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Research article

Effect of organic compost and inorganic nitrogen fertigation on spinach growth, phytochemical accumulation and antioxidant activity

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ABSTRACT

The aim of this study was to evaluate the effect of the addition of organic compost in combination with the inorganic nitrogen fertigation on growth, phytochemical accumulation, and antioxidant activity of spinach (*Spinacia oleracea* L. cv. Manatee). Soil blocked spinach seedlings (six seedlings per block), three blocks per pot (316 plants m^{-2}) were transplanted after 18 days after emergence into to 12 L pots. The treatments were: unfertilized soil, organic compost, organic compost +75 kg of N ha^{-1}, applied as ammonium sulfate; and organic compost +75 kg N ha^{-1}, applied as ammonium nitrate. The addition of organic compost to unfertilized soil increased the fresh yield. The application of inorganic N from the two sources in relation to organic compost treatment increased spinach fresh yield from 2.3 to 4.81 kg m^{-2} and shoot dry weight from 0.60 to 1,31 g $plant^{-1}$. Levels of carotenoids also increased with inorganic N addition, producing higher values in plants grown with organic compost + ammonium nitrate (31.14 mg/100 g fresh weight). However, the addition of N led to a decrease in leaf-blade total phenols from 75 to 56 mg gallic acid equivalents/100mg fresh weight. The addition of inorganic N led to a dramatic decrease in leaf-blade ferric reducing antioxidant activity. This effect was higher with ammonium sulfate application. The application of organic compost and inorganic nitrogen had no influence on the petiole's phytochemical accumulation and antioxidant activity.

1. Introduction

The addition of organic composts to soil enhances soil fertility and contributes towards achieving sustainable plant production. It reduces the volume of organic residues in landfill and reduces greenhouse gas emissions while it increases carbon sequestration (Favoino and Hogg, 2008; Marmo, 2008, Smith et al., 2001) thus promoting the circular economy. The addition of organic composts to soil also enhances soil structure, water holding capacity, soil exchange capacity and biological activity (Bastida et al., 2008; Hargreaves et al., 2008). European Union Directives stipulate that all member states must divert increasing quantities of untreated organic waste material from landfill sites in order to reduce the production of greenhouse gases caused by the anaerobic breakdown of organic matter (Smith et al., 2001). Organic composts increase nutrients content in soil and their availability for plants (Boldrin et al., 2009) and may contribute towards reducing inorganic nitrogen fertilization, which has negative environmental impacts (Graham et al., 2017). However, organic composts typically have high salinity, neutral to alkaline in pH and present a low nitrogen content. Although there are currently organic composts enriched with organic nitrogen, mainly used in organic farming, the nitrogen is released slowly and irregularly. The use of soil organic compost combined with inorganic nitrogen could provide a strategy for overcoming the irregular release of nitrogen by compost, and producing high yields. Fertigation allows for the use of nitrogen during the growth cycle, which can lead to a reduction in the variability of nitrogen availability in soil and an increase in nitrogen use efficiency (Machado and Serralheiro, 2017). It also contributes to reducing N_2O emissions, what currently is extremely important (Gruda et al., 2019a, b). In general, the best way to reduce N_2O emissions caused by inorganic N fertilization is by increasing N use efficiency (Millar et al., 2014). Combining organic, and inorganic nutrient sources is increasingly favored as a means of improving nutrient use efficiency by matching soil

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nutrient availability with crop demand (Graham et al., 2017; Wu and Ma, 2015).

Spinach cultivation requires considerable amounts of nitrogen so that the crop grows well, and leaves acquire the dark green color sought by consumers. Production usually involves the use of inorganic nitrogen fertilizers in quantities ≥150 kg N ha^{-1}, being one of the most critical elements in plant growth and quality (Rouphael et al., 2018). Nitrogen plays a significant role in biosynthesis of secondary plant metabolites such as phenols, ascorbate, and glutathione as well as antioxidant enzymes such as glutathione reductase (Argyropoulou et al., 2015; Ibrahim et al., 2012; Domínguez-Valdivia et al., 2008).

However, little is known about the influence of addition of organic composts in combination with inorganic nitrogen fertigation on the yield and quality of spinach. Therefore, the aim of this study was to evaluate the effect of organic compost and inorganic nitrogen fertigation (ammonium nitrate and ammonium sulfate) in quantities lower than usual, on soil electrical conductivity (EC_e) and pH, growth, and phytochemical accumulation in spinach.

2. Materials and methods

2.1. Growth conditions

A pot experiment was conducted in a greenhouse located at the "Herdade Experimental da Mitra" (38°31′52″N; 8º01′05″W), University of Évora, Portugal. The greenhouse was covered with polycarbonate and had no supplemental lighting. Air temperatures inside of the greenhouse ranged from 4 to 27 °C, and solar radiation ranged from 34 to 248 W m^{-2}·d^{-1} (Figure 1).

The experiment was carried out in plastic pots. Each 12-L plastic pots (21-cm high x 26.5-cm diameter) filled with 14 kg of luvisol sandy loam soil obtained from the Mitra Research Farm in Évora, Portugal.

The soil presented 1.5 % organic matter content, a bulk density of 1.3 g·cm^{-1}, a pH of 7.2 (1 soil: 2.5 water), an EC_e of 0.25 dS m^{-1}, 35 mg NO_3 kg^{-1}, 158 mg K·kg^{-1}, 162 mg P·kg^{-1}, 7.57 meq Ca^{2+}/100g, and 1.67 meq Mg^{2+}/100g.

Four treatments were carried out: unfertilized soil (S), organic compost (OC); organic compost +75 kg of N ha^{-1} applied as ammonium sulfate (21% NH_4–N. and 60 % SO_3) (OC + AS); and organic compost +75 kg N ha^{-1} applied as ammonium nitrate (16.9% NO_3–N and 16.7% NH_4–N) (OC + AN). Treatments were arranged in a randomized complete block design with six replicate pots per treatment.

Ten days prior to transplanting, 124 g of a commercial organic compost of the manure of the poultry, sheep and horses, from controlled aerobic fermentation (nutrofertil, Nutrofertil Nutrição e Fertilizantes, Lda, Portugal) in pellets, certificate for organic farming was added at each pot and mixed with upper 10 cm of the soil (≈18 g of organic compost per kg/soil).

The physicochemical characteristics of the compost were the following: organic matter (52%), moisture (12%), total carbon (30.5), pH (6.5), electrical conductivity (6.4 dS m^{-1}), total nitrogen (6.4%), organic nitrogen (6.4%), P_2O_5 (2.5%), K_2O (2.4%), CaO (8.4 %), MgO (0.3%), b (0.0020%) and relation C:N (5). The EC [1 soil: 5 water (by volume)] of the saturated extract of the soil of the upper 10 cm of the pot mixed with organic compost was 0.66 dS m^{-1}± 0.04.

Soil-blocked spinach *(Spinacia oleracea* L. cv. Manatee) seedlings (six seedlings per block), three blocks per pot (316 plants m^{-2}) were transplanted (on 23 -1- 2018) after 18 days following emergence into to twelve L pots.

