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# Optimising fertigation of hydroponically grown sowthistle (*Sonchus oleraceus* L.): The impact of the nitrogen source and supply concentration

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## ABSTRACT

The efficient use of water and nutrients in leafy vegetable crops can be improved by effective water management, whereas the form of the supplied nitrogen may affect their quality and productivity. Sowthistle (*Sonchus oleraceus* L.), a widespread weed collected and consumed as a wild vegetable in the past, currently attracts a high interest for commercial cultivation due to its high nutritional value. The present study evaluated the effects of different nitrogen concentrations: N50: 50 mg $L^{-1}$; (N 3.6 mmol $L^{-1}$), N100: 100 mg $L^{-1}$; (N 7.1 mmol $L^{-1}$), N200: 200 mg $L^{-1}$; (N 14.3 mmol $L^{-1}$), and N300: 300 mg $L^{-1}$; (N 21.4 mmol $L^{-1}$) and different ammonium/total-N ratios (Nr: 0.01, 0.05, 0.10, 0.15) on growth, physiological parameters, antioxidant capacity and nutrient accumulation in the different plant parts of sowthistle, grown in Nutrient Film Technique hydroponic system. Total N was ammonium N plus nitrate N. Plant growth, total phenols, and antioxidant capacity were higher at the two intermediate N concentrations, whereas flavonoid and nitrogen content, as well as irrigation water productivity increased only with 200 mg N $L^{-1}$ (N 14.3 mmol $L^{-1}$), compared to the other three N concentrations. The accumulation of nitrogen in leaves and roots was lower, while leaf stomatal conductance was enhanced by increased N concentrations in the nutrient solution. Higher Nr ratios than $\geq 0.05$ decreased plant dry matter, total phenols, flavonoids, and antioxidant capacity in leaf extracts, and negatively affected nitrogen translocation from roots to leaves. Sowthistle plants treated with a Nr of 0.05 exhibited a less intense oxidative stress, with decreased lipid peroxidation and hydrogen peroxide production, and increased superoxide dismutase and catalase activities, compared to those treated with higher Nr ratios. Increased Nr resulted in the accumulation of phosphorus and magnesium in leaves while the highest irrigation water productivity was obtained in plants grown with a Nr of 0.05. In conclusion, to increase yield, nutritional value and efficiency of water and nitrogen use in sowthistle grown in closed hydroponic systems, a N concentration of 200 mg $L^{-1}$ of N (N 14.3 mmol $L^{-1}$) and a Nr of 0.05 are suggested.

## 1. Introduction

Agriculture is the largest consumer of the freshwater resources. Agricultural sector places an adverse impact on available water resources and on environment, potentially resulting in soil erosion and water quality degradation. This is evident in Mediterranean areas, where water is frequently scarce while it's quality is deteriorating in many irrigation zones (Thompson et al., 2007). On top of that, the main factors that limit crop production, water use effectiveness and nitrogen (N) uptake are irrigation and nitrogen fertilisation (Liu et al., 2020). Therefore, the effective water management in agriculture is of high research interest. Additionally, the unexpected climate change could worsen future water shortage (Mancosu et al., 2015). The misuse of chemical fertilisers, particularly nitrogen fertilisers, in agricultural production systems has also become a major concern, damaging water resources with nitrates and polluting the air with gaseous emissions (ammonia, nitrous oxide, nitric oxide). The excess levels of nitrates in water are harmful to both human health and ecosystems, causing oxygen depletion and eutrophication. The European Commission is alarmed and has established policy legislations such as the Nitrate Directive (ND, Directive 91/676/EEC) and the European Water Framework Directive (WFD, Directive 2000/60/EEC) to protect water bodies from agricultural nitrate pollution, with a nitrate threshold concentration of 50 mg $L^{-1}$ for European groundwater (European Communities, 1991). Despite the increasing pressure to reduce nitrate contamination of water bodies, the excessive fertilisation and irrigation remain a problem in many parts

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of the EU, with high potential of nitrate leaching loss. Indeed, given the high temperatures and the moisture levels inside a greenhouse, unreasonable and excessive irrigation and nitrogen fertiliser application may result in soil degeneration, nitrogen leaching, and low water and nitrogen use efficiency (Wu et al., 2019). The shift from soil-grown crops to soilless cultures and more specifically, to closed hydroponic systems with recirculation of nutrients and water, is an effective strategy to decrease nitrate and phosphate emissions and sustain water resources (Savvas and Neocleous, 2019).

In plant tissue, nitrogen is the fourth most abundant element after carbon, oxygen, and hydrogen. It is a structural component of amino acids, which are the building blocks of animal and human tissues, enzymes, and numerous hormones. Plants absorb N in the form of ammonium ($NH_4^+$) and nitrate ($NO_3^-$) ions. By altering the ammonium/nitrate ratio in the nutrient solution (NS) provided to the plants while maintaining the total N content constant, the total cation to anion absorption ratio may be significantly modified. Total cation to anion uptake ratio will shift the pH of the NS and this will have a direct impact on the uptake of macro- and micronutrients from the NS. Excessive nitrogen fertiliser application results in an imbalanced growth pattern in plant roots and shoots, which can greatly reduce crop yield and nitrogen fertiliser use efficiency, while polluting the environment (Liu et al., 2020). The application of the ideal dose of nitrogen fertiliser could significantly increase crop productivity by promoting photosynthesis, enhancing dry matter accumulation, net assimilation rate, and nutrient uptake (Mahajan and Pal, 2022).

Excessive ammonium ion can be toxic to plant cells, decoupling electron flow and possibly causing oxidative phosphorylation, resulting in decreased plant development (Wenceslau et al., 2021). Moreover, the excess ammonium in plant tissue not only consumes protons which are required for several functions (such as photosynthesis, respiration and transpiration), but also disturbs the balance of cations, since they compete each other (Wenceslau et al., 2021). For soilless cultivation, the current recommendation is that $NH_4^+$-N should not exceed the 25% of the total nitrogen input (Sonneveld, 2022). However, this can be tailored to the plant species and/or to the selected soilless cultivation system (closed and open hydroponic system) (Savvas et al., 2006). The addition of ammonium to the NS also prevents nitrate accumulation in plant tissue, which is a major concern for human health. The effects of ammonium to nitrate ratio in the NS, and literally, ammonium to total nitrogen ratio (Nr= $NH_4^+$/total N), have attracted research interest, not only for vegetables, as *Lactuca sativa* (Savvas et al., 2006) and *Fragaria* x *ananassa* (Tabatabaei et al., 2008), but also for medicinal plants, as *Prunella vulgaris* (Zhu et al., 2014) and *Withania somnifera* (Praveen and Murthy, 2013).

Different plant species have different responses to $NH_4^+$, to the environmental factors as temperature, light intensity, pH, and to nutrients concentration in the growing media (Kotsiras et al., 2005). The form of nitrogen supplied to a crop may significantly affect plant morphology and chemical composition; $NH_4^+$ decreases tissue content of potassium (K), calcium (Ca), and magnesium (Mg), and increases the content of phosphorus (P), sulphur (S), and organic N (Santamaria and Elia, 1997). According to a series of studies, nitrogen form—rather than total nitrogen concentration—is more important, with significant impacts on the overall crop yield as well as on the marketability of the fresh produce (Chatzigianni et al., 2018; Perner et al., 2011).

The majority of plant species prefer $NO_3^-$ to $NH_4^+$, despite the fact that $NO_3^-$ absorption and assimilation have a higher energetic cost (Boschiero et al., 2019). This is due to the fact that plants can be harmed by the high concentrations of $NH_4^+$ in the solution ($>$ 0.5 mM), which results in lower biomass production (especially at low pH levels) (Boschiero et al., 2019; Szczerba et al., 2008). On the other hand, one critical feature is the concentration of nitrates inside the edible plant tissue, which in high concentrations appears as a potential health hazard.

The Mediterranean basin is thriving with different ecotypes of wild plant species that have been traditionally used for medicinal and therapeutic purposes by locals, for hundreds of years. Numerous studies have been conducted to evaluate the significance of these species in human diets because they were historically employed as famine foods during tough historical periods (Guarrera and Savo, 2016; Petropoulos et al., 2019, 2016). Most of these species are generally hand-harvested from the wild by locals. There are reports mentioning that these species contain high levels of bioactive compounds (such as flavonoids, tannins, vitamins, alkaloids and phenolic acids) that contribute to a healthy and diverse diet, maintain or improve human physical condition, and provide essential nutrients and microelements (Nobela et al., 2022; Sánchez-Mata et al., 2012). However, increased demand for such food products has created a market niche for commercial exploitation of indigenous species in order to meet consumer demands for year-round product availability and to avoid the risk of genetic erosion caused by irrational harvesting from wild populations (Petropoulos et al., 2018). Therefore, there have been publications about native species' cultivation practices and researches on how those practices may affect their chemical profile and the content of bioactive compounds (Chatzigianni et al., 2018; Klados and Tzortzakis, 2014), as well as for their potential commercial cultivation (Martinez et al., 2015).

The genus *Sonchus,* member of Asteraceae family, comprises of about 60 species, while three of which have become common weeds over the world. There is *Sonchus arvensis* (perennial sowthistle) and the annual species *S. oleraceus* (common sowthistle) and *Sonchus asper* (spiny sowthistle) (Ahmad et al., 2021). In the case of *S. oleraceus*, leaves, stems and flowers are consumed fresh or boiled/steamed, as salad, as an ingredient in soups and in vegetable pies (Gatto et al., 2011). The nutritional value of *Sonchus* species is highly explored and appreciated, as they possess significant antioxidant potential (Khan, 2012), high content in polyphenols (Khan, 2012; Petropoulos et al., 2019), flavonoids (such as luteolin, apigenin and quercetin) (Huo and Qin, 2008), organic acids (such as oxalic acid) (Petropoulos et al., 2019), and earned the right to be referred as nutraceutical (Nobela et al., 2022). According to Gatto et al. (2011) the main phenolic compounds detected in wild *S. oleraceus* and *S. asper* were mainly chlorogenic acid and cichoric acid. Moreover, the main tocopherol detected in *S. oleraceus* was α-tocopherol, while β-, γ-, δ-tocopherols were present in lower content (Morales et al., 2014; Petropoulos et al., 2019).

*Sonchus* species are part of human diet in several places of the world and may play a significant role in alleviating a variety of human ailments such as inflammation (Jain and Singh, 2014), bacterial infections (Jimoh et al., 2011), diabetes (Chen et al., 2019), diarrhoea and enteritis (Juhaimi et al., 2017), tumour (Huyan et al., 2016), to name a few. *Sonchus* sp. extracts exhibited significant antioxidant (Qiao et al., 2021), antibacterial (Petropoulos et al., 2019; Xia et al., 2011) and antifungal properties (Gatto et al., 2011; Petropoulos et al., 2019) and have been successfully used as a natural sanitiser and anti-browning agent (plant extract) on fresh-cut potatoes (Qiao et al., 2021).

