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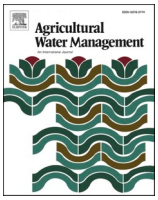

Wild edible plant species grown hydroponically with crop drainage water in a Mediterranean climate: Crop yield, leaf quality, and use of water and nutrients.

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ABSTRACT

There is an increasing interest in the cultivation of wild edible plants (WEP) in consideration of their quality attributes and salt tolerance, which makes these species good candidates for cascade cropping systems (CCS). In these systems, saline effluents from a salt-sensitive donor crop are used to irrigate a receiving crop with greater salt tolerance. The objective of this study was to evaluate two WEP species, *Picris hieracioides* (PH) and *Plantago coronopus* (PC) as candidate crops for CCS. Both species were grown hydroponically with saline effluent from a semi-closed substrate culture of tomato (the donor crop). Both PH and PC were grown in floating system for 36 days during spring using one of the following nutrient solutions: i) standard nutrient solution (CNS, control); ii) NaCl-enriched (50 mmol L^{-1}) standard nutrient solution (SNS); iii) effluent from tomato substrate culture (TE); iv) artificial effluent (ATE), i.e. a nutrient with ion concentrations and salinity level (approximately 50 mmol L^{-1} NaCl) very close to those of TE. Compared with CNS, leaf production was significantly reduced in both TE (−33.6%) and ATE (−33.6%) plants of PH, and only in TE (−23.3%) plants of PC. In both species, leaf Na content increased in SNS (+858.1% in PH; +279.4% in PC), TE (+704.7% in PH; +226.3 in PC) and ATE (+697.7% in PH; +229.4% in PC) plants compared with the controls. Leaf antioxidant capacity was positively correlated with total phenol content and, in PC, increased in SNS (+74.3%), TE (+53.9%) and ATE plants (+37.7%) compared with the controls. In conclusion, both PH and PC could be grown in CCS with saline greenhouse hydroponic effluents since the moderate reduction of leaf production could be partially compensated by reduced production costs because of zero costs for fertilisers. The growth inhibition observed in both WEPs species cultivated with the hydroponic effluent was primarily due to its high salinity with minor or no effects due to the suboptimal nutrient levels and/or the presence of phytotoxic root exudates or microbial metabolites.

1. Introduction

In greenhouse soilless culture, the waste of water and nutrients with drainage water and the consequent environmental impact can be strongly reduced by the adoption of closed-loop systems (Massa et al., 2020). In these systems, the recirculating nutrient solution is normally discharged, at least partially, when the electrical conductivity (EC) and/or the concentration of some potential toxic ion (e.g., sodium) reaches a maximum acceptable threshold (semi-closed systems; Massa et al., 2010). Instead of conventional wastewater treatments before discharge in the environment, there are several strategies for sustainable management of the effluents from open- and semi-closed soilless cultures, such as phytoremediation using constructed wetlands and the re-use for algae culture (Richa et al., 2020) or cascade cropping systems (CCS; Massa et al., 2020). In CCS, the effluent from a donor crop is used for the fertigation of one or more receiving crops with higher tolerance to salinity.

The first studies on greenhouse CCS date back to 20 years ago (Incrocci et al., 2003), but a renewed interest has recently arisen for these systems applied to greenhouse soilless (e.g. Avdouli et al., 2021; García-Caparrós et al., 2021a; Katsoulas et al., 2020; Santos et al., 2022) or soil-bound crops (Santos et al., 2022) or in open field (Muñoz et al., 2017).

The goal of CCS is to take advantage of the residual water and nutrients of hydroponic effluents and to make them safer for the environment, thus reducing the costs of fertilisers and water depuration. In

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addition, the relatively high salinity of effluents may improve the quality attributes of receiving crops, for instance increasing the fruit dry matter (Incrocci et al., 2003) or the content of several compounds that are associated with the organoleptic, nutritional, and nutraceutical quality of many fruit and leafy vegetables (Avdouli et al., 2021). The re-use of hydroponic effluents has several drawbacks, however, since they may have high salinity and abnormal nutrient mutual ratios (Samiotis et al., 2022) and/or contain phytotoxic roots exudates (Avdouli et al., 2021; Hosseinzadeh et al., 2017), microbial metabolites (Salazar et al., 2021), and residues of plant protection products (Santos et al., 2022). Moreover, long-term fertigation of soil-grown crops with drainage water from soilless culture can result in soil degradation and environmental pollution (Santos et al., 2022).

Many wild edible plant (WEP) species are halophyte or salt-tolerant glycophyte (Isayenkov, 2019; Lombardi et al., 2022) and therefore are good candidates for the role of receiving crops in CCS. There is a growing interest in the cultivation of WEPs in consideration of their nutritional and nutraceutical properties due to their high content of phytochemicals (Ceccanti et al., 2020). *Picris hieracioides* L. (PH; Hawkneed oxtongue) and *Plantago coronopus* L. (PC; Buck's horn) are two WEPs that could be grown in CCS.

Picris hieracioides is a biennial or short-lived perennial weed belonging to the family of *Asteraceae* (Gillbank, 2014). This species has been rediscovered thanks to the renewed interest in traditional food (Ceccanti et al., 2018) and the nutraceuticals and medicinal properties of its leaves (Ceccanti et al., 2020) due, for instance, to the content of numerous terpenoid glycosides that can be used against fever and inflammation (Uchiyama et al., 1990).

Plantago coronopus is a perennial herb that belongs to the family of Plantaginaceae and is considered a facultative halophyte (Bueno et al., 2020, 2021; Ltaeif et al., 2021). Its leaves are commonly consumed fresh in mixed salads and appreciated for its salty taste and high nutritional value due to a high content of phenolic compounds, essential amino acids, and minerals (Jdey et al., 2017; Koyro, 2006).

Very few papers have been published on the hydroponic cultivation of PC (e.g. Bueno et al., 2020; Bueno et al., 2021; Ceccanti et al., 2022; Chu and Brown, 2021) and to the best of our knowledge no work has been conducted on PH, apart from the study conducted by Ceccanti et al. (2020).

The goal of the present work was to investigate the performance of PH and PC grown hydroponically with the drainage water from a greenhouse substrate culture of tomato, an important greenhouse crop that is widely cultivated in soilless systems. In separate and parallel experiments, both PH and PC were grown in floating systems under the typical climate conditions that occur in spring in Mediterranean greenhouses, using the following nutrient solutions: a genuine or artificial effluent from a semi-closed tomato substrate culture, and a standard solution containing negligible (<1.0 mmol L^{-1}) or high (50 mmol L^{-1}) NaCl concentration. In a preliminary experiment, this high NaCl concentration had induced a limited reduction of plant growth in both PC and PH, and no symptom of salt toxicity was detected in either species. Based on the results reported in the literature on CCS or found in the preliminary experiment, we hypothesized that:

i. both species can be grown with saline effluents from greenhouse hydroponics with limited reduction of leaf production;
ii. the growth inhibition induced by saline hydroponic effluents is mainly due to high salinity, with no or minor effects due to the abnormal ion composition and/or the putative presence of root and/or microbial exudates;
iii. the use of saline nutrient solution improves leaf quality in both species.

2. Materials and methods

2.1. Plant material and growing conditions

The trials were conducted in an experimental glasshouse at the University of Pisa, Italy (lat. 43°42'42"48 N, long. 10°24'52"92 E), in spring 2021 under natural light. Air temperature and relative humidity, and solar radiation were monitored by a weather station located inside the greenhouse. Basic information on the experiment is reported in Table 1.

Seeds of PH and PC were purchased from Gargini Sementi (Lucca, Italy) and sown in 240-cell polystyrene trays with rockwool plugs. The trays were placed in a growth chamber at 25 °C for five days and seedlings of PC and PH were planted, respectively, 26 and 40 days after sowing in polystyrene raft boards floating in 50-L plastic tanks (water depth 25 cm) with stagnant nutrient solution. Each tank hosted 24 plants and there were four tanks per m^2; therefore, the crop density was approximately 96 plants m^{-2} of ground area. In all the tanks, the nutrient solution was continuously aerated and dissolved oxygen was above 6 mg L^{-1} during the whole experiment.

