection for the different abiotic and biotic ecosystem components. All authors have read and agreed to the published version of the manuscript.

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