In previous studies *S. oleraceus* exhibited high tolerance to heavy metals as cadmium (Cd), by increasing the plant dry matter content, but also high transfer capacity and moderate Cd accumulation in tissues (Fang et al., 2019). Xiao et al. (2021) covered the *S. oleraceus* phytoremediation properties towards lead (Pb), while Stylianou et al. (2020) reviewed the remediation of *Sonchus* by accumulating copper (Cu), nickel (Ni), Cd and Pb of grown on oxidised waste dumps in Cyprus.

Despite the well-known health related properties of the species as referred above, there is a lack of knowledge on the *Sonchus* cultivation management and the commercial exploitation of sowthistle as a cultivated vegetable. At the moment, the species has a minor but promising economic interest on soilless culture and the interesting part in the current study's findings is primarily in gaining new insights about the concentration and source of N in soilless culture on reducing nitrate pollution in groundwater, the irrigation water productivity, the agronomic efficiency of N, and the related plant growth parameters/properties.

## 2. Material and methods

### 2.1. Cropping system, plant material and growth conditions

Two independent experiments were conducted at the experimental farm of the Cyprus University of Technology, Limassol, Cyprus, in a plastic multi-span greenhouse. The greenhouse is oriented North-South, and the cover material is made of transparent polyethylene sheets (anti-drip, resistance to UV radiation, 88% light transmission). The greenhouse is equipped with an automated climate control system (ventilation, shading, and cooling premises) and a hydroponic installation, constructed according to the principles of NFT (Nutrient Film Technique). Twenty-four twin white plastic NFT channels (each of 4 m long, 8 cm wide, 6 cm deep) were aligned with 24 catchments (60 L) to form 24 independent hydroponic units (three replicate units in each experiment). Each unit consisted of a twin plastic channel set and was supported with an individual water replenishment tank (60 L). The nutrient solution (NS) absorbed by the plants was replenished through automatic refill of water from the replenishment tank and the adjustment of the electric conductivity (EC) and pH took place on a daily basis by adding appropriate amounts of stock solutions of fertilisers. Each hydroponic unit accompanied 14 plants giving a final plant density of 25 plants $m^{-2}$. Since no hydroponic studies on *Sonchus* exist, the most relevant plant density for sowthistle is the one for lettuce cultivation, where the plant density is 120,000–148,000 plants per ha, depending on the hydroponic infrastructures/dimensions.

Two experiments were conducted in the above NFT systems on sowthistle (*Sonchus oleraceus* L.). In the first experiment (Exp. 1; N concentrations), the effect of different N concentrations was examined; four concentrations of N (including both $NO_3^-$-N and $NH_4^+$-N) at a constant $NH_4^+$/total-N ratio of 0.055 in all treatments, were applied of N50: 50 mg N $L^{-1}$ (N 3.6 mmol $L^{-1}$; $NO_3^-$-N 3.4 mmol $L^{-1}$ + $NH_4^+$-N 0.2 mmol $L^{-1}$), N100: 100 mg N $L^{-1}$ (N 7.1 mmol $L^{-1}$; $NO_3^-$-N 6.7 mmol $L^{-1}$ + $NH_4^+$-N 0.4 mmol $L^{-1}$), N200: 200 mg N $L^{-1}$ (N 14.3 mmol $L^{-1}$; $NO_3^-$-N 13.5 mmol $L^{-1}$ + $NH_4^+$-N 0.8 mmol $L^{-1}$), and N300: 300 mg N $L^{-1}$ (N 21.4 mmol $L^{-1}$; (or $NO_3^-$-N 20.2 mmol $L^{-1}$ + $NH_4^+$-N 1.2 mmol $L^{-1}$). The concentrations of K and P into the nutrient solution were kept constant, at 350 mg $L^{-1}$ (K 9.0 mmol $L^{-1}$) and 70 mg $L^{-1}$ (P 2.3 mmol $L^{-1}$), respectively, based on preliminary studies and previous reports (Chrysargyris et al., 2016). In the second experiment (Exp. 2: Ammonium to total nitrogen ratio: Nr) the effects of different Nr ratios were examined, considering four Nr ratios of 0.01, 0.05, 0.10, and 0.15. The concentrations of 200 mg N $L^{-1}$ (N 14.3 mmol $L^{-1}$), 350 mg K $L^{-1}$ (K 9.0 mmol $L^{-1}$) and 70 mg P $L^{-1}$ (P 2.3 mmol $L^{-1}$), were kept constant, based on preliminary studies and previous reports (Tzortzakis et al., 2022b). Mean air temperature and humidity in the greenhouse were 27.1 °C and 46.8%, respectively, during daylights hours with minimum values of 19.0 °C and 27.9% and maximum values of 33.4 °C and 66.6%, respectively, for the cultivation period.

Seeds from commercial sowthistle were purchased from Greece (Geniki Phytotechniki Athinon SA, Athens, Greece) and were sown (28 September 2021 for both Exp. 1 and for Exp.2) in peat-perlite (90:10 v/v) based growing media (Professional peat, Gebr. Brill Substrate GmbH & Co.KG, Georgsdorf, Germany) into back plastic trays, under nursery conditions. Seedlings at the stage of the first true leaf were then transplanted into netted pots (11 days after sowing), filled with perlite and placed into the pot positions of the NFT channels, and were kept for one week under standard nutrient solution, to allow recovery from the transplanting stress.

The nutrient concentrations in the standard nutrient solution were as follows: $NO_3^-$-N = 13.7, $K^+ = 7.5$, $PO_4^{3-}$-P = 1.8, $Ca^{2+} = 3.5$, $Mg^{2+} = 1.0$, $SO_4^{2-}$-S = 1.3 and $Na^+ = 1.9$ mmol $L^{-1}$ respectively; and B = 30.0, Fe = 30.0, Mn = 5.0, Cu = 1.0, Zn = 4.0, and Mo = 0.5 μmol $L^{-1}$, respectively. After 1 week at the NFT system the plants were subjected to the modified nutrient solution, with the four N concentrations or the four Nr ratios, for 3 weeks. For the Exp.1, the 50, 100, 200 and 300 mg $L^{-1}$ of N in the nutrient solution were obtained by adding 3.4, 6.7, 13.5 and 20.2 $NO_3^-$-N mmol $L^{-1}$, adequate concentrations of $NH_4^+$-N and $SO_4^{2-}$, while keeping the ratio of $NH_4^+$/total N constant at 0.055 (the $NH_4^+$-N/$NO_3^-$-N was 0.058) and similar EC values (Table S1). For Exp. 2, the Nr of 0.01, 0.05, 0.10 and 0.15 ratios were achieved by differentiate the $NH_4^+$-N, $NO_3^-$-N and $SO_4^{2-}$ concentrations, while the total N was constant of 200 mg $L^{-1}$ (N 14.3 mmol $L^{-1}$) and similar EC values (Table S2). All the solutions were checked on a daily basis and adjusted accordingly, by using $H_2SO_4$ (5% v/v) for adjusting down the pH as needed (due to the alkalinity of water), and by adding relevant modified nutrient solutions for the Exp. 1 and Exp. 2 for adjusting the electric conductivity. The target pH and EC of the nutrient solution were 5.8 and 2.3 dS $m^{-1}$ respectively. All chemical reagents used for the plant tissue analysis were purchased from Sigma Aldrich (Germany) except if mentioned differently.

### 2.2. Plant growth and tissue analysis

For each experiment, 168 sowthistle plants were used. Following three weeks of plant growth with four N concentration nutrient solutions (Exp. 1) and with four ammonium/total N ratios (Exp. 2), six individual plants -from different replication plots- for each treatment were considered for detailed plant growth analysis. Plant height (cm), leaf number, shoot plant biomass and root fresh (g) and dry weight (g) were determined.

### 2.3. Plant physiological and photosynthetic related parameters

Leaf stomatal conductance was measured with a Delta-T AP4 porometer (Delta-T Devices-Cambridge, UK) and expressed as mmol $H_2O$ $m^{-2}$ $s^{-1}$. Leaf photochemistry features were also assessed; relative chlorophyll content values were obtained with an optical chlorophyll metre (SPAD-502, Minolta, Osaka, Japan), measuring leaf transmittance in the red (650 nm; the measuring wavelength) and infra-red (940 nm; a reference wavelength used to adjust for non-specific differences between samples) regions of the electromagnetic spectrum. These transmittance values are used by the device to derive a relative SPAD metre value (typically ranging from 0 to 50) that is proportional to the amount of chlorophyll in the sample. Moreover, leaf chlorophyll fluorescence of PSII (Fv/Fm) was measured with the OptiSci OS-30p Chlorophyll Fluorometer (Opti-Sciences, Hertfordshire, UK). Additionally, fresh plant tissue (six replications/treatment; each replication was a pool of two plants tissue; 0.1 g) was used for chlorophyll extraction. Leaf parts were incubated in a heat bath (at 65 °C) for 30 min, in the dark, with 10 mL dimethyl sulfoxide (DMSO). Photosynthetic leaf pigments, chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (t-Chl) and carotenoids content were then calculated (mg $g^{-1}$ fresh weight) (Richardson et al., 2002; Wellburn, 1994).

### 2.4. Plant tissue and nutrient ion concentration analysis

At the end of the experiment, samples from leaves and roots were used to determine the mineral content, in four replications per treatment (three pooled plants per replication). First, leaf and root tissue were dried to constant weight (at 65 °C for 4 d) and then milled at $< 0.42$ mm. Sub samples ($\sim 0.4$ g) were ash burned in porcelain cups, in a furnace (Carbolite, AAF 1100, GERO, Lilienthal, Germany) at 450 °C for 6 h. The ash was then digested with 10 mL hydrochloric acid (2 M HCl). Determination of potassium, sodium, and phosphorous content was performed according to Chrysargyris et al. (2019b) while magnesium and calcium by an atomic absorption spectrophotometer (PG Instruments AA500FG, Leicestershire, UK). Nitrogen was determined by the Kjeldahl method (BUCHI, Digest automat K-439 and Distillation Kjeldahl K-360, Flawil, Switzerland). Data were expressed in g $kg^{-1}$ of dry weight. Nitrate ($NO_3^-$) content was determined in sowthistle leaves according to the colorimetric method described by Cataldo et al. (1975), by the nitration of salicylic acid, and results were expressed as g of $NO_3^-$ per kg of fresh and dry weight.

