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Effects of Municipal Solid Waste Compost Supplemented with Inorganic Nitrogen on Physicochemical Soil Characteristics, Plant Growth, Nitrate Content, and Antioxidant Activity in Spinach

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Abstract: In this study, we evaluated the effects of municipal solid waste compost supplemented with inorganic N on the physicochemical properties of soil, plant growth, nitrate concentration, and antioxidant activity in spinach. Experiments were carried out in neutral and acidic soils that were low in organic matter. A fertilized soil was used as a control, while four compost treatments—two compost rates of 35 and 70 t ha^{-1}, supplemented or not with inorganic N (92 kg N ha^{-1} as $Ca(NO_3)_2$)—were applied by fertigation. The addition of compost increased the soil organic matter content and pH in both soils. The compost supplementation with N greatly increased the shoot dry weight and spinach fresh yield by nearly 109%. With the highest compost rate and 43% N applied, the yield increased in both soils, similar to results obtained in fertilized soil (3.8 kg m^{-2}). The combined application of compost and N could replace inorganic P and K fertilization to a significant extent. The compost application at both rates and in both soils considerably decreased shoot Mn concentrations.

Keywords: soil organic matter; acidic soil; pH; nitrogen; nutrient uptake; photosynthetic pigments; antioxidant activity; *Spinacia oleracea*

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1. Introduction

Soil organic matter decline and soil acidity are global problems for crop production. Almost half of the European soils have low organic matter content, principally in southern Europe, France, the United Kingdom, and Germany [1,2]. The decline of soil organic matter can be accentuated by climate change and the increase of intensive farming. Soil acidification affects up to 40% of the world's arable soils [3,4].

In Portugal, most soils have low organic matter content due to climatic conditions, poor agricultural practices, and low soil pH [5]. In these soils, plants grow poorly because of low water availability. In addition, the combination of H_3O^+, Al, and Mn toxicities lead to a lack of essential nutrients [3,6]. Spinach plants grown in these soils without fertilization show reduced growth and leaf chlorosis, probably due to a lack of nutrients, especially nitrogen, resulting in plant death [7,8].

Municipal organic wastes, when collected separately and properly composted, produce high-quality municipal solid waste compost (MSWC) for agriculture, with low heavy metal content and high organic matter content [9–11].

The separate collection of bio-waste and compost is increasing in European Union countries. All EU Member States will be obliged to collect bio-waste separately in the coming years [12]. MSWC can be used to preserve and enhance SOM pools and reduce soil acidity and inorganic nutrient inputs. Soil pH is one the most decisive factors affecting plant nutrition, metal solubility, nutrient movement, and microbial activity. Compost application generally increases soil pH [13]. Increased soil pH is regarded as a major advantage when MSW compost is used [14].

MSWC can reduce soil acidity by increasing hydronium (H_3O^+) concentrations in soil, since mature MSW composts usually have high pH [10], with adsorption of organic anions and the corresponding release of hydroxyl ions [15]. Soil organic matter offers many negatively charged sites to bind H_3O^+ in acidic soil or from which to release H_3O^+ in basic soil, in both cases pushing soil solutions towards neutral [16]. The main constraint to plant growth in soils amended with MSW compost is soil nitrogen availability [7,14,17], because nitrogen is released from MSWC slowly and irregularly. In order to enhance N availability and avoid reductions in crop yield, the addition of compost must be supplemented by inorganic nitrogen.

Therefore, the aim of this study was to evaluate the effects of the rate of MSWC supplemented or not with inorganic nitrogen on the physicochemical properties of the two soils with low organic matter content (a neutral (pH 7.1) and an acidic (pH 5.5)) on plant growth, nitrate concentration, and antioxidant activity in spinach.

2. Materials and Methods

2.1. Growth Conditions

The study was conducted in a greenhouse located at the "Herdade Experimental da Mitra" (38°31′52″ N; 8°01′05″ W), University of Évora, Portugal. The greenhouse was covered with polycarbonate and had no supplemental lighting. Air temperatures inside the greenhouse ranged from 5 to 35 °C (Figure 1) and outside solar radiation ranged from 76.6 to 262.8 $W \cdot m^{-2} \cdot d^{-1}$ [18].