Irrigation was based on soil water measurements. Volumetric soil water content was measured daily (9:00–10:00) using a soil-moisture probe (SM105T delta devices UK). Plants were watered by hand when average volumetric water content reading, in the first 0.1 m the soil, in the center of the pots, in three pots treated with organic compost and N fertilizer was ≤20 % (Volumetric soil water content at the field capacity and at the wilting point were approximately 35 and 12%, respectively).

Nitrogen was applied by fertigation, once a week, in five equal fertilizer applications, starting at transplanting and finishing in the week before harvest. The irrigation water presented a low EC_w (0.1 dS m^{-1}). Starting from transplantation, air temperature at the plant canopy level was monitored hourly with sensors connected to a data logger (Datalogger HI141 - Hanna Instruments) (Figure 1). The weeds were regularly removed from the pots manually.

2.2. Measurements

The plants were harvested at 38 days after transplantation. The shoots of the plants were cut off 1 cm above the substrate surface. Four representative plants (shoots) from each pot were washed, oven-dried at 70 °C for 2–3 days, weighed, ground. Plant growth in unfertilized soil was not enough to collect plants (shoots) for analysis.

After harvesting of plants, three soil cores were collected at random from each pot using a 3-cm diameter x 0.1 m deep soil probe in order to analyze soil, NO_3–N, pH and electrical conductivity (EC_e).

Soil nitrate was measured using an ion-specific electrode and meter (Crison Instruments, Barcelona, Spain), using the method outlined by Prazeres (2005). Soil pH was measured in 1:2.5 soil-water suspensions using a potentiometer (pH Micro 2000 Crison). EC_e was measured in 1:5 soil-water aqueous extracts using a conductivity meter (LF 330 WTW, Weilhein, Germany).

12 recently fully expanded leaves per pot were collected for nutrient analysis at harvest. The leaves were oven-dried at 80 °C for 3 d, ground so that they would pass through a 40-mesh sieve and analyzed for N, P, K, Ca, Mg, S, Fe, B, Cu, Mn, and Zn.

In order to determine photosynthetic pigments content, 1.000 g of leaf blade from three treatments and five replicates was macerated in a

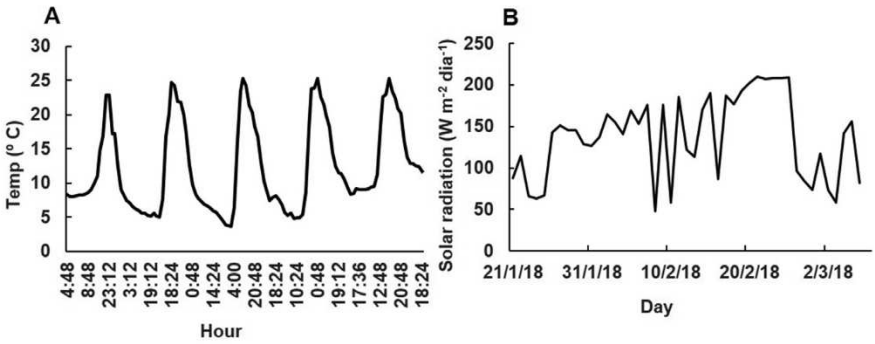

Figure 1. Diurnal changes in air temperature inside the greenhouse at the plant canopy level (A). The pattern illustrated is for temperature measured from 26 February to 2 March. Solar radiation outside greenhouse during the experimental period, (B) data obtained from a weather station located at Mitra research station (ICT, 2019).

mortar and homogenized in 8 mL of methanol:water solution (90:10 (v/v), PP-extract) for 1 min and then centrifuged at 4 °C at 6,440 *g* for 5 min. The methanol extracts were stored in aliquots at -20 °C for later use. Chlorophyll a and b and carotene were determined in PP-extract in accordance with Lichtenthaler and Buschmann (1987). Calculation of chlorophyll and carotene concentrations was performed using appropriate equations (Lichtenthaler and Buschmann, 1987):

Chl *a* (μg/mL) = 16.82 A665.2–9.28 A652.4;

Chl *b* (μg/mL) = 36.92 A652.4–16.54 A665.2;

Cc (μg/mL) = (1000 A470–1.91Chl a – 95.15Chl b)/225,

where A = Absorbance, Chl *a* = Chlorophyll *a*, Chl *b* = Chlorophyll *b*, Cc = Carotene.

In order to determine total phenol compounds (TPC), ascorbic acid (AsA) content and antioxidant activity (FRAP, DPPH), 1,000 g of leaf blade or petiole sample from each pot was macerated in a mortar and homogenized in 8 mL of methanol:water solution (80:20,v/v) for 1 min and then centrifuged for 5 min, at 4 °C at 6,440 *g*. The methanol extracts were stored in aliquots at -20 °C for later use in determining the content of total phenol compounds, ascorbic acid, and antioxidant activity.

TPC content was determined using Folin–Ciocalteau's phenol reagent as described earlier (Bouayed et al., 2011), reading the absorbance at 760 nm. TPC content expressed as milligram of gallic acid equivalents (GAE) per 100 g of fresh weight (FW) was calculated using a calibration curve (GAE, n = 6 concentrations from 0 to 50 mg/L).

AsA content was determined in accordance with the method of Cai and Tang (1999), incubating the sample (extracts or standard suitably diluted) in a mixture containing 5 % TCA in ethanol, 0.4 % H_3PO_4, 0.5 % β-phenanthroline in ethanol and 0.03 % $FeCl_3$ in ethanol, warmed at 30 °C, for 90 min. Absorbance of Fe(II)–β-phenanthroline complex formed was read at 534 nm. AsA concentration was calculated using a calibration curve (ascorbic acid, n = 6 concentrations from 0 to 30 mg/L).

Scavenging antioxidant activity (DPPH) was determined by measuring the ability of plant extracts to capture the stable organic radical $DPPH^{\bullet}$ (2, 2-diphenyl-1-picryl-hydrazyl, violet) and its conversion into a stable product, DPPH-H (diphenyl-picryl hydrazine, yellow). Aliquots of an extemporaneous methanol solution of 0.03 g/L $DPPH^{\bullet}$, kept in the dark, were added to a known volume of sample or standard solution. The reduction of $DPPH^{\bullet}$ to DPPH-H was followed by reading the absorbance at 515 nm, at 25 °C, for 180 s. Antioxidant activity reported as milligram of GAE per 100 g of FW was calculated using a calibration curve (GAE, n = 8 concentrations from 0 to 200 mg.L-1) (Brand-Williams et al., 1995).

Ferric reducing antioxidant activity (FRAP) was determined in accordance with the method of Bouayed et al. (2011). In sum, the FRAP reagent was prepared freshly by mixing 300 mM acetate buffer pH 3.6, 10 mM TPTZ solution in 40 mM HCl and 20 mM iron (III) chloride solution (10:1:1, v/v/v) and warmed to 37 °C before use. Then 0.050 mL of the sample (suitably diluted extracts or standard) was mixed with 0.950 mL of FRAP reagent. Absorbance change was read at 593 nm at 37 °C, for 180 s. The reducing power of iron present in the samples reported as milligram of Trolox equivalents per 100 g of FW was calculated using a calibration curve (Trolox solution, n = 8 concentrations from 0 to 1120 mgL^{-1}). For all determinations, a Genesys10S UV/Vis spectrophotometer was used.