At the experiment completion, drainage solution was sampled. The concentration of major cations ($K^+$, $Na^+$, $Mg^{2+}$, $Ca^{2+}$ and $NH_4^+$) were determined using ion chromatography (ICS-3000, Dionex Aquion, Sunnyvale, CA, USA) and an IonPac CS19 (4 × 250 mm, Dionex, Corporation) analytical column, while P concentration was determined based on vanadate/molybdate (yellow method), by measuring the absorbance at 470 nm (Multiskan GO, Thermo Fischer Scientific, Waltham, MA, USA), according to Chrysargyris et al. (2019b). The nitrate concentrations at the drainage solution were determined spectrophotometrically at 210 nm and 275 nm (Multiskan GO, Thermo Fischer Scientific, Waltham, MA, USA), as described previously (Tzortzakis and Economakis, 2005). In order to eliminate the organic interferences, the absorbance of the sample was measured at 275 nm where nitrate does not absorb, and the absorbance at 275 nm was subtracted from the absorbance of 210 nm to give the corrected nitrate absorbance. Data were expressed in mmol $L^{-1}$ of nutrient solution.

The total amount of water used by the plants during the cropping period was recorded and water uptake (mL $plant^{-1}$ $day^{-1}$) was calculated. The total amount of N used by the plants was recorded, by adding the N inputs from the stock solutions in the NS and subtracting the N remained at the drainage solution, at the end of the cropping period. Nitrogen uptake (mg $plant^{-1}$ $day^{-1}$) was calculated.

Irrigation water productivity ($WP_I$) was calculated by the ratio between the marketable yield produced by a crop along the growing season and the irrigation water applied (IWU) in the same period, as described by Fernández et al. (2020):

$$\text{Irrigation Water Productivity} = \frac{\text{Yield (kg per ha)}}{\text{IWU (m}^3\text{ per ha)}} \tag{1}$$

The nitrogen use efficiency was indicated by the Agronomic Efficiency of N ($AE_N$) and was calculated by the ratio of yield to N supply, as described by Ladha et al. (2005):

$$\text{Agronomic Efficiency of N} = \frac{\text{Yield (kg per ha)}}{\text{N (kg per ha)}} \tag{2}$$

Both $AE_N$ and $WP_I$ expressed on area basis (ha) and on plant basis (per plant) as presented in Table 6.

The N accumulation rate (AR), bioaccumulation coefficient (BAC), translocation factor (TF) and tolerance index (TI) of *Sonchus* plants were calculated by using the equations described by Benimeli et al. (2010), Amin et al. (2019) and Azooz et al. (2012).

The N accumulation rate was calculated as the sum up of N content in each plant tissue x plant DW (dry weight) divided by the number of days under the different N concentrations by the total plant DW (Benimeli et al., 2010).

$$\text{Accumulation rate (g per kg DW x day)} = \frac{([\text{N}]\text{ leave} \times \text{DW leave} + ([\text{N}]\text{ root} \times \text{DW root}))}{\text{Days} \times (\text{DW leave} + \text{DW root})} \tag{3}$$

The N bioaccumulation coefficient was calculated as the ratio of N content in plant tissue to that of N concentration in nutrient solution, according to Amin et al. (2019):

$$\text{N bioaccumulation coefficient} = \frac{\text{N content in plant tissue (g per kg DW)}}{\text{N concentration in nutrient solution (g per L)}} \tag{4}$$

The translocation factor was calculated as the ratio of N content in plant tissue to that of N content in plant roots according to Amin et al. (2019):

$$\text{Translocation factor} = \frac{\text{N content in plant tissue(g per kg DW)}}{\text{N content in plant root(g per kg DW)}} \tag{5}$$

Nitrogen tolerance index was calculated as the quotient of the plant growth-related factor (i.e. total biomass, plant height, leaf number) of plants grown under increased nitrogen (or Nr) treated and lower N (or Nr) conditions according to the following the equations described by Benimeli et al. (2010) and Azooz et al. (2012), with the following modifications:

$$\text{Tolerance index (\%)} = \frac{\text{Growth} - \text{related factor of high N (or Nr)} - \text{treated plants} \times 100}{\text{Growth} - \text{related factor of low N (or Nr)} - \text{treated plants}} \tag{6}$$

### 2.5. Total phenolics, total flavonoids, antioxidant activity, ascorbic acid and total soluble sugars

Polyphenols were extracted from six samples (two individual plants were pooled/sample) for each treatment. Methanolic extracts of the plant tissue (0.7 g) were stored at −20 °C until analysis of total phenolic and flavonoids content. The total antioxidant activity was assessed by the 2,2-diphenyl-1-picrylhydrazyl (DPPH), the ferric reducing antioxidant power (FRAP) and the 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) methods.

Total phenolic content measurement was performed with the Folin–Ciocalteu method (at 755 nm; using a microplate spectrophotometer -Multiskan GO, Thermo Fischer Scientific, Waltham, MA, USA), as described previously (Tzortzakis et al., 2011). Results were expressed in gallic acid equivalents (mg GA $g^{-1}$ FW). The content of total flavonoids was determined using the aluminium chloride colorimetric method (Meyers et al., 2003) as modified in Chrysargyris et al. (2016). The absorbance was measured at 510 nm. The total flavonoid content was expressed as rutin equivalents (mg rutin $g^{-1}$ FW).

Free radical-scavenging activity was determined as descripted previously (Chrysargyris et al., 2019a). Briefly, the DPPH radical scavenging activity of the leaf extracts was measured at 517 nm while the FRAP activity was measured at 593 nm, as described in Chrysargyris et al. (2019a). The ABTS assay was implemented according to the methodology described by Wojdyło et al. (2007). Results were expressed as Trolox ((±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) equivalents (mg trolox $g^{-1}$ of fresh weight).

Ascorbic acid (AA) was determined by the 2,6-Dichloroindophenol titrimetric method as described previously (AOAC, 2007). Plant tissue (0.5 g) was extracted in 10 mL oxalic acid 4.0% and was titrated by the dye solution until the colour changed. Data were expressed as µg of AA per gram of fresh weight.

Total soluble solids (TSS) concentration was determined from the juice obtained from plant tissue (n = 6) with a temperature-compensated digital refractometer (model Sper Scientific 300017, Scottsdale, Arizona, USA) at 20 °C, and results were expressed in percentage (%).

### 2.6. Lipid peroxidation, hydrogen peroxide, and enzymes antioxidant activity

The content of hydrogen peroxide ($H_2O_2$) was determined by employing the method described by Loreto and Velikova (2001). Six samples (two individual plants were pooled/sample) were used for this analysis from each treatment. The $H_2O_2$ concentration was measured using standards of 5–1000 µM of $H_2O_2$ and the calibration curve was plotted accordingly. Samples and standards were measured at 390 nm and the results were expressed as μmol $H_2O_2$ $g^{-1}$ fresh weight.

Lipid peroxidation, in terms of malondialdeyde content (MDA), was assessed according to De Azevedo Neto et al. (2006). The absorbance was recorded at 532 nm and corrected for non-specific absorbance at 600 nm. MDA content was determined using the extinction coefficient of 155 mM $cm^{-1}$. Results were expressed as nmol of MDA $g^{-1}$ fresh weight.

The activity of the antioxidant enzymes for superoxide dismutase (SOD) (EC 1.15.1.1) and for catalase (CAT) (EC 1.11.1.6) was assayed as described previously (Chrysargyris et al., 2017b) and the absorbance was determined at 560 nm for SOD and at 240 nm for CAT. Peroxidase activity (POD) (EC 1.11.1.6) was determined following the increase in absorbance at 430 nm as described previously (Chrysargyris et al., 2020). Results were expressed as enzyme units per mg of protein. The protein content in leaf tissue was measured using the Bradford method and bovine serum albumin (BSA) as a standard.

### 2.7. Statistical methods

Analysis of variance (ANOVA) on data was performed by IBM SPSS v.22, and results are presented as treatment mean ± standard error (SE). Mean values represent three replicates for pH, EC and the nutrient concentrations of the drainage nutrient solution; six replicates for plant-growth related parameters (height, leaf number, shoot and root fresh and dry weight), and physiology and biochemical related parameters (leaf stomatal conductance, leaf chlorophyll content and fluorescence, total phenolics, total flavonoids, antioxidant activity, ascorbic acid, total soluble sugars, lipid peroxidation, hydrogen peroxide, and the activity of the antioxidant enzymes); four replicates for the tissue mineral determinations. Duncan's multiple range tests were performed when AVOVA rendered a significant treatment impact at $P < 0.05$. The correlation coefficients between N concentrations and Nr ratios with individual parameters tested as well as the correlation coefficients of nitrogen content in leaves and/or roots were also determined by Pearson's correlation test.

## 3. Results

### 3.1. Evolution of EC, pH and nutrients of the drainage nutrient solution

The courses of pH and EC in the drainage nutrient solution from *Sonchus* plants that were subjected to different N concentrations or to different Nr ratios is presented in Fig. 1. The EC was fluctuated from 1.9 to 2.4 dS $m^{-1}$ among the treatments at the study of different N concentrations, and from 2.0 to 2.4 dS $m^{-1}$ among the treatments at the Nr study (Fig. 1A, B). No significant differences were observed at the pH of the drainage solution among the treatments at the N concentrations study (Fig. 1C), while pH of the NS was significantly reduced on most days of the cultivation period during the Nr study, especially at the high Nr rates i.e., Nr 0.10 and Nr 0.15 (Fig. 1D). The nutrient concentrations in the drainage solutions are presented in Table S3. In Exp. 1, the nitrates stayed consistently at high concentrations, and potassium decreased at the high N concentration (i.e., 300 mg N $L^{-1}$; N 21.4 mmol $L^{-1}$). Considering Exp. 2, high concentrations of ammonium were found at the high Nr treatments, while potassium decreased at low (Nr 0.01) and high (Nr 0.15) Nr rates.

### 3.2. Growth parameters

Plant height was significantly ($P < 0.05$) increased at the N application of 300 mg $L^{-1}$ (N 21.4 mmol $L^{-1}$) compared to 50 mg $L^{-1}$ of N (N 3.6 mmol $L^{-1}$), but no differences were found compared to 100 and 200 mg $L^{-1}$ of N (N 7.1 and N 14.3 mmol $L^{-1}$, respectively) applications (Table 1). The highest number of leaves was produced at 200 mg N $L^{-1}$ (N 14.3 mmol $L^{-1}$), whereas the lowest number was observed at the low (50 mg N $L^{-1}$; N 3.6 mmol $L^{-1}$) and the high (300 mg N $L^{-1}$; N 21.4 mmol $L^{-1}$) nitrogen concentrations. Plants grown at ≥ 100 mg $L^{-1}$ N (N 7.1 mmol $L^{-1}$) had increased fresh and dry biomass while the highest root fresh weight was observed at 100 mg N $L^{-1}$ (N 7.1 mmol $L^{-1}$). The root dry weight did not differ significantly between the N concentrations.