In both species, leaves were harvested twice, 22 and 36 days after transplanting (DAT, Table 1), by cutting the shoot approximately 2 cm above the collar level. At each harvest, leaf samples were collected for growth analysis and the laboratory determinations of minerals, pigments, flavonoids, and phenols, and the total antioxidant capacity. At the end of the experiment, samples were also collected for the determination of root biomass and mineral content.

2.2. Experimental design and nutrient solutions

The intention of this study was to separate the effects of salinity from those of other defects (see Introduction) of highly saline hydroponic wastewater. Therefore, four different nutrient solutions were compared in a randomized design with three replicates, each consisting of one hydroponic tank. The nutrient solutions were the following: i) standard nutrient solution (CNS, control); ii) NaCl-enriched (50 mmol L^{-1}) standard nutrient solution (SNS); iii) effluent from tomato substrate culture (TE); iv) artificial effluent (ATE), i.e. a nutrient with ion concentrations and salinity level (approximately 50 mmol L^{-1} NaCl) very close to those of TE.

The solutions CNS, SNS and ATE were prepared by dissolving appropriate amount of technical-grade inorganic salts in tap water, which contained approximately 0.78 mmol L^{-1} Na and 0.68 mmol L^{-1} of Cl. The salinity of hydroponic effluent, which contained 29.0 mmol L^{-1} Na and 23.0 mmol L^{-1} Cl, was deliberately increased to a level similar to that of SNS by adding 21.0 mmol L^{-1} NaCl. The mineral composition, electrical conductivity (EC), and pH of the four nutrient solutions are shown in Table 2. All nutrient solutions were prepared at the beginning of the experiment and stored at 8 °C in the dark. The composition of each solution was regularly checked and did not significantly change during

Table 1
Basic information on the experiment with *Picris hieracioides* and *Plantago coronopus* plants grown hydroponically (floating system) in greenhouse in 2021.

Sowing date	*P. hieracioides*	c9 March 2021	
	P. coronopus	23 March 2021	
Transplant date		18 April 2021	
Start of treatment		26 April 2021	
		First cut	Second cut
Harvest date		10 May 2021	24 May 2021
Days of treatment		14 (22)[a]	28 (36)[a]
Mean air temperature (°C)		21.2[b]	22.8[b]
Mean daily solar radiation (MJ m^{-2} day^{-1})		13.7[b]	13.8[b]
Cumulative solar radiation (MJ m^{-2})		301.9[b]	177.5[b]

[a] The figure within brackets is the number of days after transplanting.
[b] The values were computed for the period from transplanting to the first cut.

Table 2
Mineral composition, electrical conductivity, and pH of the four nutrient solutions used in the experiment with *Picris hieracioides* and *Plantago coronopus* grown hydroponically (floating system) in greenhouse.

	Treatment abbreviation			
	CNS	SNS	TE	ATE
$N\text{-}NO_3$ (mmol L^{-1})	10.0	10.0	7.5	7.5
P (mmol L^{-1})	1.5	1.5	0.4	0.4
K (mmol L^{-1})	9.0	9.0	8.4	8.4
Ca (mmol L^{-1})	4.5	4.5	4.4	4.4
Mg (mmol L^{-1})	2.0	2.0	2.3	2.3
Na (mmol L^{-1})	0.8	50.0	50.0	50.8
Cl (mmol L^{-1})	0.7	50.1	44.0	50.7
Fe (μmol L^{-1})	40.0	40.0	22.6	22.6
B (μmol L^{-1})	40.0	40.0	20.0	20.0
Cu (μmol L^{-1})	3.0	3.0	3.3	3.3
Zn (μmol L^{-1})	10.0	10.0	15.0	15.0
Mn (μmol L^{-1})	10.0	10.0	3.8	3.8
Electrical conductivity (dS m^{-1})	2.3	6.9	6.7	6.9
pH	5.6	5.6	5.6	5.6

Note: CNS: standard nutrient solution (control); SNS: NaCl-enriched (50 mmol L^{-1}) standard nutrient solution; TE: effluent from a tomato substrate culture; ATE: artificial effluent with ion concentrations and salinity level (approximately 50 mmol L^{-1}) very close to those of TE. Mean values (n = 3) keyed by the same letter are not statistically different at 5% level after Tukey's post-hoc test.

the experiment, which lasted 36 days. Before use, the pH of all nutrient solutions was adjusted to 5.6 with dilute sulphuric acid.

The TE was collected in an independent experiment on the effects of different salinity levels (10, 40, and 70 mmol L^{-1} NaCl) on tomato growth and fruit quality. This experiment was left out of this study, which focused on PH and PC. The experiment with tomato was conducted using grafted plants; the scion was Pisanello, a landrace variety quite popular in Tuscany, Italy, and the rootstock was Maxifort. The closed-loop hydroponic unit consisted of eight rockwool slabs (Grodan, Rockwool B.V., Roermond, The Netherlands), each hosting three plants, with a crop density of 3.2 plant m^{-2}. The volume of the nutrient solution in the mixing tank and the total volume of the recirculating solution were 130 L (17.3 L m^{-2}) and 250 L (33.3 L m^{-2}), respectively. The mineral composition of the starter and refill nutrient solutions, used in the first stage of the experiment, are reported in Table 3. During the first phase of the experiment, the crop was protected against pests (whiteflies, fungus gnats, thrips and leafminers) using beneficials and *Bacillus thuringiensis* (one distribution) and against diseases (grey mould and late blight) using two fungicides containing cyprodinil and fludioxonil or dimetomorf and copper sulphate (two applications). The TE consisted of a blend of the nutrient solutions discharged from six separate hydroponic units after 24 days of recirculation, on occasion of the first discharge before the salinity treatments were applied, when tomato plants were 73 days old (from the sowing date) and the second truss was in bloom.

2.3. Determinations

2.3.1. Crop yield and growth

Crop yield was determined by recording the fresh weight (FW) of all the leaves collected at first and second harvest in each hydroponic tank. Leaf dry weight (DW) was determined after drying fresh leaves in a ventilated oven at 70 °C till constant weight. At each harvest, a subsample of fresh leaves was collected from each tank to determine leaf area and then succulence; each sample consisted of three individual plants. Leaf area was measured using a digital planimeter (DT Area Meter MK2, Delta T-Devices, Cambridge, UK) and leaf succulence was calculated as the ratio between leaf FW and LA. Root DW was also determined at the end of the experiment.

2.3.2. Mineral content of plant organs and nutrient solutions

Dry samples of leaves or roots were digested with a mixture (5:2) of nitric acid (65%) and perchloric acid (35%) at 240 °C for 1 h. Minerals were determined in mineralized samples as follows: K, Ca, Mg, Na, Cu, Fe, Mn, and Zn by atomic absorption spectroscopy; P by UV/VIS spectrometry. The NO_3^- content was also measured spectrophotometrically in dry leaf samples extracted with distilled water (100 mg DW in 20 mL) at room temperature for 2 h, using the salicylic-sulphuric method. These methods were also used to measure the concentration of nutritive elements and Na in the filtered samples of nutrient solutions collected during the experiments. Dry plant samples were also used for the determination of organic N by the Kjeldahl method.

2.3.3. Water and mineral uptake

Plant water uptake was determined by measuring the volume of the nutrient solutions used to refill the hydroponic tanks at first harvest time, and of the tap water used for the replenishment at the end of the experiment (second harvest). Water added to each hydroponic unit was assumed to be equal to the plant water uptake because the tank was completely covered by the polystyrene tray and therefore direct evaporation was negligible.

Plant mineral uptake was determined based on the dry biomass and mineral content of leaves collected at each harvest and in the roots at the end of the experiment. The apparent mineral uptake was calculated based on the volume and the mineral composition of the nutrient solutions used to refill the hydroponic tanks (Table 1) and of the residual solutions at the end of the experiments (Table S1).

The use efficiency of water (WUE) and nutrients was calculated as the ratio between crop yield and total crop uptake of water and each nutrient.