Samples of 1 g of spinach leaf blade or petiole were macerated in liquid N_2 (Air Liquide, Portugal) and homogenized in 5 mL of 0.12 mM phosphate buffer pH 7.2 for the extract preparation used in the determination of glutathione (GSH) and glutathione reductase (GR) enzyme activity. The supernatant obtained by means of the centrifugation of this extract for 15 min at 15,000 *g* at 4 °C, was collected and stored in aliquots at -20 °C for further use in determining GSH content, GR activity and protein content.

Glutathione was assayed in accordance with the method of Hissin and Hilf (1976), based on the reaction of o-phthalaldehyde (OPT) as a fluorescent reagent with GSH at pH 8.0. The fluorescence of products was determined at 420 nm with the excitation at 350 nm, at 25 °C, using GSH as a standard in a single-beam Shimadzu RF-5001PC fluorimeter.

GR activity was determined in accordance with Goldberg and Spooner (1987) in a reaction mixture containing 15 mM EDTA, 635 mM GSSG and a suitable volume of leaf blade or petiole extract (0.5–0.2 $mg.mL^{-1}$ protein) in 0.12 mM phosphate buffer pH 7.2. The reaction was started with the addition of 9.6 mM NADPH. The oxidation of NADPH was determined by reading the absorbance at 340 nm for 360 s, at 37 °C in a double-beam Hitachi-U2001 spectrophotometer with temperature control. GR activity was calculated based on the slope of the reaction curves, using a molar absorptivity coefficient value of 6.22 $mM^{-1}.cm^{-1}$ for NADPH. GR activity was expressed in terms of nmol min^{-1}/mg protein. Protein content of the extract was determined in accordance with the Lowry et al. (1951), using a calibration curve (bovine serum albumin, BSA; n = 6 concentrations from 0 to 200 $\mu g.mL^{-1}$).

2.3. Data analysis

Data were processed by means of the variance analysis using SPSS Statistics 25 software (Chicago, Illinois, USA). Means were separated at the 5% level using Duncan's new multiple range test.

3. Results and discussion

3.1. Soil

pH, soil electrical conductivity (EC_e) and nitrate-N of the soil after plants harvest were significantly affected by the treatments (Table 1). Soil pH decreased with the N fertilizer application and was the lower when ammonium sulfate was used than when ammonium nitrate was used (Table 1). Hydronium ion (H_3O^+) increase in the root medium was due to the ammonium plant uptake and/or its nitrification. The values of the pH in OC + AS and OC + AN were lower than the suitable range to spinach (6.5–7.0) which could result in some plant nutrient uptake disturbance, which was not verified in the present study, which may be due the presence of humic substances of the organic compost. Organic composts contain humic substances (Humic acids, fulvic acids and humins) (Guo et al., 2019) which neutralize the pH effect. Humic substances improve micronutrient transport and availability (Tan, 2014). In the present study, soil nitrate concentration and EC_e increased with N fertilizer use and were higher when ammonium sulfate was applied than when ammonium nitrate was used. The values of nitrate and the decrease of the soil pH indicates the occurrence of a high rate of nitrification since the ammonium nitrification contributes to soil acidification. The conditions for the nitrification were favorable; soil temperature was relatively high during the day (Figure 1) and there was a high level of soil moisture, an increase in microbial activity due to the application of compost and a low C:N of the compost, all of which contributes to rapid mineralization of N and reduces its immobilization.

The EC_e of the soil plus organic compost addition decreased since plantation (EC_e = 0.66 d*S* m^{-1}) to harvest (0.46 d*S* m^{-1}) (Table 1) which happened due to the plant nutrient uptake, and it may also have occurred ion leaching to the soil below 10 cm.

Salinity levels increased with the use of N fertilizers, but the average values of the EC_e were much lower than soil salinity threshold (electrical conductivity that is expected to cause the initial significant reduction in the maximum expected yield) (EC_t) of the spinach (2 d*S* m^{-1}) (Machado and Serralheiro, 2017).

3.2. Plant growth and yield

In unfertilized soil, spinach plants did not grow well or died. The leaves of the plants were very small and chlorotic presenting a yellowish

Table 1. Effect of organic compost and inorganic nitrogen fertigation on pH, electrical conductivity (EC_e) and nitrate–N concentration in soil collected at harvest (38 DAT) in the depth of 0–10 cm.

Treatments	pH	Electrical conductivity ($dS \cdot m^{-1}$)	Nitrate-N (mg kg^{-1})
Soil	7.00 a	0.23 c	4.98 d
OC	7.05 a	0.46 b	16.9 c
OC + AS	5.48 c	0.87 a	74.3 a
OC + AN	5.96 b	0.51 b	46.4 b
Significance	***	***	***

Means followed by different letters within a column are significantly different. *, **, *** Significant at $p < 0.05$, 0.01 and 0.001 levels, respectively. NS = not significant. (OC- organic compost, OC + AS - organic compost +75 kg of N ha^{-1} applied as ammonium sulfate, OC + AN- organic compost +75 kg N ha^{-1} applied as ammonium nitrate).

Table 2. Effect of organic compost and inorganic nitrogen fertigation on shoot dry weight and fresh yield of spinach.[a]

Treatments	Shoot dry weight		Fresh yield
	(g/plant)	(%)	(Kg m^{-2})
Soil[1]	-	-	0.054c
OC	0.60 b	8.62 a	2.38 b
OC + AS	1.31 a	7.71 b	4.93 a
OC + AN	1.16 a	7.75 b	4.71 a
Significance	***	***	***

Means followed by different letters within a column are significantly different. *, **, *** Significant at $p < 0.05$, 0.01 and 0.001 levels, respectively. NS = not significant. (OC- organic compost, OC + AS - organic compost +75 kg of N ha^{-1} applied as ammonium sulfate, OC + AN- organic compost +75 kg N ha^{-1} applied as ammonium nitrate).

[1] Plant growth in soil treatment was not enough to collect leaf samples for analysis.

color, probably due to nitrogen deficiency. The addition of organic compost to soil significantly increased the plant growth (Table 2). Weinfurtner (2001) also reported a positive effect of organic compost on yield.

However, plants grown only with organic compost had lesser shoot dry weight and yield (fresh weight) than those grown with compost and nitrogen fertilization (Table 2). The application of inorganic nitrogen (75 kg N ha^{-1}) by fertigation as ammonium nitrate or ammonium sulfate led to a significant increase in fresh yield by 97.8 and 107 % respectively, indicating that the nitrogen availability in soil is essential for achieving high yields in spinach.

The high increase in fresh yield due to the addition of inorganic N weekly could be related to the increase of availability of nitrogen throughout the growing season.

In *Cichorium spinosum* the availability of nitrogen in growth media throughout the growing season increased the yield (Petropoulos et al., 2018). The high fresh yield (≈48.2 t ha^{-1}) in treatments with compost + N fertigation application, shown that spinach yield is very dependent on the nitrogen fertilizer rate. It also indicates that the application of compost + N fertigation allowed obtaining a high spinach fresh yield. Therefore, can be a strategy to reduce greenhouse emissions due to the use of the compost and at the reduction of the inorganic nitrogen application, as pointed out by Gruda (2019).