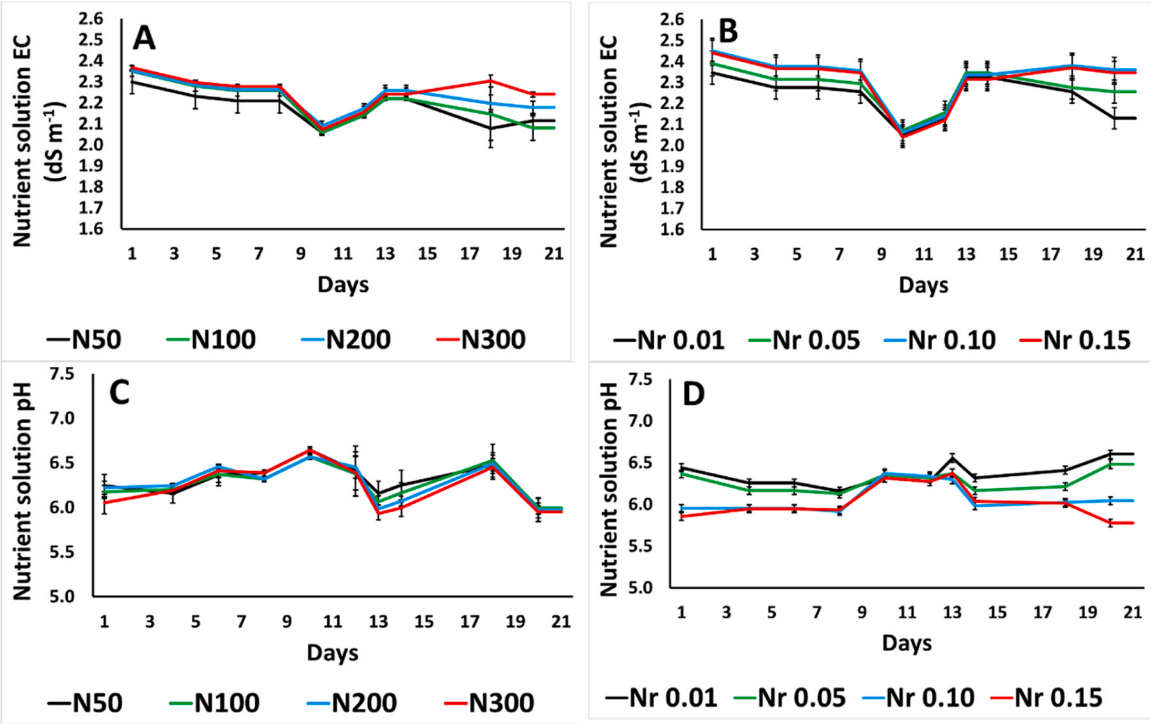

**Fig. 1.** Effect of increasing (A,C) nitrogen (N) concentration (50–100–200–300 mg $L^{-1}$, or 3.6–7.1–14.3–21.4 mmol $L^{-1}$; as N50, N100, N200, N300) and (B,D) ammonium to total nitrogen ratio ($NH_4^+$/Total N=Nr 0.01–0.05–0.10–0.15) in the (A,B) electrical conductivity (EC) and (C,D) pH of the drainage nutrient solution from *Sonchus* plants grown hydroponically in NFT system. Error bars show SE (n = 3). Total N is the sum of $NO_3^-$-N and $NH_4^+$-N.

**Table 1**
Effect of increasing total nitrogen (N) concentration (50–100–200–300 mg $L^{-1}$; as N50, N100, N200, N300) and ammonium to total nitrogen ratio ($NH_4^+$/Total N=Nr 0.01–0.05–0.10–0.15) on plant height, number of leaves, above-ground plant (leaves) and roots fresh weight (FW), dry weight (DW) and dry matter content (DM) of *Sonchus* plants grown hydroponically in NFT system. Total N is the sum of $NO_3$-N and $NH_4^+$-N. Significant differences ($P < 0.05$) among N concentrations or Nr ratios are indicated by different letters. Values are means of six replicates for each treatment.

| N concentration | Plant height (cm) | Number of leaves | Above-ground plant FW (g) | Root FW (g) | Above-ground plant DW (g) | Root DW (g) | Above-ground plant DM (%) | Root DM (%) |
|---|---|---|---|---|---|---|---|---|
| N50 | 18.0b | 9.0b | 6.2b | 0.72b | 0.48b | 0.05 | 7.9 | 8.4a |
| N100 | 19.6ab | 11.2ab | 10.1a | 2.54a | 0.82a | 0.12 | 7.6 | 5.4b |
| N200 | 18.8ab | 13.2a | 10.5a | 1.24ab | 0.89a | 0.10 | 8.3 | 6.4b |
| N300 | 20.7a | 9.2b | 10.0a | 1.01b | 0.82a | 0.05 | 7.9 | 5.5b |
| $NH_4^+$/Tot N=Nr | | | | | | | | |
| Nr 0.01 | 18.9 | 12.3 | 9.4a | 2.12a | 0.91a | 0.12a | 8.3 | 5.5 |
| Nr 0.05 | 17.0 | 11.8 | 7.7ab | 0.63c | 0.73ab | 0.04c | 8.6 | 7.1 |
| Nr 0.10 | 16.7 | 10.5 | 6.4b | 0.74c | 0.57b | 0.06bc | 9.1 | 7.8 |
| Nr 0.15 | 17.3 | 10.7 | 6.6b | 1.45b | 0.59b | 0.08b | 9.2 | 5.5 |

The N concentrations of N50-N100-N200-N300 are referring to 50 mg N $L^{-1}$ (or $NO_3$-N 3.4 mmol $L^{-1}$ + $NH_4^+$-N 0.2 mmol $L^{-1}$), 100 mg N $L^{-1}$ (or $NO_3$-N 6.7 mmol $L^{-1}$ + $NH_4^+$-N 0.4 mmol $L^{-1}$), 200 mg N $L^{-1}$ (or $NO_3$-N 13.5 mmol $L^{-1}$ + $NH_4^+$-N 0.8 mmol $L^{-1}$), and 300 mg N $L^{-1}$ (or $NO_3$-N 20.2 mmol $L^{-1}$ + $NH_4^+$-N 1.2 mmol $L^{-1}$).

Considering the ammonium to total nitrogen ratio (Nr), the higher above-ground and root biomass was found at low Nr (0.01), and this was also reflected in the increased dry weight of the plant tissue (Table 1).

### 3.3. Physiological parameters

Different concentrations of N ranging from 50 to 300 mg $L^{-1}$ (N 3.6–21.4 mmol $L^{-1}$) resulted in similar levels of leaf chlorophyll fluorescence (indicating maximal quantum yield of PSII photochemistry ($F_v/F_m$)) and relative chlorophyll content (SPAD values). Furthermore, the content of Chl a, Chl b, total chlorophylls and carotenoids were not influenced by the tested N concentrations (Table 2). Leaf stomatal conductance increased (up to 37.6%) in N concentrations ≥ 100 mg $L^{-1}$ (N 7.1 mmol $L^{-1}$) in comparison to 50 mg $L^{-1}$ of N (N 3.6 mmol $L^{-1}$).

Similarly, Nr ratio ranging from 0.01 to 0.15 did not affect chlorophylls or carotenoids content. However, leaf stomatal conductance at Nr 0.10 increased (up 25.8% and 39.1%) in comparison to Nr 0.01 and Nr 0.05, respectively.

Total phenolic and total flavonoid content, as well as the antioxidant activity of sowthistle were affected by the N concentrations (Table 3). The highest content of total phenols was found at 100 mg $L^{-1}$ of N (N 7.1 mmol $L^{-1}$), followed by the 200 mg $L^{-1}$ of N (N 14.3 mmol $L^{-1}$). Total flavonoids content increased at 200 mg N $L^{-1}$ (N 14.3 mmol $L^{-1}$) but decreased at ≤ 100 mg $L^{-1}$ (N 7.1 mmol $L^{-1}$) and at 300 mg N $L^{-1}$ (N 21.4 mmol $L^{-1}$). Moreover, antioxidant activity, indicated by DPPH, FRAP and ABTS, of sowthistle increased at 100 mg N $L^{-1}$ (N 7.1 mmol $L^{-1}$) (including 200 mg N $L^{-1}$ -N 14.3 mmol $L^{-1}$- for DPPH). Ascorbic acid content increased at ≥ 200 mg N $L^{-1}$ (N 14.3 mmol $L^{-1}$), while TSS decreased at ≥ 100 mg $L^{-1}$ of N (N 7.1 mmol $L^{-1}$).

Total phenolic content increased at Nr 0.01 in comparison to Nr ≥ 0.10. Moreover, at the lowest Nr ratio of 0.01, the total flavonoids content and antioxidant activity as assayed by FRAP exhibited increased values in comparison to the Nr 0.05 and Nr 0.15 (Table 3). The higher ascorbic acid content was found at Nr 0.10 in comparison to Nr 0.01 and Nr 0.15. The TSS decreased at ≥Nr 0.05.

The different N concentrations and/or the Nr ratio affected the tested damage indicators, $H_2O_2$ and MDA, and the activities of antioxidant enzymes of the treated sowthistle plants (Table 4). In the case of different N concentrations, the production of $H_2O_2$ in the leaf tissue increased at 100 mg N $L^{-1}$ (N 7.1 mmol $L^{-1}$) application compared to the 50 mg $L^{-1}$ (N 3.6 mmol $L^{-1}$) and ≥ 200 mg N $L^{-1}$ (N 14.3 mmol $L^{-1}$) (Table 4). SOD activity increased in plants grown at 300 mg $L^{-1}$ of N (N 21.4 mmol $L^{-1}$) application compared to the plants grown at 100 and 200 mg $L^{-1}$ of N (N 7.1 and 14.3 mmol $L^{-1}$). The opposite results were found in the case of CAT activity, whereas CAT activities increased at 100 and 200 mg N $L^{-1}$ (N 7.1 and 14.3 mmol $L^{-1}$), compared to the lower (50 mg $L^{-1}$; N 3.6 mmol $L^{-1}$) or higher N (300 mg $L^{-1}$; N 21.4 mmol $L^{-1}$) supply concentrations. POD activity was high at the 50 mg N $L^{-1}$ (N 3.6 mmol $L^{-1}$) in comparison to higher N concentrations (≥ 100 mg $L^{-1}$) (N 7.1 mmol $L^{-1}$).