2.3.4. Leaf quality attributes

Sampled fresh leaves were rapidly cut in small discs and 5 mL of methanol 99% v/v were added to each sample (100 mg), which was extracted by sonication for 60 min and then maintained for 24 h at 4 °C. The methanol extract was used to determine spectrophotometrically the concentrations of chlorophyll a, chlorophyll b, and carotenoids according to Wellburn and Lichtenthaler (1984). To determine the flavonoid content, 0.1 mL of the methanol extract was added to 0.06 mL of $NaNO_2$ (5%), 0.04 mL of $AlCl_3$ (10%) and after five minutes to 0.4 mL of NaOH and 0.2 mL of H_2O; afterwards, the absorbance was read at 510 nm and the results were expressed as mg catechin g^{-1} FW (Kim et al., 2003). The same methanol extracts were used to determine the content of total phenols using the Folin-Ciocalteau reagent (Kang and Saltveit,

Table 3
Mineral composition of the starter and refill nutrient solution, used in the first stage of the tomato experiment, from which the effluent was collected for the experiment with *Picris hieracioides* and *Plantago coronopus*.

	$N\text{-}NO_3$ (mmol L^{-1})	P (mmol L^{-1})	K (mmol L^{-1})	Ca (mmol L^{-1})	Mg (mmol L^{-1})	Na (mmol L^{-1})	Cl (mmol L^{-1})	Fe (μmol L^{-1})	B (μmol L^{-1})	Cu (μmol L^{-1})	Zn (μmol L^{-1})	Mn (μmol L^{-1})	Mo (μmol L^{-1})
Starter solution	11.0	1.2	8.0	5.0	2.5	8.0	7.1	40.0	30.0	1.0	8.0	10.0	1.0
Refill solution	11.0	1.0	6.0	4.0	1.0	8.0	7.1	20.0	20.0	1.0	5.0	10.0	1.0

2002). The total phenol content was calculated using the calibration curve containing 0, 50, 100, 150, and 250 mg gallic acid L^{-1}, and expressed as mg of gallic acid equivalent (GAE) kg^{-1} FW. The total antioxidant capacity was measured in the methanol extracts with the ferric reducing ability of plasma (FRAP) assay (Benzie and Strain, 1996) and expressed as mmol L^{-1} Fe(II) kg^{-1} FW.

2.4. Statistical analysis

Data were tested for homogeneity of variances using Levene's test and then subjected to 1- or 2-way ANOVA with harvest date and nutrient solution as variables. Mean values were separated by Tukey's post-hoc test ($P < 0.05$). Statistical analysis was performed using JMP Statistical Software.

3. Results

3.1. Picris hieracioides

3.1.1. Crop yield

The leaves harvested at the first cut accounted for 59.9%, 67.7%, 78.0% and 78.0% of total yield, respectively, in CNS, SNS, TE and ATE plants, with no significant differences across the treatments (Fig. 1, left). Crop yield, leaf and total DW did not differ significantly in CNS and SNS plants while, compared with the controls, they were significantly reduced in plants grown with TE (respectively, −33.6%, −39.7% and −43.4%) or ATE (respectively, −33.6%, −34.8% and −35.0%), with no significant differences between these treatments (Table 4). The root DW was significantly lower in SNS (−39.4%), TE (−45.5%) and ATE (−39.4%) plants than in the controls (Table 4). Leaf area index was also reduced in plant grown with SNS (−27.1%), TE (−39.2%) and ATE (−43.6%), with no significant differences between SNS and TE and between TE and ATE (Table 4).

3.1.2. Plant mineral content

There were significant differences between the controls and the plants grown with saline nutrient solutions as regards the leaf content (expressed on a DW basis) of all the mineral elements apart from P. Significant differences were also found between SNS, TE and ATE plants for Ca, Mg, Na, Mn, and Zn (Table 5). No significant differences were revealed between TE and ATE plants for the leaf content of all the elements, apart from the content of Mn, which was much lower (−49.2% on average) in TE plants than in ATE plants (Table 5).

On average, K leaf content was significantly higher in the controls than in SNS, TE and ATE plants, while leaf Ca content was higher in TE plants than in those grown with CNS or SNS (Table 5). The CNS plants showed, on average, a significantly higher leaf Mg content than other groups of plants. The lowest and highest leaf Na content was found, respectively, in CNS and SNS plants. Total leaf N content was slightly but significantly higher in TE plants than in the controls. Higher leaf Zn content was also measured in plants grown with TE and ATE with respect to CNS and SNS plants. Leaf Fe content was higher in SNS and ATE plants than in the controls.

The leaf K/Na molar ratio (calculated on a DW basis) was much higher in CNS (9.32) compared with the other treatments (0.69, on average) without significant differences between SNS, TE and ATE (Fig. S1, left).

On average, the leaves collected at the second cut contained less K, Mn and Fe, and more N, Mg and Na than the leaves of the first harvest (Table 5).

Total root N content was significantly higher in TE plants than in the others (Table S2). The root content of Mg and Zn was lower in SNS and CNS plants than those grown with the hydroponic effluents (Table S2). The roots of SNS, TE and ATE plants contained more Ca (with no significant difference between SNS and CNS) and Na and less K than the roots of the controls (Table S2). Besides, a lower content of K was detected in the roots of TE and ATE plants compared with SNS plants (Table S2).

3.1.3. Water and mineral uptake

Total water uptake was significantly greater in CNS plants (+31.2%, on average) than in those grown with SNS, TE, and ATE, with no significant differences across these treatments (Table 6).

Table 4
Total crop yield (fresh leaves), leaf, root and total dry weight (DW) production, and leaf area index (mean value of the index determined at first and second cut) of *Picris hieracioides* plants grown hydroponically (floating system), for 36 days, in greenhouse with different nutrient solutions.

Nutrient solution	Yield (kg m^{-2})	Leaf DW (kg m^{-2})	Root DW (kg m^{-2})	Total plant DW (kg m^{-2})	Leaf area index
CNS	3.01 a	0.267 a	0.033 a	0.297 a	2.91 a
SNS	2.66 a	0.221 ab	0.020 b	0.240 ab	2.12 b
TE	2.00 b	0.161 b	0.018 b	0.168 b	1.77 bc
ATE	2.00 b	0.174 b	0.020 b	0.193 b	1.64 c
ANOVA					
Nutrient solution	***	**	*	**	***

Note: CNS: standard nutrient solution (control); SNS: NaCl-enriched (50 mmol L^{-1}) standard nutrient solution; TE: effluent from a tomato substrate culture; ATE: artificial effluent with ion concentrations and salinity level (approximately 50 mmol L^{-1}) very close to those of TE. Mean values (n = 3) keyed by the same letter are not statistically different at 5% level after Tukey's post-hoc test. Significance level: *** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$; ns = not significant.

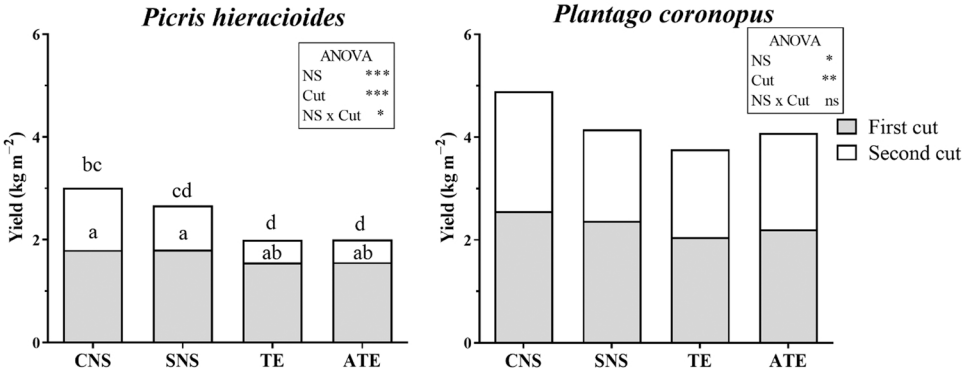

Fig. 1. Crop yield (fresh leaves) of *Picris hieracioides* and *Plantago coronopus* plants grown hydroponically (floating system) in greenhouse with different nutrient solutions (NS). Plants were harvested twice during the experiment, 22 and 36 days after transplanting; the values inside the bar are the percent contribution of each harvest to the total crop yield. CNS: standard nutrient solution (control); SNS: NaCl-enriched (50 mmol L^{-1}) standard nutrient solution; TE: effluent from a tomato substrate culture; ATE: artificial effluent with ion concentrations and salinity level (approximately 50 mmol L^{-1}) very close to those of TE. Mean values (n = 3) keyed by the same letter are not statistically different at 5% level after Tukey's post-hoc test. Significance level: *** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$; ns = not significant.