The addition of nitrogen led to a decrease in shoot dry weight (%) ($p < 0.001$) (Table 2). In spinach, Elia et al. (1998) also reported that the increase in nitrogen fertilizer resulted in a decrease in dry matter percentage.

N fertilizer source did not affect the total fresh weight or shoot dry weight. However, some researchers reported a decrease in yield when ammonium sulfate fertilizer is used (Stagnari et al., 2007; Krężel and Kołota, 2014), which was not the case in the present study probably due to the favorable conditions for nitrification and the humic acids added by organic composts as previously mentioned. According to Marschner (2012) the negative effects of ammonium nitrogen are associated with changes in pH in the root medium and toxicity of free $NH4^{+}$.

3.3. Leaf nutrients

Spinach leaf nutrients concentration in plants grown in OC, OC + AN, and OC + AS, except for nitrogen, calcium, magnesium, and manganese did not differ (Table 3). The application of nitrogen led to an increase in leaf nutrient concentration of N, Ca^{2+} and Mg^{2+}. Leaf N concentration in plants of the OC was ≈21 % lower than in the plants fertilized with nitrogen fertilizer (Table 3). The increase in leaf calcium and magnesium concentration may be related to an increase in the availability of nitrate in root medium, since is know that when the nitrate is taken by plants roots, calcium and magnesium uptake is enhanced (Jones, 2016). The application of ammonium sulfate led to an increase in leaf concentration of Mn^{2+} and Zn^{2+}, this may be due to the higher soil acidification (Table 3) which may have contributed to greater solubility of Mn^{2+}, and Zn^{2+}. Ammonium nitrification and ammonium uptake by roots acidify their root zones (Marschner, 2012). In sorghum, Szydelko-Rabska et al. (2014) also reported an increase in the leaf Mn^{2+} and Zn^{2+} when fertilization included ammonium in the form of ammonium sulfate. In addition, differences in leaf nutrients concentrations, except for P were within the sufficiency ranges proposed by Mills and Jones (1996) and Campbell (2000) (Table 3). In the case of leaf P, concentrations were above the sufficiency range (Table 3). This was probably due to the high availability of P (162 mg $P \cdot kg^{-1}$) in soil and the addition of organic compost to the soil. The addition of organic compost to soil can increase water-extractable soil P by direct addition, dissolution, displacing sorbed, or reducing sorption capacity for P (Adler and Sikora, 2003). Organic matter is a source of phosphorus and it may reduce P sorption (Gorgin et al., 2011). It may increase P uptake by plants since it forms complexes with organic phosphate and increases the volume of soil that plant roots explore. The humic acids increased lateral root and induce the production of smaller, but ramified secondary roots (Ouni et al., 2014). The fulvic acids present in organic composts improve the P availability in acid soils (Yang et al., 2013), which can explain that despite the nitrogen application lower the pH mainly in treatment OC + AS the leaf P concentration was not affected. The addition of municipal solid waste

Table 3. Effect of organic compost and inorganic nitrogen fertigation on the concentration of nutrients in recent fully expanded leaves sampled at harvest spinach.

Treatments	Leaf macronutrients (mg·g^{-1})					Leaf micronutrients (μg·g^{-1})				
	N	P	K	Ca	Mg	Fe	B	Cu	Mn	Zn
OC	5.98 b[1]	0.83	7.49	1.02 b	0.78 b	134.5	34.5	16.0	123 b	86.5 b
OC + AS	7.82 a	0.83	7.47	1.37 a	0.92 a	143.5	46.5	12.2	218 a	101.5 a
OC + AN	8.00 a	0.95	7.88	1.34 a	0.99 a	134.5	38.0	10.9	145 b	94.0 b
Significance	***	NS	NS	**	**	NS	NS	NS	***	*
Recommended range										
Mills and Jones (1996)	4.00–6.00	0.30–0.60	5.00–8.00	0.70–1.20	0.60–1.00	60–200	25–60	5–25	30–250	25–100
Campbell (2000) [2]	4.00–6.00	0.30–0.50	3.00–8.00	1.00–1.50	0.40–1.00	50–200	25–60	5–15	25–200	20–75

[1] Means followed by different letters within a column are significantly different. *, **, *** Significant at $p < 0.05$, 0.01 and 0.001 levels, respectively. NS = not significant.

[2] Values to spinach grown in greenhouses. (OC- organic compost, OC + AS - organic compost +75 kg of N ha^{-1} applied as ammonium sulfate, OC + AN- organic compost +75 kg N ha^{-1} applied as ammonium nitrate).

compost to soil increased P uptake (Hargreaves et al., 2008). In spinach, Maftoun et al. (2005) also reported that the addition of municipal waste compost to soil led to an increase in P uptake.

Inorganic N addition increased spinach shoot dry weight and total nutrient extraction, but the leaf nutrient concentrations of the nutrients were within the sufficiency ranges.

3.4. Photosynthetic pigments

Total chlorophyll ranged from an average of 54.35–66.74 mg/100 g FW (Table 4), these values were lower than those reported by Borowski and Michalek (2010) (100–243 mg/100 g FW) while they were within the range of those reported by Xu and Mou (2016).

The level of photosynthetic pigments [(total chlorophyll (Chl *a*+*b*), chlorophyll *a* and carotenoids)] in fresh weight and chlorophyll *a*:*b* ratio were significantly affected by the treatments (Table 4). Total chlorophyll, Chl *a* and Chl *a*:*b* ratio were lower in OC + AN than with the other treatments. Chl *b* was not affected by the treatments. Therefore, there was not a response by Chl *a* and Chl *b* content with an increased in nitrogen in leaf tissue (Table 3). On the contrary, nitrogen applied as ammonium nitrate led to a decreased in leaf Chl *a* content in fresh weight. This may be related to the ratio of NH_4^+/NO_3^- in the soil. In Swiss chard, chlorophyll *a* and *b* in fresh weight produced a significant positive quadratic response to N form ratio (Barickman and Kopsell, 2016).

Carotenoids content increased with N fertilizer application and was higher in plants grown with OC + AN (31.14 mg/100 g FW) than those OC + AS (24.84 mg/100g FW). The increase in carotenoids content as nitrogen supply increased also was reported in spinach by Xu and Mou (2016) and Gutiérrez-Rodríguez et al. (2013) and in lettuces by Qadir et al. (2017). Carotenoid content in fruit and vegetables tends to increase with higher nitrogen fertilization (Mozafar, 1993). The chlorophyll *a*:*b* ratio ranged from an average of 2.94–4.29 (Table 4). These values were within the range of those reported by Xu and Mou (2016) (3.2–4.1).

3.5. Phytochemical accumulation

Despite vitamin C being influenced by nitrogen fertilization (Lee and Kader, 2000; Salomez and Hofman, 2009), spinach leaf-blade and petiole AsA were not affected by the treatments (Figure 2A). This may be related to the fact that the response of AsA to nitrogen may be not linear and/or vary with the cultivar. A moderate amount of N was required to maintain ascorbic acid synthesis (Mozafar, 1993). In *Cosmos caudatus* for maximum ascorbic acid content, regardless of fertilizer sources, plants did not require large amounts of N fertilizer (Hassan et al., 2012). However, in spinach, grown in fall the leaf AsA content was affected by the interaction between nitrogen source (ammonium sulfate, calcium nitrate and ammonium nitrate) and cultivar (Krężel and Kołota, 2014).