Considering the effect of the ammonium to total N ratio, indicated as Nr values, MDA increased at ≥ Nr 0.10. The $H_2O_2$ production increased at Nr 0.01 compared to Nr 0.05, while the opposite was found for SOD activities, with values of SOD of 1.49 units $mg^{-1}$ protein at Nr 0.05 compared to 1.19 units $mg^{-1}$ protein at Nr 0.01. CAT activity was high at ≤ Nr 0.10 and significantly decreased at Nr 0.15 treatment. POD

**Table 2**
Effect of increasing total nitrogen (N) concentration (50–100–200–300 mg $L^{-1}$; as N50, N100, N200, N300) and ammonium to total nitrogen ratio ($NH_4^+$/Total N=Nr 0.01–0.05–0.10–0.15) on leaf stomatal conductance, relative leaf chlorophyll content, leaf chlorophyll fluorescence, chlorophyll content (a, b, total) and carotenoids of *Sonchus* plants grown hydroponically in NFT system. Total N is the sum of $NO_3^-$-N and $NH_4^+$-N. Significant differences ($P < 0.05$) among N concentrations or Nr ratios are indicated by different letters. Values are means of six replicates for each treatment.

| N concentration | Stomatal conductance (mmol $m^{-2}$ $s^{-1}$) | Relative chlorophyll content (SPAD value) | Chlorophyll fluorescence (Fv/Fm) | Chlorophyll a (mg $g^{-1}$ FW) | Chlorophyll b (mg $g^{-1}$ FW) | Total Chlorophylls (mg $g^{-1}$ FW) | Carotenoids (mg $g^{-1}$ FW) |
|---|---|---|---|---|---|---|---|
| N50 | 135.3b | 35.9 | 0.808 | 0.593 | 0.110 | 0.704 | 0.126 |
| N100 | 175.0a | 33.7 | 0.802 | 0.523 | 0.097 | 0.621 | 0.112 |
| N200 | 183.0a | 35.7 | 0.804 | 0.579 | 0.112 | 0.692 | 0.126 |
| N300 | 186.3a | 37.4 | 0.810 | 0.553 | 0.102 | 0.656 | 0.115 |
| $NH_4^+$/Tot N | | | | | | | |
| Nr 0.01 | 368.5b | 40.6 | 0.785 | 0.572 | 0.098 | 0.671 | 0.131 |
| Nr 0.05 | 407.5b | 38.2 | 0.790 | 0.548 | 0.097 | 0.645 | 0.121 |
| Nr 0.10 | 512.5a | 39.8 | 0.801 | 0.551 | 0.105 | 0.657 | 0.124 |
| Nr 0.15 | 451.2ab | 41.8 | 0.813 | 0.625 | 0.117 | 0.713 | 0.140 |

Fresh weight: FW. The N concentrations of N50-N100-N200-N300 are referring to 50 mg N $L^{-1}$ (or $NO_3$-N 3.4 mmol $L^{-1}$ + $NH_4^+$-N 0.2 mmol $L^{-1}$), 100 mg N $L^{-1}$ (or $NO_3$-N 6.7 mmol $L^{-1}$ + $NH_4^+$-N 0.4 mmol $L^{-1}$), 200 mg N $L^{-1}$ (or $NO_3$-N 13.5 mmol $L^{-1}$ + $NH_4^+$-N 0.8 mmol $L^{-1}$), and 300 mg N $L^{-1}$ (or $NO_3$-N 20.2 mmol $L^{-1}$ + $NH_4^+$-N 1.2 mmol $L^{-1}$).

**Table 3**

Effect of increasing total nitrogen (N) concentration (50–100–200–300 mg $L^{-1}$; as N50, N100, N200, N300) and ammonium to total nitrogen ratio ($NH_4^+$/Total N=Nr 0.01–0.05–0.10–0.15) on total phenols, antioxidant activity (DPPH. FRAP, ABTS), flavonoids, ascorbic acid, and total soluble sugars (TSS) of *Sonchus* plants grown hydroponically in NFT system. Total N is the sum of $NO_3$-N and $NH_4^+$-N. Significant differences ($P < 0.05$) among N concentrations or Nr ratios are indicated by different letters. Values are means of six replicates for each treatment.

| N concentration | Total Phenols (mg GA $g^{-1}$ FW) | DPPH (mg Trolox $g^{-1}$ FW) | FRAP (mg Trolox $g^{-1}$ FW) | ABTS (mg Trolox $g^{-1}$ FW) | Flavonoids (mg Rutin $g^{-1}$ FW) | Ascorbic acid (μg $g^{-1}$ FW) | TSS (%) |
|---|---|---|---|---|---|---|---|
| N50 | 0.167ab | 0.161b | 0.390b | 0.229b | 2.01b | 97.5b | 3.23a |
| N100 | 0.228a | 0.340a | 0.550a | 0.319a | 2.88b | 97.4b | 2.96b |
| N200 | 0.205a | 0.298a | 0.408b | 0.276ab | 4.28a | 134.0a | 2.53c |
| N300 | 0.153b | 0.188b | 0.362b | 0.235b | 1.83b | 145.9a | 2.60c |
| $NH_4^+$/Tot N | | | | | | | |
| Nr 0.01 | 0.216a | 0.374 | 0.606a | 0.321 | 3.30a | 75.4c | 3.26a |
| Nr 0.05 | 0.181ab | 0.264 | 0.486b | 0.269 | 2.41b | 110.9ab | 2.86b |
| Nr 0.10 | 0.164b | 0.352 | 0.521ab | 0.268 | 2.82ab | 116.1a | 2.83b |
| Nr 0.15 | 0.165b | 0.337 | 0.452b | 0.259 | 2.34b | 82.3bc | 2.87b |

Fresh weight: FW. The N concentrations of N50-N100-N200-N300 are referring to 50 mg N $L^{-1}$ (or $NO_3$-N 3.4 mmol $L^{-1}$ + $NH_{4^+}$-N 0.2 mmol $L^{-1}$), 100 mg N $L^{-1}$ (or $NO_3$-N 6.7 mmol $L^{-1}$ + $NH_{4^+}$-N 0.4 mmol $L^{-1}$), 200 mg N $L^{-1}$ (or $NO_3$-N 13.5 mmol $L^{-1}$ + $NH_{4^+}$-N 0.8 mmol $L^{-1}$), and 300 mg N $L^{-1}$ (or $NO_3$-N 20.2 mmol $L^{-1}$ + $NH_{4^+}$-N 1.2 mmol L-1).

**Table 4**

Effect of increasing total nitrogen (N) concentration (50–100–200–300 mg $L^{-1}$; as N50, N100, N200, N300) and ammonium to total nitrogen ratio ($NH_4^+$/Total N=Nr 0.01–0.05–0.10–0.15) on hydrogen peroxide- $H_2O_2$, lipid peroxidation-MDA and antioxidant enzymes activity of superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) of *Sonchus* plants grown hydroponically in NFT system. Total N is the sum of $NO_3^-$-N and $NH_4^+$-N. Significant differences ($P < 0.05$) among N concentrations or Nr ratios are indicated by different letters. Values are means of six replicates for each treatment.

| N concentration | $H_2O_2$ (μmol $g^{-1}$) | MDA (nmol $g^{-1}$) | SOD (units $mg^{-1}$ protein) | CAT (units $mg^{-1}$ protein) | POD (units $mg^{-1}$ protein) |
|---|---|---|---|---|---|
| N50 | 0.075b | 8.6 | 1.41ab | 7.5b | 0.864a |
| N100 | 0.098a | 8.5 | 1.27bc | 9.6a | 0.720b |
| N200 | 0.070b | 8.3 | 1.15c | 9.4a | 0.721b |
| N300 | 0.067b | 8.6 | 1.48a | 7.3b | 0.697b |
| $NH_4^+$/Tot N | | | | | |
| Nr 0.01 | 0.084a | 7.5b | 1.19b | 8.6a | 1.216 |
| Nr 0.05 | 0.048b | 7.3b | 1.49a | 8.1a | 1.152 |
| Nr 0.10 | 0.064ab | 8.4a | 1.42ab | 8.6a | 1.232 |
| Nr 0.15 | 0.080ab | 8.6a | 1.34ab | 5.9b | 1.049 |

The N concentrations of N50-N100-N200-N300 are referring to 50 mg N $L^{-1}$ (or $NO_3$-N 3.4 mmol $L^{-1}$ + $NH_{4^+}$-N 0.2 mmol $L^{-1}$), 100 mg N $L^{-1}$ (or $NO_3$-N 6.7 mmol $L^{-1}$ + $NH_{4^+}$-N 0.4 mmol $L^{-1}$), 200 mg N $L^{-1}$ (or $NO_3$-N 13.5 mmol $L^{-1}$ + $NH_{4^+}$-N 0.8 mmol $L^{-1}$), and 300 mg N $L^{-1}$ (or $NO_3$-N 20.2 mmol $L^{-1}$ + $NH_{4^+}$-N 1.2 mmol $L^{-1}$).

activity was not affected by the Nr ratios.

### 3.4. Leaf and root nutrient content

At Exp. 1, N concentrations $\geq$ 200 mg $L^{-1}$ (N 14.3 and 21.4 mmol $L^{-1}$) in the NS increased the nitrogen content in leaves and this was evidenced in roots as well (including the N100; N 7.1 mmol $L^{-1}$) in comparison to the low N concentration i.e., N50 (N 3.6 mmol $L^{-1}$) (Table 5). However, nitrate content in the leaves was not affected by the different N supply concentrations and averaged 2.19 g $kg^{-1}$ fresh weight. Potassium and calcium content, in both leaves and roots, remained unaffected by the N concentrations in the NS. Phosphorus content varied in leaves but not in roots. In roots, magnesium content increased at 200 mg $L^{-1}$ (N 14.3 mmol $L^{-1}$) compared to the $\leq$ 100 mg $L^{-1}$ (N 7.1 mmol $L^{-1}$), while no differences observed in the magnesium content of the leaves. Leaf sodium was significantly higher at 100 mg N $L^{-1}$ (N 7.1 mmol $L^{-1}$) supply compared with the lower or the higher concentrations of N in the NS.

In Exp. 2, a Nr of 0.15 in the NS increased nitrogen content in leaves, but decreased the nitrates content, in comparison to Nr 0.05–0.10, while nitrogen content in roots increased at Nr $\geq$ 0.10 (Table 5). Potassium content in leaves decreased but phosphorus and magnesium content increased as the N concentrations were increased in the NS. In roots, potassium content was high at the Nr of 0.10 and phosphorus and magnesium content were also increased at Nr $\geq$ 0.10. Sodium content was decreased by high Nr ratios in both leaves and roots. Calcium content did not differ in leaves and roots between the tested treatments.