Table 5
Leaf mineral content (on a dry weight basis) in *Picris hieracioides* plants grown hydroponically (floating system) for 36 days in greenhouse with different nutrient solutions. Nitrogen content included both organic and inorganic forms. Leaves were cut twice during the growing period, 22 and 36 days after transplanting.

Cut	Nutrient solution	N-total (g kg^{-1})	P (g kg^{-1})	K (g kg^{-1})	Ca (g kg^{-1})	Mg (g kg^{-1})	Na (g kg^{-1})	Cu (mg kg^{-1})	Mn (mg kg^{-1})	Fe (mg kg^{-1})	Zn (mg kg^{-1})
First	CNS	44.7 cd	7.0	78.2	29.0	6.2 a	4.7 e	10.3 a	125.0 b	140.0 ab	85.0
	SNS	43.0 d	8.2	49.3	31.6	4.0 c	37.9 b	9.8 b	130.0 b	145.0 ab	85.0
	TE	48.5 ab	7.8	47.0	39.4	3.1 d	32.6 cd	10.0 ab	100.0 c	150.0 ab	120.0
	ATE	45.7 bcd	7.6	46.3	39.0	3.4 d	31.2 d	10.0 b	135.0 b	165.0 a	110.0
Second	CNS	47.5 abc	7.1	58.6	33.1	5.8 a	3.9 e	10.0 b	95.0 c	75.0 d	70.0
	SNS	50.7 a	7.4	38.6	26.7	4.6 b	44.4 a	9.9 b	145.0 ab	115.0 bc	68.3
	TE	49.0 ab	6.4	36.4	38.0	4.3 bc	36.6 bc	10.0 ab	50.0 d	85.0 cd	136.0
	ATE	48.2 ab	7.0	35.3	33.0	4.4 bc	37.5 b	10.0 b	160.0 a	95.0 cd	120.0
Mean effects											
First		45.5 b	7.7	55.2 a	34.8	4.2 b	26.6 b	10.0	122.5 a	150.0 a	100.0
Second		48.8 a	7.7	42.2 b	32.7	4.8 a	30.6 a	10.0	112.5 b	92.5 b	98.6
	CNS	46.1 b	7.1	68.4 a	31.1 bc	6.0 a	4.3c	10.2 a	110.0 b	107.5 b	77.5 b
	SNS	46.8 ab	7.8	44.0 b	29.2c	4.3 b	41.2 a	9.9 b	137.5 a	130.0 a	76.7 b
	TE	48.7 a	7.1	41.7 b	38.7 a	3.7c	34.6 b	10.0 ab	75.0c	117.5 ab	128.0 a
	ATE	46.9 ab	7.3	40.8 b	36.0 ab	3.9c	34.3 b	10.0 ab	147.5 a	130.0 a	115.0 a
ANOVA											
Cut		* **	ns	* **	ns	* **	* **	ns	*	* **	ns
Nutrient solution		* *	ns	* **	* *	* **	* **	* *	* **	*	* **
Cut x Nutrient solution		* **	ns	ns	ns	* **	* *	*	* *	* **	ns

Note: DW: dry weight; CNS: standard nutrient solution (control); SNS: NaCl-enriched (50 mmol L^{-1}) standard nutrient solution; TE: effluent from a tomato substrate culture; ATE: artificial effluent with ion concentrations and salinity level (approximately 50 mmol L^{-1}) very close to those of TE. Mean values (n = 3) keyed by the same letter are not statistically different at 5% level after Tukey's post-hoc test. Significance level: *** P ≤ 0.001; ** P ≤ 0.01; * P ≤ 0.05; ns = not significant.

Table 6
Total uptake of water, mineral nutrients, and sodium in *Picris hieracioides* plants grown hydroponically (floating system), for 36 days, in greenhouse with different nutrient solutions.

Nutrient solution	Water (L m^{-2})	N (g m^{-2})	P (g m^{-2})	K (g m^{-2})	Ca (g m^{-2})	Mg (g m^{-2})	Na (g m^{-2})	Cu (mg m^{-2})	Mn (mg m^{-2})	Fe (mg m^{-2})	Zn (mg m^{-2})
CNS	100.0 a	13.23 a	3.06 a	20.95 a	9.29	2.03 a	1.30c	3.37 a	35.52 a	38.96	33.49
SNS	78.3 b	10.47 ab	2.69 ab	11.10 b	7.42	1.08 b	9.31 a	2.57 ab	32.77 a	35.55	25.16
TE	78.3 b	8.44 b	1.97 b	7.92 b	7.13	0.66 b	5.92 b	2.00 b	16.95 b	27.35	30.05
ATE	72.1 b	8.68 b	2.12 b	8.49 b	7.51	0.76 b	6.22 b	2.14 b	28.04 ab	32.94	29.75
ANOVA											
Nutrient solution	**	**	*	***	ns	***	***	**	**	ns	ns

Note: CNS: standard nutrient solution (control); SNS: NaCl-enriched (50 mmol L−^{1}) standard nutrient solution; TE: effluent from a tomato substrate culture; ATE: artificial effluent with ion concentrations and salinity level (approximately 50 mmol L^{-1}) very close to those of TE. Mean values (n = 3) keyed by the same letter are not statistically different at 5% level after Tukey's post-hoc test. Significance level: *** P ≤ 0.001; ** P ≤ 0.01; * P ≤ 0.05; ns = not significant.

In general, the actual uptake of all the elements considered matched the apparent uptake in all the treatments, as the divergence between the two quantities calculated with different procedures ranged between − 10.2% and 8.5% (Table S3).

Compared with the controls, SNS plants absorbed less K and Mg, and TE and ATE plants less N, P, K, Mg, Cu, and Mn (not ATE plants) (Table 6). The crop uptake of Ca, Fe, and Zn was not significantly affected by the composition of the nutrient solutions (Table 6). Sodium accumulated remarkably in SNS, TE, and ATE plants (Table 6); SNS plants showed a significantly higher Na uptake with respect to both the controls (+858.1%) and the plants grown with the hydroponic effluents (+701.2%, on average).

The use efficiency of water, N, P, Ca, Cu, and Fe did not differ across the treatments while that of K and Mg was significantly higher than in CNS in SNS, TE, and ATE plants, with no differences between these treatments (Table 7). Also, no significant differences were also found between SNS and ATE plants as regards Mn use efficiency, which in contrast increased in TE compared with SNS and ATE (Table 7). Plants grown with SNS showed a higher Zn compared with CNS, TE, and ATE plants, with no differences between the plants grown with hydroponic effluents (Table 7).

Table 7
Water and nutrient use efficiency in *Picris hieracioides* plants grown hydroponically (floating system), for 36 days, in greenhouse with different nutrient solutions.

Nutrient solution	Water (kg L^{-1})	N (kg g^{-1})	P (kg g^{-1})	K (kg g^{-1})	Ca (kg g^{-1})	Mg (kg g^{-1})	Cu (kg mg^{-1})	Mn (kg mg^{-1})	Fe (kg mg^{-1})	Zn (kg mg^{-1})
CNS	0.303	0.233	1.009	0.145 b	0.338	1.524 b	0.913	0.088 ab	0.080	0.093 ab
SNS	0.342	0.254	0.994	0.240 a	0.363	2.471 a	1.039	0.081 b	0.075	0.106 a
TE	0.257	0.237	1.023	0.254 a	0.280	3.042 a	0.998	0.118 a	0.073	0.067 b
ATE	0.277	0.232	0.947	0.237 a	0.269	2.630 a	0.941	0.072 b	0.061	0.068 b
ANOVA										
Nutrient solution	ns	ns	ns	***	ns	***	ns	*	ns	**

Note: CNS: standard nutrient solution (control); SNS: NaCl-enriched (50 mmol L^{-1}) standard nutrient solution; TE: effluent from a tomato substrate culture; ATE: artificial effluent with ion concentrations and salinity level (approximately 50 mmol L^{-1}) very close to those of TE. Mean values (n = 3) keyed by the same letter are not statistically different at 5% level after Tukey's post-hoc test. Significance level: * ** P ≤ 0.001; * * P ≤ 0.01; * P ≤ 0.05; ns = not significant.