Leaf and petiole AsA content ranges from 9.03 to 9.62 and from 4.8 to 5.3 mg of AsA/100 g Fw, respectively (Figure 2A). These values were lower than those reported by other authors (Rouphael et al., 2018; Dewhirst et al., 2017). This may be related to low light intensity inside of the greenhouse, due to the season (winter), since the outdoor light photosynthetic DLI (daily light integral-cumulative photosynthetic light during 24-h period) is low. The polycarbonate on the greenhouse also reduces solar radiation transmission and on the days before harvest solar radiation was low (Figure 1B).

Light intensity improves AsA content in leaf tissues (Proietti et al., 2004; Lee and Kader, 2000). Lower light intensities in the greenhouses lower the syntheses of glucose, therefore lowering the syntheses of ascorbic acid (Lester, 2006).

Leaf-blade TPC decreased significantly with the application of nitrogen in the form of both ammonium sulfate and ammonium nitrate (Figure 2B). In plants grown without nitrogen fertilizers, total phenols reached 74.6 mg GAE/100 FW, while in OC + AS and OC + AN, the values were 56.1 and 57.8 mg GAE/100 FW, respectively. The addition of nitrogen decreased the TPC by ≈ 33 %. Therefore, this indicates that the biosynthesis of total phenols is stimulated by low nitrogen content in the soil, as reported by Scheible et al. (2004). In spinach Xu and Mou (2016)

Table 4. Effect of combined use of organic compost and inorganic nitrogen fertigation on photosynthetic pigments.

Treatments	Total Chl	Chl *a*	Chl *b*	Chl *a*/Chl *b*	Cc
	mg/100g FW				mg/100gFW
OC	66.74 a	54.14 a	12.59	4.29 a	20.54 c
OC + AS	65.13 a	51.86 a	13.26	3.94 a	24.84 b
OC + AN	54.35 b	40.60 b	13.75	2.94 b	31.14 a
Significance	*	*	NS	**	***

Means followed by different letters within a column are significantly different. *, **, *** Significant at $p < 0.05$, 0.01 and 0.001 levels, respectively. NS = not significant (OC- organic compost, OC + AS - organic compost +75 kg of N ha^{-1} applied as ammonium sulfate, OC + AN- organic compost +75 kg N ha^{-1} applied as ammonium nitrate).

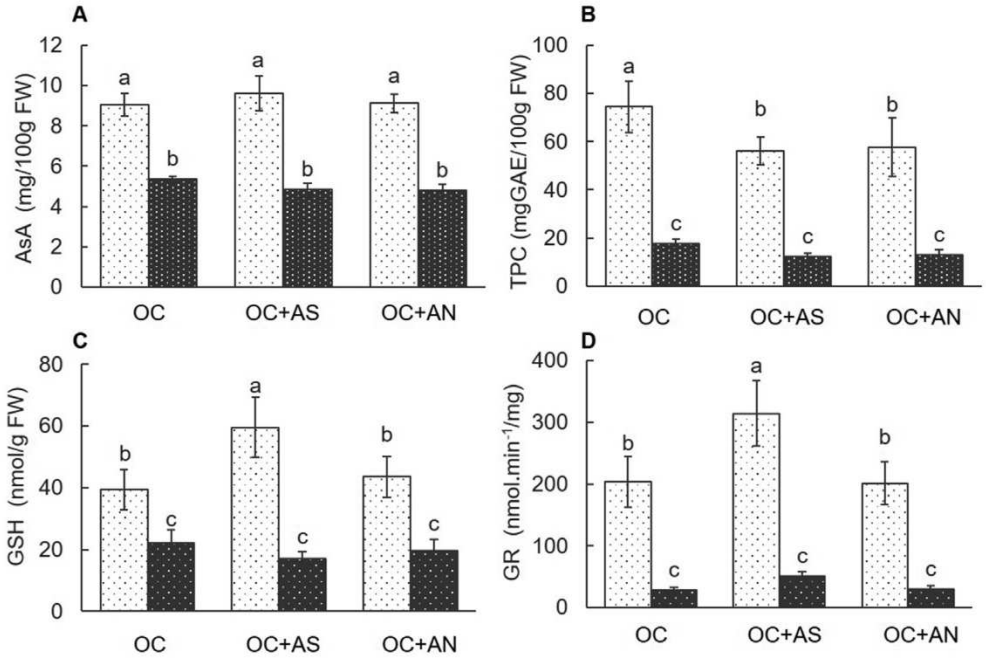

Figure 2. Effect of organic compost and inorganic nitrogen fertigation (OC - organic compost, OC + AS - organic compost +75 kg of N ha^{-1} applied as ammonium sulfate, OC + AN - organic compost +75 kg N ha^{-1} applied as ammonium nitrate) on AsA (A), TPC (B) and GSH (C) content, GR enzyme activity (D) in the leaf-blade (white bars) and petiole (black bars) of spinach. Means with different letters are significantly different at $p < 0.05$. Each bar represents the mean of five replicates, and the error bars represent ±1 SE.

also reported that nitrogen deficiency led to an increase in total phenolic contents. A TPC decreased as nitrogen level increased in root medium have been also reported in *Labisia pumila* Benth (Ibrahim et al., 2011), in sweet basil (Argyropoulou et al., 2015), basil (Nguyen and Niemeyer, 2008), mustard (Li et al., 2008), pac choi (Zhao et al., 2009) and in lettuce (Qadir et al., 2017; Galieni et al., 2015). TPC was not significantly affected by the source of nitrogen. This agrees with Domínguez-Valdivia et al. (2008), who reported that ammonium nutrition in spinach does not alter the redox status of ascorbate, glutathione, or phenolic contents.

Leaf-blade glutathione (GSH) and glutathione reductase (GR) activity levels were higher in OC + AS treatment than with OC and OC + AN treatments (Figure 2C, D). This is not in agreement with Ibrahim et al. (2012), who reported in *L. pumila* a decrease in GSH content as N applied increased. However, the higher value of GSH content was found in the plants grown with OC + AS which can be related to the presence of sulfate in the fertilizer, since GSH as an important source of non-protein thiols, is influenced by Sulphur supply (Noctor et al., 2012). This may indicate a higher level of antioxidant activity in leaf-blades of the treatment OC + AS than the other treatments since glutathione is a powerful antioxidant involved in detoxification of peroxide and lipid hydroperoxides generated by endogenous metabolism of the cells or induced by abiotic stress (Agarwal et al., 2018).

Leaf-blade GSH ranged from an average of 39.5–59.5 nmol/g FW. These values are lower than reported by Agarwal et al. (2018) (230–350 nmol/g FW) or Luwe et al. (1993) (50–320 nmol/g FW) and similar those reported by Liu et al. (2016) in *Arabidopsis thaliana* (WT) growing under conditions of limited sulfate.