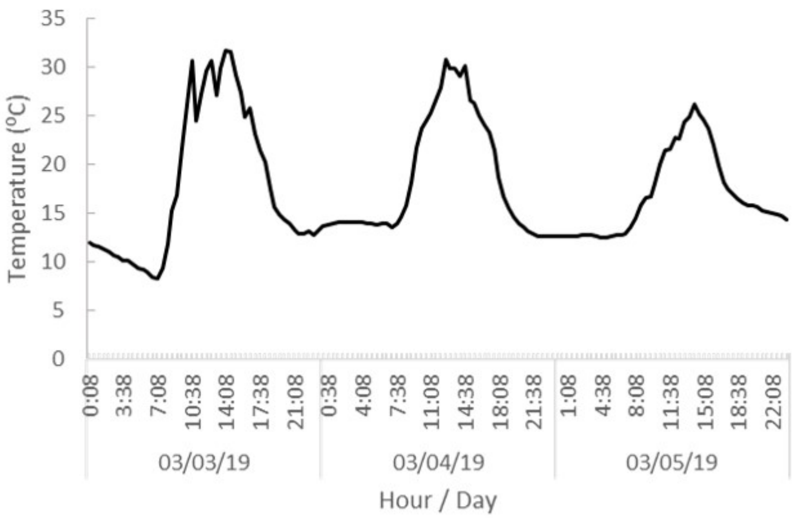

Figure 1. Diurnal changes in air temperature inside the greenhouse at the plant canopy level. The pattern illustrated is for temperatures measured from 3 to 5 March.

The experiment was carried out with two soils with low organic matter (a sand, loamy, neutral soil and a loamy sand soil that was strongly acid) (Table 1), five treatments (fertilized soil (FS) and four MSW compost treatments), two rates of MSW compost (35 and 70 t ha^{-1}), and the same rates of MSWC supplemented by nitrogen applied weekly in fertigation (35 + N, 70 + N). The rate of 30 t MSWC was chosen because it is a common rate of application. The highest rate (75 t MSWC) was calculated so as not to surpass the maximum amount of heavy metals that can be incorporated annually in the soils [19]. For the fertilized soil this was applied by fertigation 1.05 g N/pot (184 kg N ha^{-1}), while for the 35 + N and 70 + N half that amount was applied (0.53 g N/pot; 92 kg N ha^{-1}). Treatments were arranged in a randomized complete block design with six replicate pots per treatment.

In the experiments, we did not include the unfertilized treatment soil, since in previous experiments spinach plants in these unfertilized soils showed reduced growth and leaf chlorosis, probably because of lack of nutrients, especially nitrogen, resulting in plant death [7,8].

The experiment was carried out in plastic pots. Each 12 L plastic pot (21 cm height × 26.5 cm diameter) was filled with ≈14 kg of the soil from the upper layer (0–25 cm) of two different soil types obtained from the Mitra Research Farm in Évora, Portugal. The main characteristics of the soils are presented in Table 1. Ten days prior to transplanting, mature municipal solid waste organic compost (Nutrimais, Lipor, Lda, Portugal) in pellet form was added to each pot and mixed with the upper 10 cm of the soil.

In the fertilized soil (FS), we incorporated in the upward 10 cm of soil 0.17 g N, 0.35 g P_2O_5, 0.52 g K_2O, and 0.035 g MgO.

Table 1. The physicochemical properties of the soils.

	Soil	
	Neutral	**Acidic**
pH	7.19	5.50
Organic matter (%)	1.62	1.10
EC_e (d*S* m^{-1})	0.082	0.03
Bulk density (g cm^{-3})	1.39	1.47
NO_3^- (ppm)	43.6	20.5
P_2O_5 (ppm)	238.0	10.0
K_2O (ppm)	204.0	60.0
Ca (meq 100 g^{-1})	8.34	1.16
Mg (meq 100 g^{-1})	1.20	0.27
Na (meq 100 g^{-1})	0.13	0.70
CEC (meq 100 g^{-1})	9.39	5.70
K (meq 100 g^{-1})	0.49	0.11
Texture	Sand loamy	Loamy sand
Sand (%)	70.3	81.2
Loam (%)	12.3	8.00
Clay (%)	17.4	10.8

The raw materials used in the "Nutrimais" manufacturing process include horticultural products; food scraps carefully selected from restaurants, canteens, and similar establishments; forest exploitation residues (e.g., branches and foliage); and green residues (e.g., flowers, grasses, prunings). The physicochemical characteristics of the MSWC are presented in Table 2. The maximum heavy metal concentration of the MSWC was low. It was below the maximal values for the Portuguese legislation for class I compost [19] and for class A in different European countries [9].

