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Reduced nitrogen availability during growth improves quality in red oak lettuce leaves by minimizing nitrate content, and increasing antioxidant capacity and leaf mineral content

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ABSTRACT

Overuse of N in lettuce production can lead to environmental problems caused by leaching and the accumulation of harmful nitrates in edible tissues. This study investigated the effect of applied nitrogen (N) concentrations between 40 and 2400 mg·L^{-1} on growth, nitrate accumulation, mineral leaf content, and antioxidant capacity in Oak Leaf lettuce cv. "Shiraz" grown under hydroponic conditions in Australia. Yield (g FW) increased with nitrogen (N) application rate up to 1200 mg·L^{-1}, as did leaf N content, while C:N declined. Nitrogen Utilization Efficiency (NUtE) increased rapidly from 40 to 75 mg·L^{-1} applied N, leveling at 150 mg·L^{-1} with no subsequent effect of N concentrations between 400 and 2400 mg·L^{-1}. Nitrate content rose significantly with increased N, particularly at 1200 and 2400 mg·L^{-1}. Leaf total plant phenolic content (TPP) and antioxidant capacity (measured by ferric reducing antioxidant power—FRAP) were both maximal at 75 and 400 mg·L^{-1} applied N, while highest oxygen radical absorption capacity (ORAC) values were found in leaves supplied with low N (40 to 400 mg·L^{-1}). Applied N as calcium nitrate also significantly affected leaf mineral content as B, Mg, Mn, and Zn significantly decreased with increasing N. These results indicate that N applications of 1200 mg·L^{-1} or higher can result in reduced antioxidant capacity and mineral content in lettuce leaves.

Keywords: *Lactuca sativa* L.; Hydroponic; Phenolic Content; Zinc; Manganese; Magnesium

1. INTRODUCTION

In the past 50 years the use of nitrogen-phosphorus potassium-based (NPK) fertilizers has increased dramatically around the world, particularly in North America and Europe [1]. Overuse of NPK can lead to two major problems—N leaching and P runoff from agriculture is widely considered the main cause of eutrophication in fresh and salt water supplies throughout the world [2]. Secondly, leafy vegetable crops, such as lettuce, accumulate nitrates when grown with high N availability and low light [3], and this can be deleterious to human health as nitrates can be converted to harmful nitrites post-harvest [4]. Thus the efficient use of N is an important environmental and social issue [1], in addition to the potential cost savings of reduced fertilizer use [5]. Maximizing nitrogen use and nitrogen utilization efficiency (NUtE) of crop production can be achieved by 1) optimizing the supply of N to meet the requirements of a crop during growth and development [6]; 2) optimizing N supply in correlation with the desired final produce quality [7], or 3) by selecting and growing N-efficient crop genotypes [5,8].

Lettuce is considered the one of most economically important leafy vegetable crop in the world [9] and is widely consumed in Western diets. It is therefore an important source of dietary antioxidants, primarily in the form of phenolic compounds such as caffeic acid derivatives and flavonols [10]. There is increasing evidence that antioxidants contained within fruits and vegetables may protect against serious diseases, including cardiovascular disease and certain cancers, if consumed regularly [11,12]. The major flavonols contributing to antioxidant activity found in lettuce are quercetin and kaempferol derivatives [13], while isorhamnetin is less common. Quercetin has been extensively studied *in vitro*

and is known to be a potent free-radical scavenger and anti-oxidant [14,15]. Recently, the closely related compound, kaempferol, has also been shown to possess antioxidant activity in its own right, but lower than quercetin [16]. Quercetin and kaempferol are also known to act synergistically in the inhibition of cell proliferation in human gut cancer lines [17].

When grown under high N (200 $kg \cdot ha^{-1}$) other quality indices (dry matter, sugar and vitamin C content) declined in Crisphead lettuce [18]. Furthermore, Butterhead, Romaine and Oak Leaf lettuce quality, as perceived by a sensory panel, was maximized by as little as 80 $kg \cdot ha^{-1}$, significantly less than the normal recommended N application rate for field-grown lettuce in Italy [19].

Minimizing N availability in lettuce, while maintaining yield and quality, is a subject of much recent study [7, 20], but little is known of the effect of minimal N on antioxidant and mineral contents in lettuce. Nitrogen deficiency has resulted in increased flavonol accumulation in Arabidopsis [21], broccoli [22] and tomato leaves [23], but had no effect in onion bulbs [24]. Limited N also resulted in higher total plant phenolic content in basil leaves [25] while, conversely, increased N via foliar urea application resulted in an increase in free radical scavenging activity in lettuce [26].