Leaf-blade GR activity ranged from an average 230.3–365.2 $nmol.min^{-1}/g$ FW (Figure 2D). These values are similar to those reported by Agarwal et al. (2018) for spinach. Leaf-blade GR activity was higher than in the petiole, which may explain the low levels of GSH found in the petiole. Leaf-blade GR levels were positively correlated with glutathione levels ($r = 0.840$, $p < 0.01$), probably due to a greater ability of the spinach leaf-blade to regenerating glutathione via GR activity (Bartoli et al., 2017).

Leaf-blade antioxidant activity DPPH and FRAP were significantly affected by treatments (Figure 3A, B). Ammonium sulfate led to a reduction in leaf-blade antioxidant activity measured as DPPH as compared with plants grown with only compost and compost + ammonium nitrate (Figure 3A).

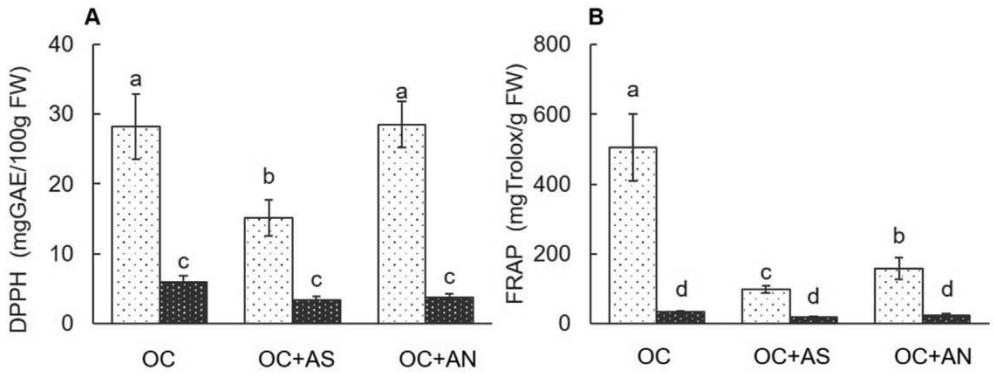

Figure 3. Effect of organic compost and inorganic nitrogen fertigation (OC - organic compost, OC + AS - organic compost +75 kg of N ha^{-1} applied as ammonium sulfate, OC + AN - organic compost +75 kg N ha^{-1} applied as ammonium nitrate) on DPPH (A) and FRAP (B) in the leaf-blade (white bars) and petiole (black bars) of spinach. Means with different letters are significantly different at $p < 0.05$. Each bar represents the mean of five replicates, and the error bars represent ±1 SE.

Petiole DPPH was not affected by the treatments and it was lower than the leaf-blades (Figure 3A). Leaf-blade antioxidant activity (DPPH) of spinach ranged from 15.2 to 28.5 mg GAE/100 g FW. These values are comparable to those reported by Machado et al. (2018) (12–23 mg GAE/100 g FW). Petiole DPPH was not affected by the treatments and it was lower than the leaf-blades.

Leaf-blade capacity for producing a reduction in ferric iron (FRAP) decreased significantly by application of N fertilizers ($p \leq 0.001$) (Figure 3B). Leaf-blade FRAP was higher with OC (505.2 mg Trolox/g FW) than with OC + AN (190 mg Trolox/g FW) and OC + AS (98.7 mg Trolox/g FW). The application of ammonium sulfate and ammonium nitrate led to a dramatic decrease in leaf-blade FRAP, indicating that nitrogen nutrition has great influence on leaf-blade capacity for producing a reduction in ferric ions.

The decrease of the FRAP as nitrogen application increases was also reported in *Labisia pumila* Blume (Ibrahim et al., 2012) and mustard (Li et al., 2008). Leaf-blade FRAP of the plants grown in OC + AN and OC + AS were higher than that reported by Sreeramulu et al. (2013) (13.81 mg Trolox/g FW) and in those grown in OC were slightly higher than those reported by Machado et al. (2018) in spinach grown in soilless crop system (substrate) (400–500 mg Trolox/g FW).

Petiole FRAP was not affected by the treatments and, as in the case of the other phytochemicals previously presented, it was lower than the FRAP antioxidant activity in spinach leaf-blade. This indicates that nitrogen source and availability in root medium have no influence on antioxidant activity measured by DPPH and FRAP.

The results of this study indicate that the combination of organic compost with inorganic nitrogen fertigation could be suitable to reduce inorganic nitrogen application in spinach production. However, further research is needed to optimize the synchrony between nitrogen availability and crop demand. Thus, our next step is to assess the influence of the rate, frequency, and timing of application of inorganic nitrogen fertigation in combination with organic compost on soil nitrogen availability, nitrogen uptake by plants, and phytochemical accumulation.

4. Conclusions

The application of inorganic nitrogen by fertigation and organic compost may contribute to reduced inorganic nitrogen application without compromising spinach fresh yield. Spinach fresh yield increased with inorganic nitrogen application but was not affected by nitrogen source.

Nitrogen fertilizer application led to an increase in carotenoids content but did not influence leaf-blade and petiole ascorbate content. However, the addition of nitrogen with the two sources led to a decrease in the leaf-blade total phenols (≈33 %) and the capacity to reducing ferric iron (FRAP). Leaf-blade FRAP decreased drastically in plants grown with organic compost plus ammonium nitrate or ammonium sulfate. Antioxidant activity (DPPH and FRAP) was higher in plants grown with ammonium nitrate than those grown with ammonium sulfate. The addition of nitrogen in both sources had no influence in the petiole's phytochemicals accumulation and antioxidant activity.

Declarations

Author contribution statement

R.M.A. Machado: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

I. Alves-Pereira, R.M.A. Ferreira: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

D. Lourenço: Performed the experiments; Analyzed and interpreted the data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

Adler, P.R., Sikora, L.J., 2003. Changes in soil phosphorus availability with poultry compost age. Commun. Soil Sci. Plan. 34 (1-2), 81–95.

Agarwal, A., Gupta, S.D., Barman, M., Mitra, A., 2018. Photosynthetic apparatus plays a central role in photosensitive physiological acclimations affecting spinach (*Spinacia oleracea* L.) growth in response to blue and red photon flux ratios. Environ. Exp. Bot. 156, 170–182.

Argyropoulou, K., Salahas, G., Hela, D., Papasavvas, A., 2015. Impact of nitrogen deficiency on biomass production, morphological and biochemical characteristics of sweet basil (*Ocimum basilicum* L.) plants, cultivated aeroponically. Agri. Food 3, 32–42.

Barickman, T.C., Kopsell, D.A., 2016. Nitrogen form and ratio impact Swiss chard (*Beta vulgaris* subsp. cicla) shoot tissue carotenoid and chlorophyll concentrations. Sci. Hortic 204, 99–105.

Bartoli, C.G., Buet, A., Grozeff, G.G., Galatro, A., 2017. A Ascorbate-glutathione cycle and abiotic stress tolerance in plants. In: Hossain, et al. (Eds.), Ascorbic Acid in Plant Growth, Development and Stress Tolerance. Springer International Publishing AG, part of Springer Nature, Cham.