3.1.4. Leaf quality

Leaf content (expressed on a FW basis) of dry matter, nitrates, and total carotenoids, flavonoids, and phenols content were not significantly affected by the composition of the nutrient solution (Table 8). The plants grown with SNS showed higher leaf Na content and FRAP index, and were more succulent than CNS, TE, and ATE (not as regards the succulence) plants (Table 8). All the measured quantities shown in Table 8 did not differ significantly in TE and ATE plants.

On average, the leaves collected at the second cut contained less pigments, phenols, and flavonoids, had a lower FRAP index, and were slightly more succulent than the leaves of the first cut (Table 8).

A significant positive correlation (R^2 =0.725; P > 0.0001; n = 24) was found between leaf antioxidant capacity and total phenol content (Fig. S2, left).

3.2. Plantago coronopus

3.2.1. Crop yield

The leaves harvested at the first cut accounted for 52.4%, 57.1%, 54.7%, and 54.2% of total yield, respectively, in CNS, SNS, TE, and ATE plants, with no significant differences across the treatments for leaf production at both cuts (Fig. 1, right).

Total crop yield, however, was significantly lower in TE plants than in the controls (−23.3%) while it did not differ in SNS, TE, and ATE plants (Table 9). Leaf area index was significantly higher in the controls (+23.5%) than in SNS, TE, and ATE, with no significant differences between these treatments. The composition of the nutrient solution did not affect leaf and total DW and root DW (Table 9).

3.2.2. Plant mineral content

There were significant differences between the controls and the plants grown with saline nutrient solutions as regards the leaf content (expressed on a DW basis) of all the mineral elements considered with the exception of Cu (Table 10). Significant differences were also found between SNS, TE, and ATE plants for N, K, Mg, Na, Mn, and Zn (Table 10). Leaf mineral content did not differ significantly in TE and ATE plants (Table 10).

Table 9
Total crop yield (fresh leaves), leaf, root and total dry weight (DW) production, and leaf area index (mean value of the index determined at first and second cut) of *Plantago coronopus* plants grown hydroponically (floating system), for 36 days, in greenhouse with different nutrient solutions.

Nutrient solution	Yield (kg m^{-2})	Leaf DW (kg m^{-2})	Root DW (kg m^{-2})	Total DW (kg m^{-2})	Leaf area index
CNS	4.90 a	0.272	0.013	0.287	2.47 a
SNS	4.15 ab	0.251	0.013	0.262	2.01 b
TE	3.76 b	0.252	0.017	0.269	1.90 b
ATE	4.08 ab	0.265	0.020	0.294	2.08 b
ANOVA					
Nutrient solution	*	ns	ns	ns	* **

Note: CNS: standard nutrient solution (control); SNS: NaCl-enriched (50 mmol L^{-1}) standard nutrient solution; TE: effluent from a tomato substrate culture; ATE: artificial effluent with ion concentrations and salinity level (approximately 50 mmol L^{-1}) very close to those of TE. Mean values (n = 3) keyed by the same letter are not statistically different at 5% level after Tukey's post-hoc test. Significance level: * ** P ≤ 0.001; * * P ≤ 0.01; * P ≤ 0.05; ns = not significant.

On average, leaf content of K, Mg, Fe, and Mn was significantly higher in the controls than in SNS, TE, and ATE plants (Table 10). Total leaf N content was slightly but significantly reduced in TE and ATE plants (Table 10). The lowest and highest leaf Na content was detected, respectively, in CNS and in SNS plants. No significant differences were found between TE and ATE plants for all the parameters shown in Table 10, apart from leaf Mg content that was higher in ATE. The leaves of TE plants contained less N, K, Mg, Na, Mn, and Fe, and more Zn compared with SNS plants (Table 10).

The leaf K/Na ratio was higher in CNS (2.13) than in the other treatments (0.37, on average) (Fig. S1, right).

On average, the leaves collected at the second cut contained less P, K, and Fe, and more N, Mg, Mn, and Zn than those of the first harvest (Table 10).

Table 8
Leaf content (on a fresh weight basis) of dry matter, nitrate, sodium, chlorophylls, carotenoids, phenols and flavonoids, antioxidant capacity (measured by ferric reducing antioxidant power), and leaf succulence in *Picris hieracioides* plants grown hydroponically (floating system), for 36 days, in greenhouse with different nutrient solutions. Leaves were cut twice during the growing period, 22 and 36 days after transplanting.

Cut	Nutrient solution	Dry matter content (%)	Nitrate (mg kg^{-1})	Sodium (mg kg^{-1})	Total chlorophylls (mg kg^{-1})	Carotenoids (mg kg^{-1})	Total phenols (mg kg^{-1})	Flavonoids (mg kg^{-1})	FRAP (mmol Fe(II) kg^{-1})	Leaf succulence (g cm^{-2})
First	CNS	8.75	2113.6	410.6	2472.5	426.7	7.11 ab	6.68	75.62 b	0.051c
	SNS	8.96	1822.7	3403.1	2080.2	365.9	7.24 a	6.71	97.78 a	0.059 bc
	TE	8.26	2145.7	2695.9	2386.7	411.4	5.79 bcd	5.30	46.76 d	0.056 bc
	ATE	9.13	2275.7	2836.9	2055.6	370.8	6.34 abc	5.60	63.53 bc	0.062 b
Second	CNS	9.31	2311.9	354.5	1811.8	254.3	4.68 d	4.23	32.24 e	0.054 bc
	SNS	6.79	1981.2	3020.6	1416.5	226.7	4.88 d	4.48	42.16 de	0.074 a
	TE	7.45	1820.5	2721.0	1371.0	202.4	5.73 cd	4.80	55.45 cd	0.058 bc
	ATE	7.42	1797.2	2786.0	1128.7	177.8	5.35 cd	4.84	53.29 cd	0.059 bc
Mean effects										
First		8.77	2089.4	2336.6	2248.7 a	393.7 a	6.62 a	6.07 a	70.92 a	0.057 b
Second		7.74	1977.7	2220.6	1432.0 b	214.3 b	5.16 b	4.59 b	45.78 b	0.061 a
	CNS	9.03	2212.7	382.7c	2142.1 a	340.4	5.90	5.46	53.93 b	0.052 c
	SNS	7.88	1902.0	3211.9 a	1748.3 ab	310.2	6.06	5.60	69.97 a	0.066 a
	TE	7.85	1983.1	2708.4 b	1878.9 ab	306.9	5.76	5.05	51.10 b	0.057 bc
	ATE	8.28	2036.5	2811.4 b	1592.1 b	274.3	5.85	5.22	58.41 b	0.061 ab
ANOVA										
Cut		ns	ns	ns	* **	* **	* **	* **	* **	*
Nutrient solution		ns	ns	* **	* *	ns	ns	ns	* **	* **
Cut x Nutrient solution		ns	ns	ns	ns	ns	* **	ns	* **	* *

Note: FRAP: ferric reducing antioxidant power; CNS: standard nutrient solution (control); SNS: NaCl-enriched (50 mmol L^{-1}) standard nutrient solution; TE: effluent from a tomato substrate culture; ATE: artificial effluent with ion concentrations and salinity level (approximately 50 mmol L^{-1}) very close to those of TE. Mean values (n = 3) keyed by the same letter are not statistically different at 5% level after Tukey's post-hoc test. Significance level: * ** P ≤ 0.001; * * P ≤ 0.01; * P ≤ 0.05; ns = not significant.

Table 10
Leaf content (on a dry weight basis) of mineral nutrients and sodium in *Plantago coronopus* plants grown hydroponically (floating system) for 36 days in greenhouse with different nutrient solutions. Nitrogen content included both organic and inorganic forms. The leaves were cut twice during the growing period. Leaves were cut twice during the growing period, 22 and 36 days after transplanting.