It is estimated that up to two-thirds of the world's population might be at risk of deficiency in one or more essential mineral nutrients [27], and the concentration of mineral elements in edible plant tissues is therefore of fundamental importance to human nutrition [28]. A recent area of research has been termed "ionomics" [29], which focuses on the quantification and characterization of the mineral elements of plant tissues [28,30,31]. The ionome is influenced significantly by developmental, environmental, and agronomical factors [27,32] which are fundamental not only to mineral nutrition of the plant, but also for increasing the concentrations of mineral elements in edible tissues for human consumption [27]. Fortification of horticultural produce and leafy vegetables in particular, could be a successful strategy for improving human diets [33], resulting in an increase in the value and quality of the produce itself [7].

This study investigates the effect of N concentrations between 40 and 2400 $mg \cdot L^{-1}$ on yield, nitrate accumulation, mineral leaf content, and antioxidant capacity in Oak Leaf lettuce cv. Shiraz grown under hydroponic conditions in Australia.

2. MATERIALS AND METHODS

2.1. Experimental Design

A greenhouse experiment was conducted in November-December 2008 at the Department of Primary Industries (DPI) Knoxfield, Victoria, Australia. Thirty *Lactuca sativa* L. var. "Shiraz" plants were grown hydroponically in 800 mL square plastic pots with perlite as growing medium. Plants were germinated on site and transplanted into pots as plugs after three weeks. Greenhouse temperature was maintained between 18˚C (night) and 24˚C (day) [34] by an evaporative cooling system. Irrigation was delivered thrice per day by an automatic system with two drippers (300 $ml \cdot day^{-1} \cdot plant^{-1}$) per pot. Saucers underneath the pots collected irrigation water and maintained medium (perlite) moisture. An especially prepared commercial lettuce hydroponic fertilizer (Hysol Twin, Duralite, Melbourne, Australia) in which all traces of N were removed from the manufacturer, was applied as base fertilizer to all pots at the industry standard rate of 1.0 to 1.2 $g \cdot L^{-1}$. Six N levels were applied as calcium nitrate ($Ca:NO_3$—19:15.5) derived N ($CaNO_3$-N) [35]. Both base fertilizers and N levels (40, 75, 150, 400, 1200, 2400 $mg \cdot L^{-1}$ of actual $CaNO_3$-N) were applied manually on alternate days throughout the experiment for a total of 13 applications of 30 ml each. Plants received a total of 15.6, 29.3, 58.5, 156, 468, and 936 mg of N per plant during the experiment, equivalent to 1.7, 3.2, 6.4, 17.2, 51.5, and 103.0 kg/ha of N. Water collected in the saucers was checked with an EC meter (NZ Hydroponics LTD, Tauranga, NZ) to avoid excessive build up of salts [36]. To avoid influencing elements absorption, EC was maintained at similar levels in all treatments by periodically flushing excess salts from the high N concentrations with de-ionized water.

2.2. Measurements

At the completion of the 30 day trial, plants were weighed for FW and immediately frozen at –20˚C and freeze-dried. Fresh and dry weight, total N and C were measured in roots and leaves from all plants. Additionally, mineral (Al, B, Ca, Cu, Fe, Mg, Mn, K, P, S, Na, Zn) and NO_3 content in leaves from all $CaNO_3$-N levels was also measured. Leaves from the 40 $mg \cdot L^{-1}$ treatment were excluded due to lack of material.

2.3. Leaf Analysis

Mineral Leaf Content: Approximately 0.5 g (DW) of freeze-dried sample from each plant was weighed into a test tube and 5mL of a 1:4 mixture of perchloric and nitric acids was added. Test tubes were left overnight at 20˚C to "cold digest". The following day the mixture was heated to 80˚C on an aluminium heating block for 30 minutes, then the temperature was increased to 150˚C for 1.5 hours, and then raised again to 185˚C for approximately 1 to 2 hours or until white perchloric acid fumes were observed. The resulting digest was made up

to 25 mL with distilled de-ionized water and the solution measured by ICP-OES VISTA (VARIAN Inc., Palo Alto, USA) against external calibrates following the suggested methodology [37].