### 3.5. Nitrogen and water uptake and use efficiency

The increase of N supplied concentrations resulted in increased nitrogen uptake but decreased $AE_N$ at $\geq$ 100 mg N $L^{-1}$ (N 7.1 mmol $L^{-1}$), in Exp 1 (Table 6). Water uptake was remained at similar levels (averaged in 288.68 mL $H_2O$ $plant^{-1}$ $day^{-1}$) among the N concentrations, while $WP_I$ was significantly higher at 200 mg N $L^{-1}$ (N 14.3 mmol $L^{-1}$) compared with rest examined N concentrations.

In Exp.2, different Nr rations did not affect the nitrogen uptake (averaged in 8.96 mg N $plant^{-1}$ $day^{-1}$) and $AE_N$ (averaged in 3.71 g DW $g^{-1}$ of N per plant) (Table 6). The water uptake increased at Nr of 0.01, when compared with the higher ratios of Nr (0.05–0.15). The highest $WP_I$ was found at Nr 0.05.

Considering the different N concentrations in Exp 1, the nitrogen accumulation rate was significantly higher at 200 mg N $L^{-1}$(N 14.3 mmol $L^{-1}$), followed by those estimated at 100 mg N $L^{-1}$ (N 7.1 mmol $L^{-1}$), 300 mg N $L^{-1}$ (N 21.4 mmol $L^{-1}$) and then at 50 mg N $L^{-1}$ (N 3.6 mmol $L^{-1}$) (Table 7). The bioaccumulation coefficient in both leaves and roots decreased as the N concentrations in the nutrient solution increased. Tolerance index (TI) increased at 300 mg N $L^{-1}$ (N 21.4 mmol $L^{-1}$) for plant height when compared with 50 mg N $L^{-1}$ (N 3.6 mmol $L^{-1}$) in the NS, while the higher TI for leaf number per plant was observed at 200 mg N $L^{-1}$ (N 14.3 mmol $L^{-1}$).

Ammonium to total N ratio affected the transport of N, as indicated by the decrease of the nitrogen accumulation rate with increasing Nr ratio in the NS (Table 7). The N bioaccumulation coefficient in leaves was decreased by the Nr $\geq$ 0.05, while in roots, the bioaccumulation coefficient was increased by the Nr $\geq$ 0.10 in comparison to the lower Nr ratios. The translocation factor of N from the roots to the leaves decreased as the Nr ratio was increased. The Nr ratio affected TI only for the total biomass of *Sonchus* plants, as TI of total biomass decreased at the Nr $\geq$ 0.05 in comparison to the lower Nr ratio of 0.01.

### 3.6. Correlation of N concentrations and Nr ratios to individual parameters

Linear correlation coefficients were calculated and reported in detail in Tables S4–S5, to analyse the contribution of the N concentrations as well as the Nr in the NS for each individual parameter. There was a

**Table 5**
Effect of increasing total nitrogen (N) concentration (50–100–200–300 mg $L^{-1}$; as N50, N100, N200, N300) and ammonium to total nitrogen ratio ($NH_4^+$/Total N=Nr 0.01–0.05–0.10–0.15) in the nutrient solution on the content of macronutrients in leaves and roots of *Sonchus* plants grown hydroponically in NFT system. Values of nitrogen-N, potassium-K, phosphorus-P, calcium-Ca, magnesium-Mg, and sodium-Na are expressed in dry weight-DW. Values of nitrates-$NO_3^-$ are expressed in dry weight (in parenthesis in fresh weight-FW). Total N is the sum of $NO_3^-$-N and $NH_4^+$-N. Significant differences ($P < 0.05$) among N concentrations or Nr ratios are indicated by different letters. Values are means of four replicates for each treatment.

| | Leaves | | | | | | | Roots | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| N concentration | $NO_3$ (g $kg^{-1}$ DW (FW)) | N (g $kg^{-1}$ DW) | K (g $kg^{-1}$ DW) | P (g $kg^{-1}$ DW) | Ca (g $kg^{-1}$ DW) | Mg (g $kg^{-1}$ DW) | Na (g $kg^{-1}$ DW) | N (g $kg^{-1}$ DW) | K (g $kg^{-1}$ DW) | P (g $kg^{-1}$ DW) | Ca (g $kg^{-1}$ DW) | Mg (g $kg^{-1}$ DW) | Na (g $kg^{-1}$ DW) |
| N50 | 27.9 (2.21) | 49.1b | 67.4 | 8.8ab | 3.85 | 0.80 | 5.62b | 39.6b | 46.7 | 9.6 | 4.23 | 0.23b | 5.26 |
| N100 | 26.0 (1.96) | 51.1ab | 67.7 | 8.2b | 4.38 | 0.78 | 6.44a | 43.0a | 49.4 | 11.5 | 4.64 | 0.21b | 4.79 |
| N200 | 28.8 (2.39) | 51.9a | 67.1 | 8.6ab | 2.47 | 0.71 | 5.59b | 41.9a | 53.2 | 10.9 | 4.40 | 0.43a | 4.43 |
| N300 | 27.6 (2.18) | 51.6a | 69.3 | 9.1a | 1.60 | 0.61 | 5.24c | 43.3a | 49.6 | 11.2 | 3.48 | 0.39ab | 5.15 |
| $NH_4^+$/Tot N | | | | | | | | | | | | | |
| Nr 0.01 | 35.3a (2.94a) | 48.5ab | 65.9a | 6.9c | 5.63 | 0.54c | 6.22ab | 38.9b | 42.3b | 9.4c | 5.43ab | 0.69c | 8.03b |
| Nr 0.05 | 28.0ab (2.41ab) | 44.9c | 62.8ab | 7.6b | 6.48 | 0.70b | 6.65a | 40.7b | 46.0b | 9.6c | 3.91ab | 0.75bc | 9.00a |
| Nr 0.10 | 23.8b (2.18ab) | 46.5bc | 60.9b | 7.9b | 3.91 | 0.69b | 5.34b | 47.3a | 56.6a | 14.7a | 2.53b | 0.79ab | 3.47d |
| Nr 0.15 | 18.8b (1.72b) | 51.2a | 52.4c | 8.9a | 4.82 | 0.85a | 5.27b | 48.5a | 42.0b | 13.5b | 6.13a | 0.85a | 6.14c |

The N concentrations of N50-N100-N200-N300 are referring to 50 mg N $L^{-1}$ (or $NO_3$-N 3.4 mmol $L^{-1}$ + $NH_{4+}$-N 0.2 mmol $L^{-1}$), 100 mg N $L^{-1}$ (or $NO_3$-N 6.7 mmol $L^{-1}$ + $NH_{4+}$-N 0.4 mmol $L^{-1}$), 200 mg N $L^{-1}$ (or $NO_3$-N 13.5 mmol $L^{-1}$ + $NH_{4+}$-N 0.8 mmol $L^{-1}$), and 300 mg N $L^{-1}$ (or $NO_3$-N 20.2 mmol $L^{-1}$ + $NH_{4+}$-N 1.2 mmol $L^{-1}$).

**Table 6**
Influence of increasing total nitrogen (N) concentration (50–100–200–300 mg $L^{-1}$; as N50, N100, N200, N300) and ammonium to total nitrogen ratio ($NH_4^+$/Total N=Nr 0.01–0.05–0.10–0.15) on nitrogen uptake, water uptake, agronomic efficiency of N ($AE_N$), irrigation water productivity ($WP_I$) of *Sonchus* plants grown hydroponically in NFT system. Total N is the sum of $NO_3^-$-N and $NH_4^+$-N. The plant density at the present study was 120,000 plants per ha. Values for $AE_N$ and $WP_I$ are expressed per plant or per ha. Significant differences ($P < 0.05$) among N concentrations or Nr ratios are indicated by different letters. Values are means of three replicates for each treatment.

| N concentration | Nitrogen uptake (mg $plant^{-1}$ $day^{-1}$) | Water uptake (mL $plant^{-1}$ $day^{-1}$) | $AE_N$ (g DW $g^{-1}$ of N per plant or kg DW $kg^{-1}$ of N per ha) | $WP_I$ (g DW $L^{-1}$ $H_2O$ per plant or kg DW $m^{-3}$ $H_2O$ per ha) |
|---|---|---|---|---|
| N50 | 3.57d | 286.3 | 6.73a | 0.082c |
| N100 | 8.09c | 283.5 | 4.84b | 0.128b |
| N200 | 15.03b | 266.4 | 3.01bc | 0.170a |
| N300 | 23.17a | 318.5 | 1.68c | 0.124b |
| $NH_4^+$/Tot N=Nr | | | | |
| Nr 0.01 | 9.64 | 333.7a | 4.55 | 0.131ab |
| Nr 0.05 | 9.10 | 218.7c | 3.86 | 0.159a |
| Nr 0.10 | 8.55 | 218.6c | 3.17 | 0.124ab |
| Nr 0.15 | 8.56 | 285.8b | 3.29 | 0.098b |

The N concentrations of N50-N100-N200-N300 are referring to 50 mg N $L^{-1}$ (or $NO_3$-N 3.4 mmol $L^{-1}$ + $NH_{4+}$-N 0.2 mmol $L^{-1}$), 100 mg N $L^{-1}$ (or $NO_3$-N 6.7 mmol $L^{-1}$ + $NH_{4+}$-N 0.4 mmol $L^{-1}$), 200 mg N $L^{-1}$ (or $NO_3$-N 13.5 mmol $L^{-1}$ + $NH_{4+}$-N 0.8 mmol $L^{-1}$), and 300 mg N $L^{-1}$ (or $NO_3$-N 20.2 mmol L-1 + $NH_{4+}$-N 1.2 mmol $L^{-1}$).

positive correlation with N (50–100–200–300 mg $L^{-1}$; N 3.6–21.4 mmol $L^{-1}$) concentrations and leaf stomatal conductance, ascorbic acid content, plant height, leaf nitrogen content, and root magnesium content. Negative correlations were found between N concentrations and the contents of leaf calcium, leaf magnesium, leaf sodium, TSS and POD activity (Table S4). Therefore, the nitrogen accumulation in leaves correlated positively to plant height, leaf stomatal conductance, biomass and root nitrogen content, and negatively to POD activity.

Considering the impact of the Nr in the NS, a positive correlation of Nr was observed between leaf stomatal conductance, plant dry matter, and the contents of leaf phosphorus, leaf magnesium, root nitrogen, root phosphorus, root magnesium and MDA production. On the other hand, the Nr ratios were negatively correlated with plant fresh and dry weight, TSS, total phenol and FRAP antioxidant activity, leaf potassium and CAT activity (Table S5). Therefore, the nitrogen accumulation in roots exhibited positive correlation with the accumulation of phosphorus and magnesium in leaves, but it was negatively correlated with plant growth (height, number of leaves, fresh biomass production), TSS and the content of potassium in leaves.