Cut	Nutrient solution	N-total (g kg^{-1})	P (g kg^{-1})	K (g kg^{-1})	Ca (g kg^{-1})	Mg (g kg^{-1})	Na (g kg^{-1})	Cu (mg kg^{-1})	Mn (mg kg^{-1})	Fe (mg kg^{-1})	Zn (mg kg^{-1})
First	CNS	46.5 a	8.1	58.4 a	38.0	9.0 b	15.9 e	10.0	105.0	155.0 a	67.0 b
	SNS	43.7 b	8.3	40.8 b	36.4	6.7c	62.2 a	10.1	60.0	103.7 bc	37.7 d
	TE	42.2 b	7.2	35.1 c	31.7	5.1 e	55.6 bc	10.1	38.3	88.3 d	50.0 c
	ATE	43.6 b	7.3	37.5 bc	31.7	5.5 de	51.6 cd	10.0	40.0	73.3 e	37.7 d
Second	CNS	48.2 a	7.9	56.7 a	38.1	10.9 a	16.1 e	10.1	94.5	107.0 b	52.5 c
	SNS	48.5 a	6.8	30.7 d	34.1	6.1 cd	59.2 ab	10.0	70.0	91.3 cd	50.3 c
	TE	47.2 a	6.5	30.2 d	29.9	6.2 cd	48.8 d	10.1	40.0	70.0 e	69.0 b
	ATE	46.9 a	7.0	31.4 d	34.4	6.7 c	53.8 bcd	10.0	75.0	75.0 e	80.0 a
Mean effects											
First		44.0 b	7.7 a	42.9 a	34.4	6.6 b	46.3	10.0	60.8 b	105.1 a	48.1 b
Second		47.7 a	7.0 b	37.3 b	34.1	7.5 a	44.5	10.0	69.9 a	85.8 b	63.0 a
	CNS	47.3 a	8.0 a	57.6 a	38.0 a	9.9 a	16.0c	10.0	99.8 a	131.0 a	59.8 a
	SNS	46.1 ab	7.5 ab	35.8 b	35.3 ab	6.4 b	60.7 a	10.0	65.0 b	97.5 b	44.0 b
	TE	44.7 c	6.9 b	32.6 c	30.8 b	5.6 c	52.2 b	10.1	39.2c	79.2 c	59.5 a
	ATE	45.3 bc	7.1 b	34.4 bc	33.1 ab	6.1 bc	52.7 b	10.0	57.5 b	74.2 c	58.8 a
ANOVA											
Cut		* **	* *	* **	ns	* **	ns	ns	* **	* **	* **
Nutrient solution		* **	* *	* **	*	* **	* **	ns	* **	* **	* **
Cut x Nutrient solution		* *	ns	* **	ns	* **	*	ns	ns	* **	* **

Note: CNS: standard nutrient solution (control); SNS: NaCl-enriched (50 mmol L^{-1}) standard nutrient solution; TE: effluent from a tomato substrate culture; ATE: artificial effluent with ion concentrations and salinity level (approximately 50 mmol L^{-1}) very close to those of TE. Mean values (n = 3) keyed by the same letter are not statistically different at 5% level after Tukey's post-hoc test. Significance level: *** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$; ns = not significant.

Root content of total N, K, Mg, Mn, Fe, and Zn was significantly higher in the controls than in the SNS, TE, and ATE plants, with no significant differences between these treatments for N, K, and Mn, and between ATE and SNS plants for Fe (Table S4). Root Mg content was lower in TE and ATE plants than in SNS plants (Table S4). A lower root content of P was also detected in ATE plants compared with CNS and SNS plants (Table S4). Root Na content was much lower in the controls than in the SNS, TE, and ATE plants, with no significant differences between these treatments (Table S4).

3.2.3. Water and mineral uptake

The total uptake of water, N, P, Ca, and Cu was not significantly affected by the composition of the nutrient solution and no significant differences were found between TE and ATE plants for all the quantities reported in Table 11.

The actual uptake of all the elements considered corresponded to the apparent uptake in all the treatments (Table S5), since the divergence between the two quantities was − 7.4% to 5.9%. Compared with the controls, SNS, TE, and ATE plants absorbed less K, Mg, Fe, and Mn, with no significant differences across the saline nutrient solutions for most elements (Table 11). Total Zn uptake was significantly greater in TE and ATE plants than in CNS and SNS plants (Table 11).

Sodium accumulated much less in the controls than in SNS, TE, and ATE, without significant differences across these treatments (Table 11).

The use efficiency of water, P, and Ca did not differ significantly in the four plant groups (Table 12). Also, no significant differences were found between CNS and SNS plants as regards the use efficiency of N and Cu which in contrast decreased in TE and ATE plants, with no significant differences between these treatments (Table 12). The use efficiency of K, Mg, Mn, and Fe was higher than CNS in SNS, TE, and ATE, with no differences between treatments, except for Mn. In fact, Mn use efficiency was significantly higher in TE plants than in the other treatments. Zinc use efficiency was significantly lower in the controls than in SNS plants but higher than the one calculated for TE plants (Table 12).

3.2.4. Leaf quality

Leaf nitrate content (expressed on a FW basis) and succulence were not affected by composition of nutrient solution (Table 13).

Leaf antioxidant capacity and content of dry matter, Na, phenols, and flavonoids were significantly higher in SNS (not for DW/FW), TE and ATE plants than in the controls (Table 13). No significant differences were found across SNS, TE, and ATE treatments as regards all the measured quantities shown in Table 8, apart from the DW/FW ratio, which was significantly lower in SNS plants than in those grown with TE (Table 13). The FRAP index was also significantly lower in ATE plants than in those grown with SNS.

On average, the leaves collected at the second cut contained less pigments, phenols, and flavonoids, and more nitrate and Na than the leaves of the first cut (Table 13). Besides, the second-cut leaves had a lower antioxidant capacity and higher DW/FW ratio, and were less

Table 11
Total uptake of water, mineral nutrients, and sodium in *Plantago coronopus* plants grown hydroponically (floating system), for 36 days, in greenhouse with different nutrient solutions.

Nutrient solution	Water (L m^{-2})	N (g m^{-2})	P (g m^{-2})	K (g m^{-2})	Ca (g m^{-2})	Mg (g m^{-2})	Na (g m^{-2})	Cu (mg m^{-2})	Mn (mg m^{-2})	Fe (mg m^{-2})	Zn (mg m^{-2})
CNS	94.6	13.35	2.79	16.49 a	10.97	2.97 a	4.39 b	3.10	29.18 a	40.21 a	20.60 a
SNS	80.0	11.75	2.48	9.52 b	9.33	1.78 b	15.87 a	2.71	17.10 b	26.76 b	12.87 b
TE	86.6	11.73	2.39	8.80 b	8.38	1.59 b	14.21 a	2.94	10.90c	23.01 b	18.63 a
ATE	78.2	12.63	2.60	9.79 b	9.53	1.85 b	14.97 a	3.14	16.38 b	23.36 b	18.69 a
ANOVA											
Nutrient solution	ns	ns	ns	* **	ns	* **	* **	ns	* **	* **	* **

Note: CNS: standard nutrient solution (control); SNS: NaCl-enriched (50 mmol L^{-1}) standard nutrient solution; TE: effluent from a tomato substrate culture; ATE: artificial effluent with ion concentrations and salinity level (approximately 50 mmol L^{-1}) very close to those of TE. Mean values (n = 3) keyed by the same letter are not statistically different at 5% level after Tukey's post-hoc test. Significance level: * *** $P \leq 0.001$; * * $P \leq 0.01$; * $P \leq 0.05$; ns = not significant.

Table 12
Water and nutrient use efficiency in *Plantago coronopus* plants grown hydroponically (floating system), for 36 days, in greenhouse with different nutrient solutions.

Nutrient solution	Water (kg L^{-1})	N (kg g^{-1})	P (kg g^{-1})	K (kg g^{-1})	Ca (kg g^{-1})	Mg (kg g^{-1})	Cu (kg mg^{-1})	Mn (kg mg^{-1})	Fe (kg mg^{-1})	Zn (kg mg^{-1})
CNS	0.526	0.367 a	1.758	0.297 b	0.449	1.651 b	1.584 a	0.168c	0.122 b	0.238 b
SNS	0.520	0.353 a	1.682	0.436 a	0.445	2.332 a	1.534 a	0.243 b	0.155 a	0.323 a
TE	0.436	0.321 b	1.585	0.428 a	0.450	2.372 a	1.280 b	0.348 a	0.164 a	0.202c
ATE	0.529	0.323 b	1.575	0.417 a	0.432	2.211 a	1.297 b	0.251 b	0.176 a	0.218 bc
ANOVA										
Nutrient solution	ns	* **	ns	* **	ns	* **	* *	* **	* **	* **

Note: CNS: standard nutrient solution (control); SNS: NaCl-enriched (50 mmol L^{-1}) standard nutrient solution; TE: effluent from a tomato substrate culture; ATE: artificial effluent with ion concentrations and salinity level (approximately 50 mmol L^{-1}) very close to those of TE. Mean values (n = 3) keyed by the same letter are not statistically different at 5% level after Tukey's post-hoc test. Significance level: * ** $P \leq 0.001$; * * $P \leq 0.01$; * $P \leq 0.05$; ns = not significant.