Total N and C were measured with a LECO CNS2000 (LECO Corporation, St. Joseph, USA) following the suggested methodology [38].

Nitrate Content: NO_3 content in leaves was assessed by weighing approximately 0.2 g into a plastic tube filled with 100 mL of distilled de-ionized water. Tubes were shaken for 20 minutes and filtered through a 0.45 um filter disk. The collected solution was analyzed following the APHA method 4500-NO_3-F [39].

Total plant phenolics: Water soluble TPP, as an assessment of antioxidant activity, were measured following the Folin-Cioccalteau (FC) method [40].

Oxygen radical absorbance capacity: antioxidant capacity, as measurement of peroxyl radical scavenging activity [41], was measured following the microplate fluorescence reading method [42] and carried out on a Varioskan Flash (Thermoscientific Corp., Melbourne, Australia).

Ferric reducing antioxidant power: the antioxidant capacity potential, as measurement of Fe(III) reducing activity [41] was performed as previously described by Benzie and Strain [43] and was also carried out on a Varioskan Flash (Thermoscientific Corp., Melbourne, Australia).

Leaf chlorophyll content was measured by SPAD (Konica-Minolta SPAD-502 Chlorophyll meter, Braeside, Australia) on two fully expanded leaves before each $CaNO_3$-N fertilization to monitor chlorophyll content.

2.4. Statistical Method

The experiment was a complete randomized block design with five replications. Data were analyzed by Anova ($p < 0.005$) with Genstat 12.0 (VSN International Ltd, Hemel Hempstead, UK). Regression curves were performed with SigmaPlot 10 (Systat Software Inc, Chicago, USA). Plants fertilized with 40, 75, and 150 mg·L^{-1} $CaNO_3$-N were harvested at 45 days after transplant due to lack of growth at 30 days. All data were converted to a per day basis for uniform comparison and then either reported as such or compared at 30 days.

3. RESULTS

3.1. Yield

Lettuce leaf yield, expressed in g FW per day, was significantly affected by N application rate (**Figure 1**), with higher N applications resulting in increased yield. **Figure 1** represents a typical rise to maximum growth

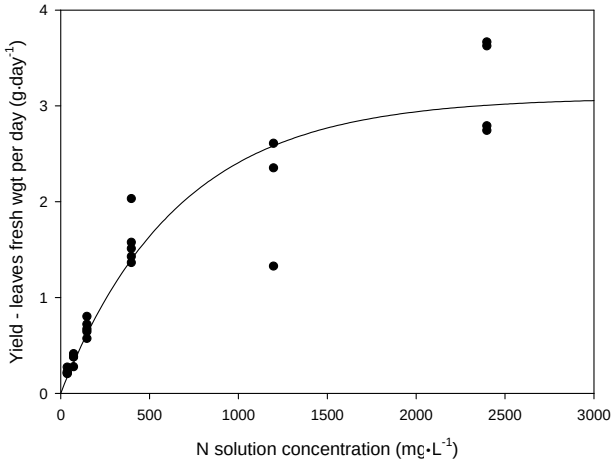

Figure 1. Lettuce yield (g·day^{-1} FW) as affected by N supplied at 40, 75, 150, 400, 1200 or 2400 mg·L^{-1}. Fitted curve is a Rise to max, equation = $3.0887(1-e^{(0.0015X)})$ $R^2 = 0.90$, $p < 0.0001$. Each data point represents a single plant.

curve for lettuce ($R^2 = 0.90$; $p < 0.0001$) with significant increases in FW as N increased from 40 to approximately 1200 mg·L^{-1}. Fresh weight continued to increase between 1200 and 2400 mg·L^{-1} applied N, but at a slower rate. Similarly, leaf N uptake, as expressed by N accumulation within lettuce leaves, also increased with greater N availability (**Figure 2(a)**) and increased more rapidly from 40 to 400 mg·L^{-1} applied N than from 400 to 2400 mg·L^{-1}. N uptake slowed markedly between 1200 and 2400 mg·L^{-1} applied N, showing a logarithmic growth curve overall ($R^2 = 0.84$, $p < 0.0001$) (**Figure 2(a)**).