Table 2. Physicochemical characteristics of the municipal solid waste compost (MSWC) and maximum values of heavy metals in class I organic composts.

Municipal Soil Waste Compost				**Maximum Values of Heavy Metals for Organic Composts of Class I** [3]	
pH[1]	8.68	Cd ($mg.kg^{-1}$)	0.35	Cd ($mg.kg^{-1}$) [3]	0.7
EC (Elec. Conductivity) ($dS.m^{-1}$) [1]	5.4	Pb ($mg.kg^{-1}$)	32	Pb ($mg.kg^{-1}$)	100
Organic Matter (%) [2]	52.47	Cr ($mg.kg^{-1}$)	22.3	Cr ($mg.kg^{-1}$)	100
Humidity (%)	10.52	Cu ($mg.kg^{-1}$)	49	Cu ($mg.kg^{-1}$)	100
Humic acids (%)	3.71	Hg ($mg.kg^{-1}$)	0.1	Hg ($mg.kg^{-1}$)	0.7
Ratio C/N	11.94	Ni ($mg.kg^{-1}$)	7.47	Ni ($mg.kg^{-1}$)	50
C (%)	29.15	Zn ($mg.kg^{-1}$)	160	Zn ($mg.kg^{-1}$)	200
N (%)	2.41	B ($mg.kg^{-1}$)	38	B($mg.kg^{-1}$)	
P_2O_5 (%)	1.49				
K_2O (%)	1.81				
CaO (%)	15.17				
MgO (%)	0.7				

[1] EC and pH were measured in extracted 1:5 compost/water, *w*/*v*. [2] Concentrations are expressed on a dry weight basis. The moisture of the compost before soil application was 14%. [3] Portuguese legislation [19].

Soil-blocked spinach (*Spinacia oleracea* L. cv. Manatee) seedlings (seven seedlings per block, three blocks per pot = 339 plants m^{-2}) were transplanted (19 February 2019) after 18 days following emergence into 12 L pots.

Plants were watered by hand daily (9–10 am) to avoid applying high volumes of water, minimizing drainage losses and preventing plants from suffering water stress.

The volume of water applied (ranged from 90 to 400 mL/pot) was adjusted to the climatic conditions (temperature and solar radiation), readings of the volumetric soil water content, and the soil water storage capacity of the soils. Volumetric soil water content was measured daily (08:00–09:00) using a soil moisture probe (SM105T delta devices UK). The irrigation water had a low EC_w (0.1 d*S* m^{-1}).

Nitrogen was applied via fertigation once a week in five equal fertilizer applications, starting at transplantation and finishing in the week before harvest. The fertilizer used to apply nitrogen was calcium nitrate (15.5% N-NO_3, 1.1% N-NH_4, and 26.5% CaO). The nutrients applied using inorganic fertilizers and MWSC in each treatment are presented in Table 3.

Table 3. Total amounts of nutrients added in each treatment.

Treat.	**Type**	**N**	**$P_2 0_5$**	**K_2O**	**CaO**	**MgO**
				Kg ha^{-1}		
FS	Inorg.[1]	214.1 [3]	61.4	91.3	294 [4]	6.14
35	Org.[2]	679.7	431.6	524.3	4545.2	202.7
70	Org.	1395.4	862.7	1047.9	9090.3	405.3
35 + N	Org. + Inorg.	679.7 + 92	431.6	524.3	4545.2 + 147 [4]	202.7
70 + N	Org. + Inorg.	1395.4 + 92	862.7	1047.9	9090.3 + 147 [4]	405.3

[1] Inorganic fertilizer; [2] MSWC; [3] 29.8 kg N ha^{-1} applied before spinach plantation and 184 kg N ha^{-1} applied via fertigation; [4] applied in fertigation.

Starting from transplantation, air temperature at the plant canopy level was monitored hourly using a T-Logger HI141 temperature sensor (Hanna Instruments) (Figure 1). The weeds were regularly manually removed from the pots.