Bastida, F., Kandeler, E., Moreno, J., Ros, M., García, C., Hernández, T., 2008. Application of fresh and composted organic wastes modifies structure, size and activity of soil microbial community under semiarid climate. Appl. Soil Ecol. 40, 318.

Boldrin, A., Andersen, J.K., Moller, J., Christensen, T.H., Favoino, E., 2009. Composting and compost utilization: accounting of greenhouse gases and global warming contributions. Waste Manag. Res. 27, 800–812.

Borowski, E., Michalek, S., 2010. The effect of foliar nutrition of spinach (*Spinacia oleracea* L.) with magnesium salts and urea on gas exange, leaf yield and quality. Acta Agrobot. 63, 77–85.

Bouayed, J., Hoffmann, L., Bohn, T., 2011. Total phenolics, flavonoids, anthocyanins and antioxidant activity following simulated gastro-intestinal digestion and dialysis of apple varieties: bioaccessibility and potential uptake. Food Chem. 128, 14–21.

Brand-Williams, W., Cuvelier, M.E., Berse, C., 1995. Use of a free radical method to evaluate antioxidant activity. LWT - Food Sci.Technol. 28, 25–30.

Cai, W.M., Tang, Z.C., 1999. Plant tolerance physiology. In: Tang, Z.C. (Ed.), Experimental Guide for Modern Plant Physiology, first ed. Science Press, Beijing, pp. 315–316.

Campbell, C., 2000. Reference Sufficiency Ranges for Plant Analysis in the Southern Region of the United States, Southern Cooperative Series Bulletin #394. www.ncagr.gov/agronomi/saaesd/scsb394.pdf.

Dewhirst, R.A., Clarkson, G.J.J., Rothwell, S.D., Fry, S.C., 2017. Novel insights into ascorbate retention and degradation during the washing and post-harvest storage of spinach and other salad leaves. Food Chem. 233, 237–246.

Domínguez-Valdivia, M.D., Aparicio-Tejo, P.M., Lamsfus, C., Cruz, C., 2008. Nitrogen nutrition and antioxidant metabolism in ammonium tolerant and sensitive. Physiol. Plantarum 132, 359–369.

Elia, A., Santamaria, P., Serio, F., 1998. Nitrogen nutrition yield and quality of spinach. J. Sci. Food Agric. 76, 341–346.

Favoino, E., Hogg, D., 2008. The potential role of compost in reducing greenhouse gases. Waste Manag. Res. 26, 61–69.

Galieni, A., Di Mattia, C., De Gregorio, M., Speca, S., Mastrocola, D., Pisante, M., Stagnari, F., 2015. Effects of nutrient deficiency and abiotic environmental stresses on yield, phenolic compounds and antiradical activity in lettuce (*Latuca sativa* L.). Sci. Hortic. 187, 93–101.

Goldberg, D., Spooner, R., 1987. Gluathione reductase. In: Bergmeyer, H.-U. (Ed.), Methods of Enzymatic Analysis, third ed. Academic Press, pp. 258–265.

Gorgin, N., Fekri, M., Sadegh, L., 2011. Impact of organic-matter application on phosphorus-desorption kinetics in two agricultural soils in southeastern Iran. Commun. Soil Sci. Plan. 42 (5), 514–527.

Graham, R.F., Wortman, S.E., Pittelkow, C.M., 2017. Comparison of organic and integrated nutrient management strategies for reducing soil N_2O emissions. Sustainability 9, 510.

Gruda, N.S., 2019. Increasing sustainability of growing media constituents and stand-alone substrates in soilless culture systems. Agronomy 9 (6), 298.

Gruda, N., Bisbis, M., Tanny, J., 2019a. Influence of climate change on protected cultivation: impacts and sustainable adaptation strategies-A review. J. Clean. Prod. 225, 481–495.

Gruda, N., Bisbis, M., Tanny, J., 2019b. Impacts of protected vegetable cultivation on climate change and adaptation strategies for cleaner production–A review. J. Clean. Prod. 225, 324–339.

Guo, X.X., Liu, H.T., Wu, S.B., 2019. Humic substances developed during organic waste composting: formation mechanisms, structural properties, and agronomic functions. Sci. Total Environ. 662, 501–510.

Gutiérrez-Rodríguez, E., Lieth, H.J., Jernstedt, J.A., Labavitch, J.M., Suslow, T.V., Cantwell, M.I., 2013. Texture, composition and anatomy of spinach leaves in relation to nitrogen fertilization. J. Sci. Food Agric. 93, 227–237.

Hargreaves, J.C., Adl, M.S., Warman, P.R., 2008. A review of the use of composted municipal solid waste in agriculture. Agric. Ecosyst. Environ. 123, 1–14.

Hassan, S.A., Mijin, S., Yusoff, U.K., Ding, P., Wahab, P.E.M., 2012. Nitrate, ascorbic acid, mineral and antioxidant activities of *Cosmos caudatus* in response to organic and mineral-based fertilizer rates. Molecules 17, 7843–7853.

Hissin, A., Hilf, P., 1976. A fluorometric method for determination of oxidized and reduced glutathione in tissues. Anal. Biochem. 74, 214–226.

Ibrahim, M.H., Jaafar, H.Z., Rahmat, A., Rahman, Z.A., 2011. The relationship between phenolics and flavonoids production with total nonstructural carbohydrate and photosynthetic rate in *Labisia pumila* Benth. under high CO_2 and nitrogen fertilization. Molecules 16, 162–174.

Ibrahim, M.H., Jaafar, Z.E., Rahmat, A., Rahman, Z.A., 2012. Involvement of nitrogen on flavonoids, glutathione, anthocyanin, ascorbic acid and antioxidant activities of malaysian medicinal plant *Labisia pumila* Blume (Kacip Fatimah). Int. J. Mol. Sci. 13, 393–408.

ICT, 2019. Atmospheric Sciences Water and Climate. http://www.ict.uevora.pt/g1/index.php/meteo-data/.

Jones Jr., J.B., 2016. Hydroponics: a Practical Guide for the Soilless Grower. CRC press, Boca Raton, 0-8493-3167-6.

Krężel, J., Kołota, E., 2014. Source of nitrogen affects the yield and quality of spinach cultivars grown for autumn harvest. *Acta Agr. Scand.* B-S P 64, 583–589.

Lee, S.K., Kader, A.A., 2000. Preharvest and postharvest factors influencing vitamin C content of horticultural crops. Postharvest Biol. Technol. 20, 207–220.

Lester, G.E., 2006. Environmental regulation of human health nutrients (ascorbic acid, β-carotene, and folic acid) in fruits and vegetables. Hortscience 41, 59–64.

Li, J., Zhu, Z., Gerendás, J., 2008. Effects of nitrogen and sulfur on total phenolics and antioxidant activity in two genotypes of leaf mustard. J. Plant Nutr. 31, 1642–1655.

Lichtenthaler, H.K., Buschmann, C., 1987. Chlorophylls and carotenoids: measurement and characterization by UV-VIS spectroscopy. Curr. Protoc. Food Anal. Chem. 4, 3.1–3.8.