Leaf NUtE, calculated as the amount of leaf FW per g of N accumulated, showed a rise to maximum growth curve ($R^2 = 0.65$, $p < 0.0001$) (**Figure 2(b)**). NUtE grew rapidly from 40 to 75 mg·L^{-1} applied N, leveling at 150 mg·L^{-1}, and with no subsequent effect of N concentrations between 400 and 2400 mg·L^{-1} (**Figure 2(b)**). This pattern was also reflected in the logarithmic decrease in the carbon to nitrogen ratio (C:N) curve ($R^2 = 0.92$, $p < 0.0001$) (**Figure 3**). C:N is inversely proportional to N leaf accumulation, decreasing with the increase of N concentration in the fertilizer solution. Total leaf nitrate increased exponentially ($R^2 = 0.84$; $p < 0.0001$) with increased N supply (**Figure 4**), particularly at application rates of 400 mg·L^{-1} N or higher, reaching a maximum at 2400 mg·L^{-1} applied N.

3.2. Phenolic Content, Antioxidant Capacity and Chlorophyll

Total plant phenolics and antioxidant capacity, as measured by both FRAP and ORAC, were significantly affected by applied N rate (**Table 1**), with the highest levels of all three indices recorded between 75 and 400 mg·L^{-1} applied N. Highest TPP was recorded at 75 and

400 mg·L^{-1}, which both had significantly higher TPP than the other N rates (**Table 1**). It is noteworthy that TPP and FRAP in leaves supplied with 150 mg·L^{-1} N were significantly lower than leaves supplied either 75 or 400 mg·L^{-1}, but the reason for this result is unknown (**Table 1**). FRAP peaked at 400 mg·L^{-1}, which was significantly higher than 40, 150 and 2400 mg·L^{-1} applied N (**Table 1**). There was no significant difference in TPP and FRAP values for leaves supplied with lowest (40 mg·L^{-1}) or highest (2400 mg·L^{-1}) N. Antioxidant capacity as measured by ORAC was also significantly lower in leaves supplied with 1200 or 2400 mg·L^{-1} N, compared with lower N application rates (**Table 1**). There were no statistical differences between chlorophyll levels measured by SPAD. Levels oscillated between 15 (40 mg·L^{-1} N) and 20 (2400 mg·L^{-1} N) SPAD units (data not shown), indicating that N application rate did not potentially affect photosynthesis.