## 4. Discussion

The present study was commissioned to provide insight into the responses of sowthistle to different concentrations and sources of N in soilless culture, focussing mainly on irrigation water productivity, agronomic N efficiency, accumulation rates of N into the plant tissue, plant growth and physiological parameters, and nitrate leaching. The agronomic efficiency of N in a closed hydroponic system is an unexplored and less understood field of research. Nitrogen concentrations and ratios of ammonium to total-N (Nr) supply were obtained by using different concentrations of $NH_4^+$-N, $NO_3^-$-N and $SO_4^{2-}$, while keeping constant the Nr rates (Nr 0.05) and EC values of the NS in the Exp. 1 and constant the N concentrations (200 mg $L^{-1}$; N 14.3 mmol $L^{-1}$) and EC values of the NS in the Exp. 2.

Effective nutrient solution management is required to control nitrate concentration and to reduce the nitrate leakage to the water resources. This is of great concern in the Mediterranean region and areas where protected crop farming is expanding (Omondi and Angel, 2023; Thompson et al., 2007). Aside from the environmental and health risks associated with nitrates, the source of N supplied to plants is critical for crop performance in terms of both plant growth and nitrate accumulation in plant tissue. Several studies have been undertaken to better understand the plant growth responses to the source of plant available inorganic nitrogen, i.e., $NH_4^+$ and $NO_3^-$ (Akl et al., 2003; Savvas et al., 2006; Tzortzakis et al., 2022b); however, the outcomes are inconsistent and largely dependent on the plant species and the cultivation practices applied. When it comes to plant growth and the synthesis/accumulation of chemical compounds, the majority of plants prefer a combination of nitrate and ammonium rather than sole $NO_3^-$ or $NH_4^+$ supply, provided that the pH of the nutrient solution in the root zone can be maintained close to neutral values (Tabatabaei et al., 2008; Zhu et al., 2014). In this regard, mixed nitrate and ammonium fertigation was used in the present

**Table 7**
Influence of increasing total nitrogen (N) concentration (50–100–200–300 mg $L^{-1}$; as N50, N100, N200, N300) and ammonium to total nitrogen ratio ($NH_4^+$/Total N=Nr 0.01–0.05–0.10–0.15) on the nitrogen accumulation rate-AR, N bioaccumulation coefficient-BAC, N translocation factor-TF and N tolerance indices-TI (as defined at Section 2.4) of *Sonchus* plants grown hydroponically in NFT system. Total N is the sum of $NO_3$-N and $NH_4^+$-N. Significant differences ($P < 0.05$) among N concentrations or Nr ratios are indicated by different letters. Values are means of three replicates for each treatment.

| | Accumulation rate-AR (g $kg^{-1}$ DW $day^{-1}$) | Bioaccumulation coefficient-BAC | | Translocation factor-TF | Tolerance indices-TI (%) | | |
|---|---|---|---|---|---|---|---|
| | | | | | Total Biomass | Plant height | Leaf No |
| N concentration | | Leaves | Roots | | | | |
| N50 | 0.68d | 928.5a | 748.1a | 1.24 | 100.0 | 100.0b | 100.0b |
| N100 | 2.13b | 483.1b | 406.6b | 1.18 | 173.4 | 106.5ab | 124.1ab |
| N200 | 2.42a | 245.2c | 197.7c | 1.24 | 183.4 | 104.3ab | 146.3a |
| N300 | 1.86c | 162.7d | 136.6d | 1.19 | 160.4 | 115.1a | 101.8b |
| $NH_4^+$/Tot N=Nr | | | | | | | |
| Nr 0.01 | 2.34a | 240.2a | 192.5b | 1.25a | 100.0a | 100.0 | 100.0 |
| Nr 0.05 | 1.25b | 213.3b | 193.5b | 1.11b | 74.8b | 91.2 | 104.9 |
| Nr 0.10 | 0.85d | 209.5b | 212.8a | 1.01c | 60.9b | 90.2 | 97.1 |
| Nr 0.15 | 1.04c | 217.6b | 206.1a | 1.05bc | 64.6b | 93.1 | 97.0 |

The N concentrations of N50-N100-N200-N300 are referring to 50 mg N $L^{-1}$ (or $NO_3$-N 3.4 mmol $L^{-1}$ + $NH_{4^+}$-N 0.2 mmol $L^{-1}$), 100 mg N $L^{-1}$ (or $NO_3$-N 6.7 mmol $L^{-1}$ + $NH_{4^+}$-N 0.4 mmol $L^{-1}$), 200 mg N $L^{-1}$ (or $NO_3$-N 13.5 mmol $L^{-1}$ + $NH_{4^+}$-N 0.8 mmol $L^{-1}$), and 300 mg N $L^{-1}$ (or $NO_3$-N 20.2 mmol $L^{-1}$ + $NH_{4^+}$-N 1.2 mmol $L^{-1}$).

study, under hydroponic conditions. Hydroponic systems have been documented to improve crop performance, including both production and quality in vegetable crops, since those cultivation systems provide precise mineral fertigation management, as well as water and fertiliser savings if the fertigation effluents are collected and recycled (Tzortzakis et al., 2022a). The results of the present study show that not only the N concentrations but also the source of N in the NS affect the features under investigation, such as plant growth, physiological and biochemical parameters, nutrient accumulation, agronomic efficiency of N and irrigation water productivity.

The increase of nitrogen concentrations in the NS increased the yield of sowthistle, which is consistent with earlier reports on lettuce yield in response to the N supply concentration in a floating-hydroponic system (Sapkota et al., 2019; Wenceslau et al., 2021). Nitrogen concentrations of $\geq$ 100 mg $L^{-1}$ (N 7.1 mmol $L^{-1}$) were effective in increasing plant biomass, since nitrogen favours vegetative growth, although there are reports indicated that N concentrations ranging from 150 to 250 mg $L^{-1}$ (N 10.7–17.8 mmol $L^{-1}$) had no effect on spearmint yield (Chrysargyris et al., 2017a). However, increasing the yield during a crop production should be considered of secondary importance, as the primary focus should be on the nitrate content in the edible parts of the crop, as well as on the preservation of water resources as a result of reduced nitrate emissions originating from crop fertigation.

As reported by Wenceslau et al. (2021), growth and plant yield losses caused by low $NO_3$-N supply concentrations combined with high concentrations of $NH_4^+$-N supply, are due to limitations in photosynthesis and concomitantly to reduced availability of carbon skeletons that are needed to assimilate ammonium. However, uptake of ammonium by plants at high levels can cause toxicity to the plants, and decrease organic acid synthesis and osmotic regulation (Wang et al., 2022). In the present study, plant growth related parameters (height, leaves, and plant fresh biomass) were almost unaffected by the increased Nr, with the exception of plant dry weight that decreased. Similarly, Savvas et al. (2006) reported no differences in fresh biomass of lettuce plants when Nr was 0.1 or Nr 0.2, in a closed hydroponic system. In contrast, Zhu et al. (2014) reported increased leaf area, photosynthetic rates and biomass in *Prunella vulgaris* (L.) grown at Nr 0.2. Song et al. (2011) reported that when the flowering Chinese cabbage was grown hydroponically under different Nr, plant height and biomass increased at low Nr ratios and decreased at high Nr ratios. Nevertheless, in the present study, the Nr used was $\leq$ 0.15, while the recommended Nr for iceberg lettuce in a floating-hydroponic system was 0.23; on top of that, higher Nr ratios (i.e., Nr 0.5) decreased yield and caused toxicity symptoms (Wenceslau et al., 2021). It is noteworthy to mention that plant sensitivity to ammonium toxicity increases as the root zone temperature increases (Ganmore-Neumann and Kafkafi, 1980), and thus the optimal Nr ratios established in the current study are valid only for the range of root zone temperature that was prevailing during the experiment, which ranged from 19.5 to 24 °C. Other authors reported enhanced yield, ultimate physiological state and increased secondary metabolite production in medicinal cannabis plants supplied with nitrates, however those observations were not evidenced with ammonium supply (Saloner and Bernstein, 2022); nitrate leakage though should be taken into consideration when sole nitrate supply is employed.

Changes in the amount of chlorophyll, the structure of the chloroplasts, and the stomatal conductance have a strong impact on the rate of net photosynthesis in leaves (Zhao et al., 2001). In the present study, the leaf stomatal conductance increased at N supply concentrations $\geq$ 100 mg $L^{-1}$ (N 7.1 mmol $L^{-1}$), while the nutrient solutions with the different N supply concentrations and Nr ratios did not affect the chlorophyll content. The similar photosynthetic rates and $F_v/F_m$ values (close to 0.8) in all treatments indicate that they had no impact on the PSII in sowthistle plants (Broetto et al., 2007). Similarly, the chlorophyll content in sweet basil plants growing in recirculating water (NFT) was not affected by different Nr ratios (Saadatian et al., 2014). However, Chrysargyris et al. (2017a) reported a significant increase of the chlorophyll content in spearmint plants grown hydroponically under high N concentrations (i.e., 250 mg N $L^{-1}$; N 17.8 mmol $L^{-1}$) in the NS, and this was ascribed to the different plant species, the different growth season, and the different hydroponic system used (NFT versus floating system).

Currently, there is an appreciably high interest in plant-based diets and the various plant tissue components contributing to improved health. This is due to the well-known nutritional benefits derived from eating polyphenol-rich plant foods. A rich diet in natural phenolic compounds can prevent tissue oxidation by scavenging free radicals and by inhibiting lipid peroxidation, thus improving the nutritional value of the food and removing potential issues brought on by an excessive intake of synthetic additives (Scherer et al., 2013). Therefore, compounds with a high phenolic content have the capacity to guard against harm from oxidative stress caused by free radicals. In the current study, increased phenol content and antioxidant capacity were found in *Sonchus* plants supplied with 100–200 mg N $L^{-1}$ (N 7.1–14.3 mmol $L^{-1}$) via the NS. This trend has been reported for other species too, as for instance for lavender grown in perlite at 200–250 mg N $L^{-1}$ (N 14.3–17.8 mmol $L^{-1}$), which exhibited increases in phenol and antioxidant content compared to plants supplied with lower N concentrations (Chrysargyris et al., 2016). Nguyen and Niemeyer (2008) proved that changes in the nitrogen fertilisation during the growing period had significant impacts on the basil phenolic compounds, in particular rosmarinic acid. Saadatian et al. (2014) reported that a Nr of 0.11 increased phenolic content and antioxidants (DPPH) compared to the higher tested ratios (Nr 0.20, Nr 0.27) in hydroponically (NFT) grown basil. In the present study, sowthistle exhibited increased phenolic content at even lower Nr ratios (Nr 0.01). This difference can be related to the plant species and to the different cultivation practices applied in the two studies. Moreover, Zhu et al. (2014) reported increased flavonoids and rosmarinic acid content in *Prunella vulgaris* (L.) grown at Nr 0.20. However, as documented in earlier investigations, the high antioxidant capacity and the increased total phenolic content are largely linked to the treatment applied and the plant species (Scherer et al., 2013).