Liu, X., Wu, F.-H., Li, J.-X., Chen, J., Wang, G.-H., Wang, W.-H., Hu, W.-J., Gao, L.-J., Wang, Z.-L., Chen, J.-H., Simon, M., Zheng, H.-L., 2016. Glutathione homeostasis and Cd tolerance in the *Arabidopsis sultr1, 1-sultre1;2* double mutant with limiting sulfate supply. Plant Cell Rep. 35, 397–413.

Lowry, O.H., Roseburg, N.J., Farr, A.L., Randell, R.J., 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193, 265–275.

Luwe, M.W.F., Takahama, U., Heber, U., 1993. Role of ascorbate in detoxifying ozone in the apoplast of spinach (*Spinacia oleracea* L.) leaves. Plant Physiol. 101, 969–976.

Machado, R.M.A., Alves-Pereira, I., Ferreira, R.M.A., 2018. Plant growth, phytochemical accumulation, and antioxidant activity of substrate-grown spinach. Heliyon 4, e00751.

Machado, R.M.A., Serralheiro, R.P., 2017. Soil salinity: effect on vegetable crop growth. Management practices to prevent and mitigate soil salinization. Horticulturae 3, 30.

Maftoun, M., Moshiri, F., Karimian, N., Ronaghi, A.M., 2005. Effects of two organic wastes in combination with phosphorus on growth and chemical composition of spinach and soil properties. J. Plant Nutr. 27, 1635–1651.

Marmo, L., 2008. EU strategies and policies on soil and waste management to offset greenhouse gas emissions. Waste Manag. 28, 685–689.

Marschner, H., 2012. Mineral Nutrition of Higher Plants, second ed. Academic Press, New York.

Millar, N., Doll, J.E., Robertson, G.P., 2014. Management of nitrogen fertilizer to reduce nitrous oxide (N_2O) emissions from field crops. Climate Change and Agriculture Fact Sheet Series. MSU Extension Bulletin E, p. 3152.

Mills, H.A., Jones, J.R., 1996. Plant Analysis Handbook II. A Practical Sampling, Preparation Analysis and Interpretation Guide. Micro Macro international, Inc., Athens, GA, 1878148001.

Mozafar, A., 1993. Nitrogen fertilizers and the amount of vitamins in plants: a review. J. Plant Nutr. 16, 2479–2500.

Nguyen, P.M., Niemeyer, E.D., 2008. Effects of nitrogen fertilization on the phenolic composition and antioxidant properties of basil (*Ocimum basilicum* L.). J. Agric. Food Chem. 56, 8685–8691.

Noctor, G., Mhandi, A., Chaouf, S., Han, Y., Neukermans, J., Marquez-Garcia, B., Queval, G., Foyer, C.H., 2012. Glutathione in plants: an integrated overview. Plant Cell Environ. 5, 454–484.

Ouni, Y., Ghnaya, T., Montemurro, F., Abdelly, C., Lakhdar, A., 2014. The role of humic substances in mitigating the harmful effects of soil salinity and improve plant productivity. Int. J. Plant Prod. 8 (3), 353–374. ISSN: 1735-8043 (Online).

Petropoulos, S., Fernandes, Â., Karkanisc, A., Antoniadisd, V., Barros, L., Ferreira, I.C.F.R., 2018. Nutrient solution composition and growing season affect yield and chemical composition of *Cichorium spinosum* plants. Sci. Hortic. 231, 97–107.

Prazeres, A.O., 2005. Comparação de metodologias laboratoriais para determinação de azoto nítrico e amoniacal em solos e águas. In: Programa e Livro de Resumos do 1º Congresso Nacional e Rega e Drenagem. Beja, PT. 59, p. 60.

Proietti, S., Moscatello, S., Colla, G., Battistelli, Y., 2004. The effect of growing spinach (*Spinacia oleracea* L.) at two light intensities on the amounts of oxalate, ascorbate and nitrate in their leaves. J. Hortic. Sci. Biotechnol. 79, 606–609.

Qadir, O., Siervo, M., Seal, C.J., Brandt, K., 2017. Manipulation of contents of nitrate, phenolic acids, chlorophylls, and carotenoids in lettuce (*Lactuca sativa* L.) via contrasting responses to nitrogen fertilizer when grown in a controlled environment. J. Agric. Food Chem. 65, 10003–10010.

Rouphael, Y., Kyriacou, M.C., Petropoulos, S.A., De Pascale, S., Colla, G., 2018. Improving vegetable quality in controlled environments. Sci. Hortic. 234, 275–289.

Salomez, J., Hofman, G., 2009. Nitrogen nutrition effects on nitrate accumulation of soil grown greenhouse butterhead lettuce. Commun. Soil Sci. Plant Anal. 40, 620–632.

Scheible, W.-R., Morcuende, R., Czechowski, T., Fritz, C., Osuna, D., Palacios-Rojas, N., Schindelasch, D., Thimm, O., Udvardi, M.K., Stitt, M., 2004. Genome-wide reprogramming of primary and secondary metabolism, protein synthesis, cellular growth processes, and the regulatory infrastructure of Arabidopsis in response to nitrogen. Plant Physiol. 136, 2483–2499.

Smith, A., Brown, K., Ogilvie, S., Rushton, K., Bates, J., 2001. Waste management options and climate change: final report to the European Commission. In: Waste Management Options and Climate Change: Final Report to the European Commission. European Commission, Louxembourg, 92-894-1733-1.

Sreeramulu, D., Reddy, C.V.K., Chauhan, A., Balakrishna, N., Raghunath, M., 2013. Natural antioxidant activity of commonly consumed plant foods in India: effect of domestic processing. Oxid. Med. Cell Longev. 369479, 1–12.

Stagnari, F., Di Bitetto, V., Pisante, M., 2007. Effect of N fertilizers and rates on yield, safety and nutrients in processing spinach genotypes. Sci. Hortic. 114, 225–233.

Szydelko-Rabska, E., Kulczycki, G., Sowinski, J., 2014. Impact of different forms of nitrogen fertilizer on the content and uptake of microelements in sorghum. J. Elementol. 19, 567–576.

Tan, K.H., 2014. Humic Matter in Soil and the Environment: Principles and Controversies. CRC Press.

Weinfurtner, K., 2001. Plant nutrition and productivity – is compost a competitive fertiliser? "Applying compost—benefits and needs". In: Seminar Proceedings, Brussels, 2001, pp. 22–23.

Wu, W., Ma, B., 2015. Integrated nutrient management (INM) for sustaining crop productivity and reducing environmental impact: a review. Sci. Total Environ. 415–427.

Xu, C., Mou, B., 2016. Responses of spinach to salinity and nutrient deficiency in growth, physiology, and nutritional value. J. Am. Soc. Hortic. Sci. 141, 12–21.

Yang, S., Zhang, Z., Cong, L., Wang, X., Shi, S., 2013. Effect of fulvic acid on the phosphorus availability in acid soil. J. Soil Sci. Plant Nutr. 13 (3), 526–533.

Zhao, X., Nechols, J.R., Williams, K.A., Wang, W., Carey, E.E., 2009. Comparison of phenolic acids in organically and conventionally grown pac choi (*Brassica rapa* L. chinensis). J. Sci. Food Agric. 89, 940–946.