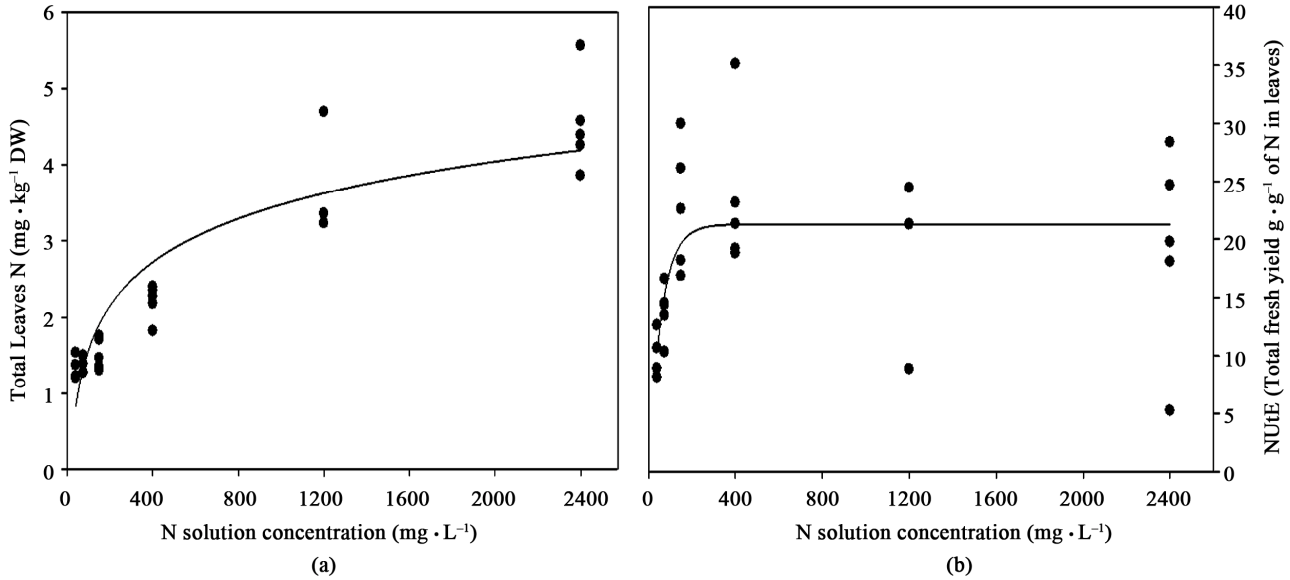

Figure 2. (a) Total N content (mg·kg^{-1} DW) and (b) Nitrogen Utilization Efficiency (NUtE) in lettuce leaves as affected by N supplied at 40, 75, 150, 400, 1200 or 2400 mg·L^{-1}. Fitted curve equations: A = –2.1973 + 0.8207lnx; R^2 = 0.84; p < 0.0001; B = Exponential rise to max = 22.5277(1−e$^{(0.0152X)}$); R^2 = 0.65; p < 0.0001. NUtE was calculated by the ratio between total fresh yield and total nitrogen accumulated in leaves. Each data point represents a single plant.

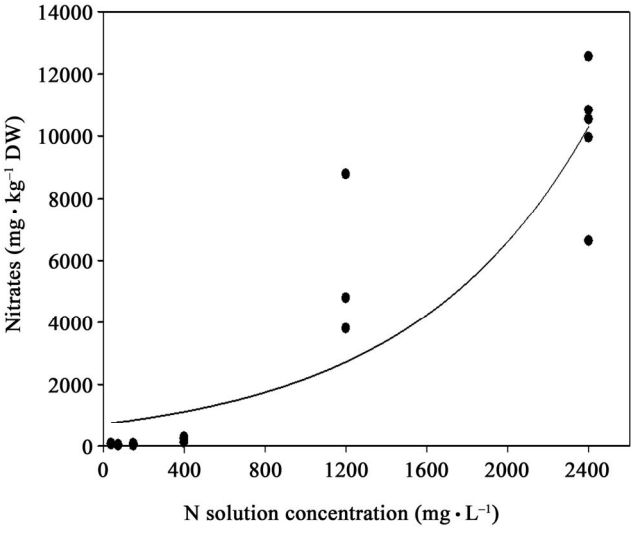

Figure 3. Total nitrate content (mg·kg^{-1} DW) in lettuce leaves as affected by N supplied at 40, 75, 150, 400, 1200 or 2400 mg·L^{-1}. Fitted exponential curve equation = 719.9728e$^{0.0011X}$ R^2 = 0.84; p < 0.0001. Each data point represents a single plant.

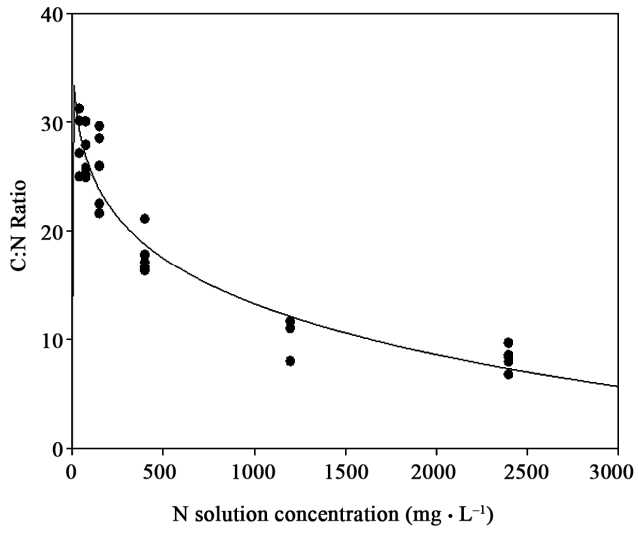

Figure 4. Carbon:Nitrogen ratio in lettuce leaves as affected by N supplied at 40, 75, 150, 400, 1200 or 2400 mg·L^{-1}. Fitted logarithmic curve equation = 35.5525 – 0.0427lnx – 0.4604lnx^2; R^2 = 0.92; p < 0.0001. Each data point represents a single plant.

Table 1. Total Plant Phenolics (TPP) and antioxidant capacity, as measured by FRAP (µmol Fe^{2+} g^{-1} FW) or ORAC (µmol Trolox equivalents 100 g^{-1} DW) in lettuce leaves supplied with N at 40, 75, 150, 400, 1200 or 2400 mg·L^{-1}. Values represent averages of five plants.