According to Cakmak (2005), the application of appropriate N concentrations for plant fertigation prevents stress reactions and, as a result, the buildup of reactive oxygen species (ROS). Plants activate detoxification mechanisms towards the ROS production by scavenging free radicals with the induction of antioxidant enzymes such as SOD, CAT, ascorbate peroxidase (APX), and glutathione reductase (GR) (Foyer and Noctor, 2011) or the activation of non-enzymatic antioxidant mechanisms such as polyphenols, ascorbic acid, proline, etc. In the present study, the increased Nr ratio in the NS increased the MDA content, indicating the presence of oxidative stress in plants exposed to Nr$\geq$ 0.10. In that sense, antioxidant enzymes (i.e., CAT and SOD) and non-enzymatic mechanisms, i.e., AA at Nr 0.10 were activated as a protective mechanism by the plant in order to detoxify the stress condition.

The different N concentrations in the NS were not associated with oxidative stress, as the MDA content remained unaffected by the tested N concentrations. The ascorbic acid content increased at 200–300 mg N $L^{-1}$ (N 14.3–21.4 mmol $L^{-1}$) and the TSS decreased at moderately high N concentrations. Presumably plants increase the production of TSS, proline, AA and total phenolics at high N supply concentrations to defend against hyperosmotic stress (He et al., 2021). Similar findings have been reported for spearmint plants exposed to different N concentrations, where the MDA content was unaffected by the different N concentrations (Chrysargyris et al., 2017a). However, $H_2O_2$ induction was eliminated initially by the activation of SOD, followed by the activation of CAT or POD, in different N concentrations. $H_2O_2$ can be removed through the ascorbate-glutathione cycle, and peroxidases and SOD are the key enzymes in this cycle (Pasternak et al., 2005). With respect to the Nr ratios, their elevation increased the MDA content, while CAT activity was found to be increased with increasing Nr ratios. While POD remained inactive after all treatments, SOD increased at Nr 0.05, trying to transform ROS to $H_2O_2$, revealing an active reaction of plant defence against the applied stress. *Portulaca oleracea* plants exposed to high Nr ratios and salinity, resulted in leaf injury, and that was related to the increased Na accumulation and the excess in $NH_4^+$ that might disturb cellular homoeostasis and increased oxidative stress (Camalle et al., 2020).

The increased N concentrations and Nr ratio affected macronutrients in both leaves and roots. Mineral contents in the present study were within levels reported as optimal (De Paula Filho et al., 2022). Total nitrogen content in leaves increased at the high Nr ratios (i.e., Nr 0.15), being in agreement with previous studies on *Prunella vulgaris* grown at high Nr ratio (Zhu et al., 2014) and in Chinese kale (*Brassica alboglabra* L. H. Bailey) grown at high Nr (Wang et al., 2022). In the present study, the accumulation of potassium, nitrates, and sodium was lower, while the accumulation of phosphorus was higher in sowthistle leaves, as the Nr increased in the NS. Similar findings were observed when Chinese kale was treated with high Nr ratios (Wang et al., 2022). This indicates that increasing Nr in the NS is an effective mean to prevent the accumulation of nitrates in the plant tissue, as their uptake is restricted due to partial substitution by $NH_4^+$-N. The decreased potassium and sodium accumulation in leaves at high Nr is correlated with the well-known competition for uptake between potassium and $NH_4^+$ (Saloner and Bernstein, 2022). Therefore, as the Nr increased in the NS, leaf magnesium increased, while leaf sodium did not. Leaf phosphorus was reduced at the lowest Nr treatment, presumably due to antagonism of phosphates with nitrates. Similar findings have been reported for hydroponically grown tomato plants under various Nr ratios (Tzortzakis et al., 2022b). Additionally, the increased concentrations of ammonium in the NS, decreased the content of several minerals in cucumber fruit (Kotsiras et al., 2002). The pH in the drainage NS had a decreased tendency as the Nr ratio was increased, but always within the accepted pH limits, and varied from 5.7 to 6.4 due to the appropriate pH correction management at the NS. A decline of the pH values along with the increased Nr ratio in the NS has been reported previously in studies on lettuce (Savvas et al., 2006) and *Cichorium spinosum* (Chatzigianni et al., 2018).

In the present study, the content of leaf and root nitrogen increased at the high N concentrations and Nr ratios in the NS. Nitrates content in leaves varied from 1.7 to 2.9 g $kg^{-1}$ of fresh weight, ranging to similar content with previous reports for lettuce (Savvas et al., 2006). Due to the human health concern from the increased nitrate content in fresh produce, especially in leafy vegetables, the strategy of increased Nr in the NS may alleviate the nitrate accumulation in plant tissue. This is one of the known advantages of soilless culture and appropriate NS management. Moreover, the vital role of chloride ions as competitive anion to the $NO_3^-$ has been previously reported in hydroponics (Neocleous et al., 2021). When spearmint was subjected to different N concentrations, ranging from 150 to 250 mg N $L^{-1}$ (N 10.7–17.8 mmol $L^{-1}$), macronutrients content were not affected, in contrast to micronutrients that were significantly affected, thus impacting several plant secondary metabolism pathways, including essential oils synthesis and composition, as they act as activators or components of vital enzymes for these pathways (Chrysargyris et al., 2017a).

In the current study, the N concentrations affected not only plant growth and secondary metabolism but also agronomic efficiency of N ($AE_N$). Even though the $AE_N$ decreased as the N concentrations increased in the NS, the higher nitrogen accumulation rates were found at 200 mg N $L^{-1}$ (N 14.3 mmol $L^{-1}$), indicating the sustainable use of N at this concentration in the NS, for hydroponically grown sowthistle. The maximum irrigation water productivity ($WP_I$) was found at 200 mg N $L^{-1}$ (N 14.3 mmol $L^{-1}$). Due to the increased constrains of the excess use of fertilisers and water in agriculture, cultivation practices that result in increased $AE_N$ and $WP_I$ are recommended. The recommended concentration of 200 mg N $L^{-1}$ (N 14.3 mmol $L^{-1}$) is for cultivation in a closed hydroponic system. Moreover, the plant density in hydroponic cultivation is related to the greenhouse infrastructure, and the present findings are reflected in plant density of 120,000 plants per ha. In the case of the different Nr ratios in the NS, increased $AE_N$ and $WP_I$ were observed at Nr 0.01 and Nr 0.05, respectively. Considering the current trend for water and fertiliser savings due environmental and health related concerns, Nr of 0.05 can meet this demand more efficiently, as Nr 0.05 has high $AE_N$ too. In a closed hydroponic system, the loss of nitrogen could be related to the plant N metabolism or might be caused when the composition of the nutrient solution during plant cultivation varies (Wang et al., 2022). At the end of the experiment, nitrates remained in high concentrations at the high N treatment (i.e., 300 mg N $L^{-1}$; N 21.4 mmol $L^{-1}$), while ammonium was relatively high concentrations with high Nr values in the NS. However, detailed measurement of the variation of the $NH_4^+$ and $NO_3^-$ concentrations during plant growth, could provide more information regarding the nitrogen metabolism and the N losses in gaseous forms, including $NH_3$, $N_2O$, and NO (Wang et al., 2022).

Nitrogen accumulation rate decreased as the Nr ratio was increased in the NS, while the amount of nitrogen bioaccumulation was lower in leaves and higher in roots at the higher Nr ratios. The decreased translocation of N from roots to leaves, as the Nr ratio was increased, resulted in decreasing plant biomass and more oxidative stress as the MDA production was increased. The TF and BAC indices are important factors in determining how plants respond in terms of phytoremediation and element accumulation in specific organs. Both parameters in hyperaccumulator species are greater than one, as in sowthistle leaves in the present study. These important indicators provide new insights for the management of nutrients in closed hydroponic systems and contribute to a better understanding of N accumulation in plant tissue. The TF decreased as the Nr increased, being almost 1.0 at Nr 0.10 and Nr 0.15, indicating that little N was translocated from roots to leaves at these Nr values. Sowthistle leaves are the plant part that is mostly consumed. Therefore, prevention of N accumulation in leaves is important to manage the nitrates accumulation in leaf tissue. This was evidenced in the 46.7% reduction of nitrate content at Nr 0.15 compared to the Nr 0.01 application.

The accumulation of nitrogen in leaves and roots under different N concentrations and Nr ratios induced changes in plant growth and in the accumulation of other nutrients in the plant organs. Nitrate metabolism is less efficient energetically than ammonium metabolism. That is because nitrate must be reduced to ammonium in order to be assimilated into organic compounds, and nitrate absorption and reduction are energy-intensive processes (Saloner and Bernstein, 2022). Therefore, oversupply of N or unbalanced ammonium to nitrate sources may negatively influence plant metabolism and function, affecting root ion balance and inhibiting or promoting uptake of other mineral nutrients.

## 5. Conclusions

The current study demonstrated that sowthistle, an under-exploited vegetable, can be successfully grown in soilless culture systems. Furthermore, the current study showed that the growth and nutritional value of sowthistle grown hydroponically is influenced by the N supply concentration and the N source (Nr: $NH_4^+$-N/total-N ratio). Intermediate N supply concentrations of 100–200 mg $L^{-1}$ (N 7.1–14.3 mmol $L^{-1}$) promoted plant growth (leaf number and biomass production) and enhanced the defence mechanisms of the plant against oxidative stress as indicated by the increased content of phenolics, flavonoids, ascorbic acid, the higher total antioxidant capacity, as well as the low levels of oxidative stress indicated by the increased CAT activity. The highest agronomic efficiency of N (AEN) and irrigation water productivity (WPi), combined with high yield and high nutritional value in sowthistle grown in a closed hydroponic system, were observed at 200 mg N $L^{-1}$ (N 14.3 mmol $L^{-1}$) in the supplied nutrient solution. The recommended ratio of ammonium to total nitrogen (Nr) was 0.05; plants grown at this Nr ratio exhibited lower stress profile (decreased MDA and increased detoxification response), high levels of leaf mineral nutrients and high WPi. The optimal N concentration and Nr ratio found in the current study are based on two different single factorial studies. However, a full-scale factorial study would provide the optimum combination of N concentration and Nr ratio.

## 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

No data was used for the research described in the article.

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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.2023.108528.

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