2.2. Measurements

The plants were harvested 38 days after transplantation. The shoots of the plants were cut off 1 cm above the substrate surface. Ten representative plants (shoots) from each box were washed, oven-dried at 70 °C for 2–3 days, weighed, ground so that they would pass through a 40-mesh sieve, then analyzed for N, P, K, Ca, Mg, Na, B, Cu, Mn, and Zn. Total N was analyzed by using a combustion analyzer (Leco Corp. St. Josef, MI, USA). The K and Na were analyzed by flame photometry (Jenway, Dunmow, UK). The P and B were analyzed using a UV/Vis spectrometer (Perkin Elmer lamba25). The remaining nutrients were analyzed using an atomic absorption spectrometer (Perkin Elmer, Inc., Shelton, CT, USA)

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

Soil nitrate was measured using an ion-specific electrode and meter (Crison Instruments, Barcelona, Spain), using the method outlined by [20]. 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). Organic carbon (%) was measured using a sulfur and carbon determinator (SC-144 DR, Leco Inc, St. Joseph, MI, USA). Organic matter (%) was estimated from organic carbon (%) using the conversion factor 1.72 (organic matter (%) = total organic carbon (%) × 1.72) [21].

Leaf samples from ten treatments and five replicates were stored at −80 °C for NO_3^- determination according to [22]. Briefly, portions (0.1000 g) of spinach leaves were suspended in 10 mL of distilled water. The samples were oven-dried at 65 °C for 48 h, macerated in a mortar, homogenized in a test tube with 10 mL of distilled water, agitated in a vortex, and incubated for 1 h at 45 °C in a shaking water bath. Filtrated extracts in Whatman 40 filter paper were then mixed with salicylic acid in 5% sulfuric acid (1:4), incubated for 20 min at room temperature, and mixed with 9.5 mL of 2 M sodium hydroxide. The concentration of NO_3^- in the solution was then determined by reading the absorbance at 338 and 440 nm using a calibration curve (NO_3^-, n = 6 concentrations between 0 and 500 mg/L)

In order to determine the photosynthetic pigment content, 1.000 g of spinach leaf from each treatment was macerated in a mortar and homogenized in 8 mL of methanol/water solution (90:10 (*v*/*v*), M90-extract) for 1 min, then centrifuged at 4 °C at 6440× *g* for 5 min. Chlorophyll a and b and carotenoids were quantified in aliquots of M90-extract via UV-Vis spectrophotometry, using the appropriate equations [23]:

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 = carotenoids.

In order to determine free radical scavenging antioxidant activity (DPPH), 1000 g of leaf sample from each treatment was macerated in a mortar and homogenized in 8 mL of methanol/water solution (80:20, *v*/*v*) for 1 min, then centrifuged for 5 min at 4 °C and 6440× *g* (M80 extract). Aliquots of methanol extracts were stored at −20 °C for later use. Antioxidant activity was determined by measuring the ability of M80 spinach extracts to scavenge the violet-colored stable organic radical 2,2-diphenyl-1-picryl-hydrazyl ($DPPH^\bullet$), converting it into the yellow-colored stable product diphenyl-picryl hydrazine (DPPH-H). Aliquots of an extemporaneous methanol solution of 0.03 g/L $DPPH^\bullet$, which were kept in the dark, were added to a known volume of sample (M80 extract) or standard solution. The reduction of $DPPH^\bullet$ to DPPH-H was followed by reading the absorbance at 515 nm and 25 °C for 180 s. Antioxidant activity, reported as milligrams of GAE (gallic acid equivalent) per 100 g of FW, was calculated using a calibration curve (GAE, n = 8 concentrations from 0 to 200 $mg \cdot L^{-1}$) [24].

2.3. Data Analysis

Data were processed via analysis of variance using SPSS Statistics 25 software (Chicago, IL, USA), licensed to the University of Évora. Means were separated at the 5% level using Duncan's new multiple range test.

3. Results and Discussion

3.1. Soil Physicochemical Properties

Soil moisture values at depths of 0 to 5 cm were not affected by the interactions between the treatments. The moisture content values for all samplings were significantly greater in the neutral soil (Figure 2). The addition of MSWC to the soil, as compared to fertilized soil, significantly increased the moisture contents at 24 and 31 days after plantation (DAP) (Figure 2). On the last sampling date, soil moisture content increased up to 12.3 %. Moisture content was not affected by the MSWC rate when combined or not with nitrogen. This could be due to a higher plant water uptake caused by the increase of the yield due to the combined application of MSWC and nitrogen (Table 4).