N solution concentration (mg·L^{-1})	TPP (mg GAE g^{-1} DW)	FRAP (µmol Fe^{2+} g^{-1} DW)	ORAC (µmol Trolox equiv 100 g^{-1} DW)
40	26.24 b*	259.7 cd	3369 a
75	32.19 a	335.5 ab	3631 a
150	21.07 b	186.7 d	3283 a
400	32.18 a	396.2 a	3384 a
1200	26.43 ab	328.8 abc	2647 b
2400	24.91 b	293.8 bc	2635 b
lsd	5.68	74.8	508

* = Values with different letters in the same column are significantly different at $p < 0.05$.

Table 2. Lettuce leaf macronutrient composition in dry weight (DW) as affected by N supplied at 75, 150, 400, 1200 or 2400 mg·L^{-1}. Values represent averages of five plants.

N solution concentration (mg·L^{-1})	Ca	Mg	P	K	Na	S
	g 100 g^{-1} DW					
75	0.33 c*	0.40 a	0.59	5.8 a	0.10	0.21
150	0.31 c	0.36 a	0.63	5.1 b	0.09	0.24
400	0.34 c	0.24 c	0.58	5.6 ab	0.07	0.21
1200	0.52 b	0.26 bc	0.57	6.0 a	0.08	0.24
2400	0.77 a	0.30 b	0.54	5.7 ab	0.09	0.23
lsd	0.07	0.05	0.09	0.63	0.02	0.03

* = Values with different letters in the same column are significantly different at $p < 0.05$.

Table 3. Lettuce leaf micronutrient composition (DW) as affected by N supplied at 75, 150, 400, 1200 or 2400 mg·L^{-1}. Values represent averages of five plants.

N solution concentration (mg·L^{-1})	Al	B	Cu	Fe	Mn	Zn
	mg·kg^{-1} DW					
75	20.3 b*	75.3 a	11.0 a	67.2 b	471 a	125 a
150	27.1 b	76.2 a	10.1 ab	69.1 b	378 b	121 a
400	28.8 ab	47.1 b	9.9 ab	83.6 ab	175 c	57 b
1200	55.7 a	45.4 bc	9.7 ab	108.4 a	121 d	59 b
2400	8.2 b	37.1 c	8.6 b	69.4 b	106 d	42 b
lsd	26.45	7.91	1.75	26.20	32.71	18.83

* = Values with different letters in the same column are significantly different at $p < 0.05$.

3.3. Leaf Mineral Content

Tables 2 and **3** show lettuce leaf mineral composition as affected by N solution concentration. Out of the 12 elements measured by ICP only P, Na and S were not significantly affected by N application rates. Ca accumulated in leaves with increased N rate. The highest level of Ca accumulation was reached with 2400 mg·L^{-1} of applied N, followed by 1200 mg·L^{-1} and with no significant difference between 400, 150 and 75 mg·L^{-1} (**Table 2**). B, Cu, Mg, Mn, and Zn all declined significantly at the increase of N concentrations. K did not show a clear trend related to N applications. Metals Fe and Al content did peak at 1200 mg·L^{-1} N, which was significantly different from 75, 150 and 2400 mg·L^{-1} N, while 400 was similar to all other treatments (**Table 3**).

4. DISCUSSION

Increased fertilizer utilization efficiency can be achieved through either improved fertilizer management practices and/or by cultivating crops and cultivars that genetically acquire or use elements more efficiently [44,45]. In the present study, we have illustrated that N application rates

can be reduced significantly without a significant reduction in quality as expressed by antioxidant capacity and mineral content, while yield was marginally reduced. Increasing N concentration in hydroponic fertilizer solutions is known to stimulate yield, with our data following the classic growth N response curve [46] (**Figure 1**). However there were no statistical differences in yield between N rates of 400 mg·L^{-1} and the commercial standard rate (**Figure 1**). These results imply that reducing N rates in hydroponic lettuce cultivation below the industry standard rate of 1000 - 1200 mg·L^{-1} should not reduce yield significantly, potentially allowing a more environmentally sustainable production. This finding was also corroborated by the NUtE data (**Figure 2(b)**) which reached a plateau between 150 and 400 mg·L^{-1} N, while N leaf accumulation continued above 1200 mg·L^{-1} N (**Figure 2(a)**). This indicates that the maximal usage NUtE (from a yield perspective) in lettuce leaves of this variety was between 150 and 400 mg·L^{-1}. Reduction of N fertilization rates to increase NUtE is one method suggested to improve environmentally sustainable agriculture [6,28]. Recent research in this area is also attempting to select for higher N-efficient plants with regards to uptake, in combination with improved nitrogen efficiency [44,47]. Our results indicate that NUtE in lettuce leaves could be improved with N application rates significantly lower than commonly applied in Australia. In fact chlorophyll levels did not change in the present study (data not shown), indicating that the lowest applied N rate (40 mg·L^{-1}) was sufficient to maintain adequate photosynthesis.