Table 13
Leaf content (on a fresh weight basis) of dry matter, nitrate, sodium, chlorophylls, carotenoids, phenols and flavonoids, antioxidant capacity (measured by ferric reducing antioxidant power), and leaf succulence in *Plantago coronopus* plants grown hydroponically (floating system) for 36 days in greenhouse with different nutrient solutions. Leaves were cut twice during the growing period, 22 and 36 days after transplanting.

Cut	Nutrient solution	Dry matter content (%)	Nitrate (mg kg^{-1})	Sodium (mg kg^{-1})	Total chlorophylls (mg kg^{-1})	Carotenoids (mg kg^{-1})	Total phenols (mg kg^{-1})	Flavonoids (mg kg^{-1})	FRAP (mmol Fe (II) kg^{-1})	Leaf succulence (g cm^{-2})
First	CNS	5.33	1689.6	844.3c	986.9	169.0 ab	3.25	2.61	25.95	0.096
	SNS	5.78	1929.7	3591.9 ab	1032.6	172.2 ab	4.24	4.00	42.60	0.107
	TE	6.44	1776.0	3576.4 ab	1269.3	220.3 a	4.34	3.64	39.18	0.099
	ATE	5.98	1635.7	3081.6 b	1107.9	220.8 a	4.14	3.46	34.93	0.097
Second	CNS	5.76	2499.0	924.0 c	722.3	97.3 c	1.90	1.20	10.05	0.102
	SNS	6.25	2736.3	3703.5 a	975.3	155.7 abc	3.17	2.08	20.17	0.099
	TE	7.06	2620.8	3440.7 ab	902.9	130.0 bc	2.50	1.76	16.25	0.099
	ATE	7.12	2580.6	3838.2 a	877.0	110.9 bc	2.33	1.78	14.65	0.100
Mean effects										
First		5.88 b	1757.8 b	2773.5 b	1099.2 a	195.6 a	3.99 a	3.43 a	35.66 a	0.100
Second		6.55 a	2609.2 a	2976.6 a	869.4 b	123.4 b	2.48 b	1.71 b	15.28 b	0.100
	CNS	5.55c	2094.3	884.1 b	854.6 b	133.1 b	2.58 b	1.91 b	18.00c	0.099
	SNS	6.01 bc	2333.0	3647.7 a	1003.9 ab	163.9 ab	3.71 a	3.04 a	31.38 a	0.103
	TE	6.75 a	2198.4	3508.5 a	1086.1 a	175.1 a	3.42 a	2.70 a	27.71 ab	0.099
	ATE	6.55 ab	2108.1	3459.9 a	992.5 ab	165.8 ab	3.23 a	2.62 a	24.79 b	0.099
ANOVA										
Cut		* **	* **	*	* **	* **	* **	* **	* **	ns
Nutrient solution		* **	ns	* **	* *	*	* **	* **	* **	ns
Cut x Nutrient solution		ns	ns	*	ns	*	ns	ns	ns	ns

Note: FRAP: ferric reducing antioxidant power; CNS: standard nutrient solution (control); SNS: NaCl-enriched (50 mmol L^{-1}) standard nutrient solution; TE: effluent from a tomato substrate culture; ATE: artificial effluent with ion concentrations and salinity level (approximately 50 mmol L^{-1}) very close to those of TE. Mean values (n = 3) keyed by the same letter are not statistically different at 5% level after Tukey's post-hoc test. Significance level: *** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$; ns = not significant.

succulent.

A significant positive correlation (R^2 =0.893; $P > 0.0001$; n = 24) was found between leaf antioxidant capacity and total phenol content (Fig. S2, right).

4. Discussion

4.1. Effect of salinity

In the present work, both PH and PC showed good adaptation to the cultivation in floating raft system with standard nutrient solution or greenhouse hydroponic effluents and gave satisfactory leaf production (on average, 2.42 ± 0.14 kg m^{-2} in PH, Table 4; 4.22 ± 0.15 kg m^{-2} in PC, Table 9) in consideration of the short growing period (36 days). All the plants grew well and healthy and the cultivation could probably have been prolonged for another two or three weeks, thus allowing for another harvest or two.

The addition of NaCl (50 mmol L^{-1}) to the standard nutrient solution did not significantly impact either total plant dry biomass or crop yield in both species. A slight but significant reduction of leaf area index and root DW in SNS plants was observed only in PH, which in general showed less salt tolerance than PC, as expected in consideration of the halophytic nature of PC (Ltaeif et al., 2021). In PC plants grown in pots or in a gravel/hydroponic system and irrigated with NaCl-enriched nutrient solution, growth inhibition was observed at much higher concentrations (100 mmol L^{-1}, Bueno et al., 2020; 250 mmol L^{-1}, Koyro, 2006; 600 mmol L^{-1}, Ltaeif et al., 2021) than those tested in the present work. To the best of our knowledge, no study has been conducted to investigate the salinity response of PH in soilless culture.

Our findings may be the results of the growing system adopted in the experiments. Hydroponic systems such as deep culture and floating raft system can alleviate the stress induced by NaCl salinity. In hydroponics, indeed, the root uptake of both water and nutrients is facilitated and appropriate management of the nutrient solution can prevent the salinity build-up in the root zone that may occur in soil- or substrate-grown plants (Atzori et al., 2019). In basil, for instance, salt tolerance was much greater in nutrient film technique than in rockwool cubes (Faliagka et al., 2021).

In contrast to crop yield, the salinity of the nutrient solution influenced both water and mineral relations, although during the experiment

no plant of either species showed clear symptoms of nutrient deficiency (e.g., leaf chlorosis) and the level of nutritive elements in leaf tissues were consistently within the adequate ranges reported for leafy vegetables (Hochmuth et al., 2012). The higher leaf Zn content detected in TE and ATE plants of both species than in those grown with CNS or SNS, could be explained by the higher Zn level in the hydroponic effluents (Table 2).

Water uptake was reduced by the use of the saline nutrient solution only in PH (Table 6) due to an inhibition of both root and leaf growth (Table 4), and the effect of NaCl depended on the nutrient considered (Tables 5, 6, 10, and 11). The lower Mg content in SNS plants is in agreement with previous findings. For instance, leaf Mg content was drastically reduced by NaCl salinity in sorghum (Kausar and Gull, 2019). The limited effects of salinity on mineral nutrition were likely the results of the supply of a culture solution rich in all the essential nutrients and the better root water uptake, which are typical of hydroponic cultivation. Plant mineral nutrition is barely affected by salinity when the concentration of nutritive element in the root zone is optimal (Hu and Schmidhalter, 2005).

As expected, the most important effect of NaCl salinity was on leaf content of K and Na. In fact, leaf K content markedly decreased while Na noticeably accumulated in both leaf and root tissues of SNS plants of both species. As a consequence, the K/Na molar ratio significantly decreased in SNS plants compared with the controls, to a much larger extent in PH than in PC (Fig. S2). Many molecular and physiological processes, including the control of the flux of K, Na, and Cl for osmotic adjustment and ion homeostasis, are involved in the plant response to NaCl salinity (Assaha et al., 2017). The reduction of leaf K content in both species induced by NaCl can be ascribed to the Na-induced inhibition of K uptake (Marschner, 2011). In this work, the halophyte PC showed a greater affinity for Na than PH, as leaf Na content was invariably higher in PC than in PH.

Growing plants with SNS also affected leaf quality, which was assessed by determining the succulence and the content of dry matter and some nutraceutical substances. Due its influence of leaf texture, succulence affects sensory quality (Rana, 2015) and leaf dry matter content is often positively correlated with the shelf-life of leafy vegetables (Clarkson et al., 2003).

Leaf Na content was much higher in SNS plants than in the controls. However, a serving dose of 100 g of fresh leaves of PH or PC grown with SNS would provide 321 and 365 mg of Na, respectively, which is in both cases is much lower than the safe and adequate daily intake of Na (2000 mg day^{-1}; European Food Safety Authority, 2019). In contrast, leaf nitrate content was not affected by salinity in either species and was invariably lower than the maximum level established, for instance, by the European Parliament and Council of the European Union (2011) for lettuce and spinach grown in greenhouse in spring and summer (4000 and 3500 mg kg^{-1} FW, respectively).