Accumulation of N in leaves, especially in the form of nitrates, is an important negative quality trait for leafy vegetables [9]. Low N availability generally results in minimal nitrate accumulation in lettuce [20], while high N (>150 kg·ha^{-1}) significantly increased nitrate accumulation [9]. Our results (**Figure 3**) confirm these observations, with negligible nitrate recorded in lettuce leaves supplied with ≤400 mg·L^{-1} N (**Figure 4**). The European Community has set upper nitrate limits in lettuce leaves of between 3500 and 4500 mg·kg^{-1} FW [48]. Our data shows 2400 mg·L^{-1} N resulted in a mean nitrate content of 10,000 mg·kg^{-1} DW, which translates in the order of 700 mg·kg^{-1} FW, therefore quite within the limits of the acceptable daily intake [49].

High nitrogen availability is known to inhibit phenolic production and subsequently antioxidant capacity, in a range of leafy vegetables [7]. In lettuce, fertilization treatments that resulted in relatively high soil N content caused a reduction in phenolic content, specifically coumaric acid and antioxidant capacity [50]. Highest antioxidant capacity (measured by FRAP and DPPH) and TPP in Chinese cabbage were recorded in plants grown in soil under 0 mg/kg applied N, with higher N application rates resulting in lower TPP and antioxidant capacity [51]. Similarly highest phenolic content in basil grown hydroponically was found after minimal N application (0.1 mM) [25], with lowest antioxidant capacity in leaves grown under the highest N availability. Our data partially agrees with these studies in that highest TPP and antioxidant capacity were recorded in plants supplied with low N, specifically ≤400 mg·L^{-1} (**Table 1**). However, plants supplied with the lowest N (40 mg·L^{-1}) were not significantly different with respect to TPP or FRAP from those supplied with 2400 mg·L^{-1} N. TPP and FRAP peaked at 75 or 400 mg·L^{-1} applied N (**Table 1**), while antioxidant capacity, as measured by ORAC, was maximal at 40 to 400 mg·L^{-1} N (**Table 1**). Thus, there appears to be a lower effective limit for N supply of approximately 75 mg·L^{-1} N, below which TPP and antioxidant capacity declined. Furthermore, there was no significant difference in TPP, FRAP and ORAC values in leaves supplied with 400 mg·L^{-1} N and 75 mg·L^{-1} (**Table 1**), while yield was significantly greater in plants supplied with 400 mg·L^{-1} N (**Figure 1**). Maximizing antioxidant capacity in lettuce cv. "Shiraz" can be therefore be achieved with 400 mg·L^{-1} N

A variety of stresses are known to result in an increase in the phenolic synthesis pathway in plants [52]. Wounding, drought, nutrient deficiency and high light intensity all resulted in increased phenolic compounds in lettuce leaves due to upregulation of the phenylpropanoid pathway [53]. Accumulation of the flavonols quercetin and kaempferol, which would result in both increased TPP and antioxidant capacity, is known to be induced by N depletion through enhanced synthesis [54]. Low N availability specifically stimulated phenolic synthesis in tomato leaves [21]; and fruit [55]. Nitrogen-depleted *Matricaria* rosettes showed a significant increase in PAL activity and a concomitant increase in phenolic content [56]; an increase in PAL and TPP was also seen in yarrow leaves grown with low N (0.1 mM) for 4 months [57].