High nitrate accumulation in plant leaves is due to a disproportion between its uptake and assimilation and depends on both plant species and growing conditions (Colla et al., 2018). Sodium chloride salinity generally impairs the root uptake and leaf accumulation of nitrate due to the antagonistic inhibition of chloride (Rouphael et al., 2018). In several species grown hydroponically, leaf nitrate level was lower in plants grown with nutrient solution containing 40 or 60 mmol L^{-1} NaCl in comparison with NaCl-free solution (Takahama et al., 2020). However, leaf nitrate content was greater in salinized plants of Swiss chard and sea beet than in non-salinized plants because of higher DW/FW (Puccinelli et al., 2022).

In both PC and PH, the DW/FW ratio and the content of leaf pigments were not significantly affected by NaCl salinity, which in contrast increased the antioxidant capacity. In both PH and PC, the antioxidant capacity was closely correlated with the content of total phenols (Fig. S2), which indeed increased in SNS plants with respect to the controls, albeit this increase was not significant in PH. A strong positive correlation between the total antioxidant capacity and the content of total phenols was found in many studies conducted with plants grown under saline (e.g. Hossain et al., 2022; Tareq et al., 2021) or non-saline conditions (e.g. Piluzza and Bullitta, 2011; Puccinelli et al., 2021).

Salinity stress is known to activate the antioxidant defence system that protects the plant against the salt-induced oxidative damage and depends on both enzymatic and non-enzymatic antioxidants, such as phenols and flavonoids (Hasanuzzaman et al., 2021). Salinity stress increases the leaf content of phenols and flavonoids in many plant species (e.g. Hossain et al., 2022; Taârit et al., 2012; Tareq et al., 2021) including PC (Boestfleisch et al., 2014; Bueno et al., 2020, 2021).

Leaf succulence significantly increased in SNS plants compared with the controls only in PH (Table 8). Leaf succulence is a typical trait of halophytes grown under saline conditions (Hernández, 2019), as it allows efficient water storage and the dilution of accumulated salts (Munns and Tester, 2008; Schiattone et al., 2017). In our work, however, leaf succulence was not affected by the composition of the nutrient solution in the halophyte PC and significantly augmented only in PH plants grown with SNS compared with the controls (Table 13).

4.2. Agronomic, economic, and environmental implications of cascade cropping systems

The experiment design adopted in this work allowed the separation of the effects of NaCl salinity from those due to the other defects of hydroponic drainage water, such as the reduced level of several nutrients (N, Fe, B, Mn, and in particular P; Table 3) and the possible presence of root and microbial metabolites originated in the donor crop. There were no or minor differences between SNS plants and those grown with hydroponic effluents for all the measured quantities. Moreover, a significant difference between TE and ATE plants was found in both species only for leaf Mn content (in the roots of PH as well), which was significantly higher in ATE than in TE. Therefore, the reduction of crop yield in TE and ATE plants was primarily due to the high effluent salinity, as found in a previous work on CCS, in which the drainage solution from a round-tomato crop was used for the cultivation of cherry tomato plants (Incrocci et al., 2003). Our findings also suggest that both WEP species, in particular PC, could be grown hydroponically with N and P concentration of the nutrient solution much lower than those recommended for soilless culture (Raviv et al., 2019).

The main advantages of CCS are saving both water and fertilisers and reducing of the pollution caused by the nutrients dissolved in the effluents from the donor crop. In greenhouse crops, fertilisers are reported to account for up to 9% of the total production costs (Martínez-Alvarez et al., 2020; Martínez-Granados et al., 2022), which are more or less equal to the total revenue (Martínez-Granados et al., 2022; Torrellas et al., 2012). The incidence of fertilisation on running cost has recently augmented because the price of fertilisers has markedly increased in the last few years (Eardley, 2022). For example, in 2022 the price of ammonia nitrate, potassium chloride, and phosphate fertilisers was higher, respectively, by 152%, 165%, and 124% than the price in 2021 (AHDB, 2022). Therefore, the moderate yield reduction observed in this (−33% in PH and −23% in PC) and other studies on CCS (e.g. Avdouli et al., 2021; Elvanidi et al., 2020; García-Caparrós et al., 2021b; Santos et al., 2022) grown with hydroponic effluents could be compensated, at least partially, by the zero cost for fertilisation and water depuration. Moreover, the application of CCS reduces the water (García-Caparrós et al., 2018b) and carbon (Muñoz et al., 2010) footprint of products, thus improving farm sustainability and green marketing.

Implementing the CCS concept on a commercial scale is not simple and straightforward, however. In addition to the difficulties already mentioned in the Introduction, the design of a CCS requires an appropriate sizing of the cultures carried out in sequence, specifically a precise definition of the area cultivated for the donor crops and the recipient crop(s). The dimensioning of a CCS can consider the water (García-Caparrós et al., 2018a) or nutritional (Rufí-Salís et al., 2020) demand of the crops. García-Caparrós et al. (2018a) proposed a simple

equation for appropriate dimensioning of CCS with two or three soilless cultures in sequence; the equation considers the water absorbed by each crop and the leaching requirement (namely, the drainage fraction), which is generally determined by the salinity of the irrigation water and is very seldom lower than 20–30% (Massa et al., 2020). Using this approach and the volume of drainage water from a semi-closed substrate culture of tomato with a ceiling EC of 4.5 dS m^{-1} for partial discharge of the recirculating nutrient solution (Massa et al., 2010), it would be necessary 0.96 or 0.87 m^2 of PH or PC, respectively, for each m^2 of the donor crop to completely reuse its effluents.

Rufí-Salís et al. (2020) extensively discussed the possible options for optimal CCS dimensioning taking into account the amount of nitrogen leached by the primary crop (tomato) and the one absorbed by the secondary crop (lettuce). These authors concluded that the best strategy depends on the grower's priorities and the availability of water for both the primary and secondary crops.

Models exist for predicting water and nutrient requirements of greenhouse crops (e.g., Carmassi et al., 2007; Gallardo et al., 2016, 2009) and the volume and ion composition of the drainage water from soilless culture (e.g., Katsoulas et al., 2015; Massa et al., 2011; Neocleous and Savvas, 2022), and could be used for optimal design of CCS.

How the drainage water from the primary crop is used must also be considered when the CCS is scaled up. In this regards, two main options exist: the effluent from the donor crop is collected and stored before the distribution to the secondary crops, likewise in this and other studies (Choi et al., 2011; Incrocci et al., 2003; Santos et al., 2022), or is used directly as proposed by other authors (e.g. García-Caparrós et al., 2021a, 2018b; Katsoulas et al., 2020; Muñoz et al., 2010; Rufí-Salís et al., 2020). The first option is suitable for a CCS consisting of a semi-closed culture of the donor crop (i.e with periodical discharge, say every one or more weeks) and a receiving crop with short cycle, as in this study. This system would require large tanks to store the effluent but facilitates the control and adjustment of its composition (Incrocci et al., 2003). When the donor crop is cultivated in free-drain systems, the best option is to irrigate the receiving crop with the effluents produced daily. In this system, the adjustment of the mineral composition and salinity of the drainage water, which change during the growing season, would be more difficult.

There are also regulatory issues regarding the use of hydroponic effluents for fertigation of secondary crops. In the European Union, for instance, greenhouse effluents are considered industrial wastewater and their use for crop irrigation needs specific authorization (EEC, 1991; Santos et al., 2022). Hydroponic wastewater may also contain active principles of plant protection products (Boye et al., 2022; Santos et al., 2022; Vermeulen et al., 2017) that are not registered for use on the secondary crops.

5. Conclusions

Picris hieracioides and *Plantago coronopus* could be hydroponically grown on saline effluents from greenhouse soilless cultures with a moderate reduction of crop yield, which could be compensated for by the better leaf quality induced by high salinity and the zero cost for fertilisers and the treatment of the donor crop's wastewater. Both species could be grown with nitrogen and phosphorus concentrations of the nutrient solution lower than those recommended for soilless culture. Therefore, these two wild edible species are good candidates as receiving crops in cascade cropping systems.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agwat.2023.108275.

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