The phenolic compounds quercetin, kaempferol, isorhamnetin and anthocyanins are all commonly found in leafy vegetables and contribute significantly to antioxidant capacity [58]. Our results indicate that a similar increase in phenolic compounds (TPP in **Table 1**) was seen under low N nutrition, but the largest increase was not at the lowest N rate (40 mg·L^{-1}) but between 75 and 400 mg·L^{-1}. It is not known why TPP and FRAP values were significantly lower at 150 mg·L^{-1} compared with either 75 or 400 mg·L^{-1}. Despite this anomaly, it is possible to grow lettuce plants at 400 mg·L^{-1} N and achieve high antioxidant capacity with a yield not particularly depressed when compared with plants grown under 1200

$mg \cdot L^{-1}$—currently the industry standard N application rate for lettuce plants in Australia.

It is not certain, that the increase in phenolic compounds was solely responsible for increased antioxidant capacity. In lettuce leaves, the major contributor to antioxidant capacity, as measured by DPPH was ascorbic acid (57% - 63%) [50]. Furthermore, a good correlation between TPP and antioxidant capacity was found in only 5 out of 10 lettuce varieties studied by Heimler *et al.* [59] most likely due to interference by ascorbic acid. Ascorbic acid in spinach is also known to increase with low N availability [60], therefore we can speculate that the high FRAP and ORAC levels at applied N of 400 $mg \cdot L^{-1}$ or less in the present study (**Table 1**) could be due at least in part to higher ascorbic acid levels, as well as the observed significant increase in TPP at 400 and 75 $mg \cdot L^{-1}$ applied N. Ascorbic acid levels were not recorded in our study. Conversely, Chiesa *et al.* [9] found high N (150 $kg \cdot ha^{-1}$) increased ascorbic acid content in lettuce, indicating this area requires further investigation.

The Carbon/Nutrient Balance Theory (CNB) [61] predicts that production of carbon-based secondary metabolites (e.g. phenolics) decreases with increased N as C is needed primarily for plant growth and development. This hypothesis supposes that a decrease in N supply causes a restriction in photosynthesis and plant growth and reduces the demand for amino acids for protein synthesis. In lettuce plants, however, it seems that the theory is only partially accurate, as highest TPP and antioxidant capacity was found not at the lowest N availability, as predicted by the model, but at more "intermediate" rates, vis. 75 or 400 $mg \cdot L^{-1}$.

In our experiment, it appears that the ionome [27,28, 32] of lettuce cv. "Shiraz" was affected by varying N rate. Nitrogen was applied as calcium-nitrate, thereby increasing the N concentration in the solution at the same time as Ca. This resulted in an increased accumulation of Ca in leaves (**Table 2**) agreeing with Neeser *et al*. [33] who reported increasing Ca in the fertilizer solution was an effective method to biofortify lettuce for this element. Our results indicate a further influence of increased N and Ca availability on other essential minerals. **Tables 2** and **3** show that B, Mg, Mn, and Zn all decreased significantly with increasing N and Ca applications. This is possibly due to 2^+ cation absorption-competition interactions between Ca and Mg, Mn, and Zn or the 1^- anion competition between borate and nitrate [46]. More research is necessary to better identify specific ion interactions and correlations with plant physiological pathways.

In lettuce several factors can influence leaf mineral content. High nutrient solution electrical conductivity (EC) has been reported to diminish Fe and Zn and increase Mn concentrations, while N, P, and K were not affected [62]. Gent [36], however, reported only a small increase in the accumulation of nitrates with increased EC. Nitrogen form in the fertilizer solution had no effect on nitrate accumulation, with organic forms stimulating K absorption [35]. In our trials EC was maintained as similar as possible in all treatments by flushing excess salts from the high N concentrations with deionised water periodically, therefore reducing the influence on the elements absorption to immediately after fertilization.

5. CONCLUSIONS

In conclusion, leaf total plant phenolic content (TPP) and antioxidant capacity (measured by FRAP) were both maximal at 75 or 400 $mg \cdot L^{-1}$ applied N, while highest ORAC values were found in leaves supplied with low N (40 to 400 $mg \cdot L^{-1}$). Applied N also significantly affected leaf mineral content: Ca rose with increased N, while B, Mg, Mn, and Zn significantly decreased. These results indicate that $CaNO_3$-N applications of 1200 $mg \cdot L^{-1}$ or higher can result in reduced antioxidant capacity and mineral content in lettuce leaves. It is possible to reduce N concentration in the hydroponic solution for lettuce production in Australia without having a detrimental effect on yield. This in addition will increase lettuce quality reducing nitrate content, increasing essential elements such as Mn, Zn, Mg, and Ca and increasing antioxidant capacity.

6. ACKNOWLEDGEMENTS

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