Soil temperature values at 10 cm depth were not significantly affected by the treatments, nor by their interaction. The temperature values measured at 10:00 to 11:30 on the different sampling dates ranged from 18.0 to 24.5 °C, probably increasing during the day. These temperatures are favorable for organic matter mineralization (Figure 2).

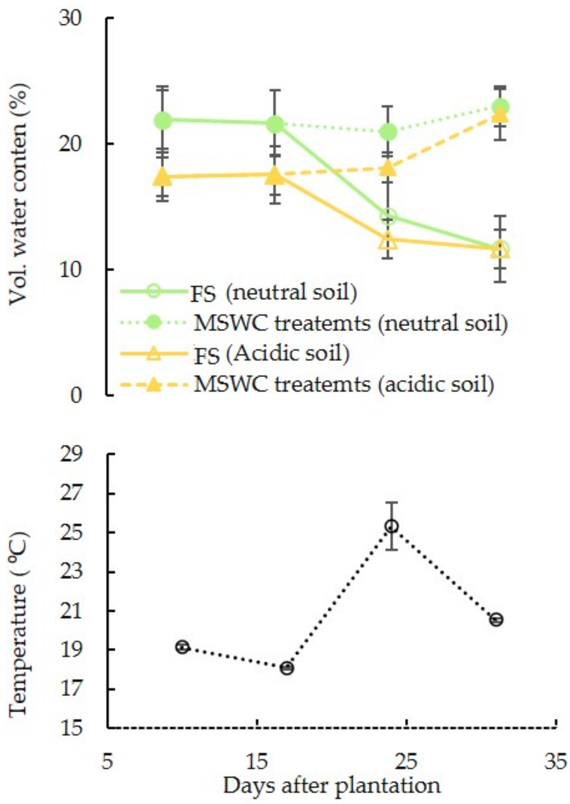

Figure 2. Effects of soil and MSWC supplemented or not with inorganic nitrogen on soil volumetric water content (%) values at 0–5 cm depth and soil temperature at 10 cm depth. Each symbol represents the mean of six replicates, while the error bars represent ±1 standard error.

Soil organic matter content (SOM), pH, EC_e, and nitrate values were significantly affected by the interactions between treatments ($p < 0.001$), indicating that the responses of soil to the addition of MSW compost differed. Despite the SOM, pH, and EC_e in both soils, in relation to fertilized soil, these components increased with the addition of MSWC supplemented or not with inorganic nitrogen (Figure 3). In neutral soil, SOM increased with the rate of MSWC (Figure 3a). However, in acidic soil, SOM only increased significantly with MSWC when supplemented with inorganic nitrogen (Figure 3a).

The SOM values in neutral and acidic soils with the addition of 70 t MSWC ha^{-1} were higher than 3.5 and 2%, respectively. Regarding soil before spinach plantation, the addition of 35 and 70 t MSWC to neutral soil increased the average SOM contents by 1.15 and 2.25%, respectively. However, in acidic soil, for the same rates of MSWC the increases of SOM were only 0.45 and 0.90% respectively. This could be because of the soil characteristics (e.g., bulk density) or the different organic matter decomposition rates.

The addition of MSWC to the soils at both rates, supplemented or not with inorganic nitrogen, increased soil pH values relative to those before plantation and only fertilized with nitrogen (Figure 3b). Increases in soil pH with the addition of MSWC have also been reported by other authors [11,25].

However, regarding neutral soil, the amount of compost had no significant influence on soil pH, while in the acidic soil it significantly increased with the amount of the compost (Figure 3b). Paradelo and Barral (2017) also reported that the soil pH of acidic soils increased with the addition of MSWC [26].

Soil pH increases in the neutral and acidic soils ranged on average from 0.61 to 0.89 and from 1.09 to 1.85, respectively (Figure 3b). The difference in the magnitude of the pH increases may be due to the initial soil pH and cation exchange capacity (CEC) of the soils, and also due to the increase in the soil buffer capacity caused by the addition of MSWC. The humic acids in MSWC intensify the CEC and buffering capacity of the soil [27].

The addition of 35 and 70 t of MSWC ha^{-1} to neutral soil increased the soil pH from 7.17 to average values of 7.7 and 8.0, respectively (Figure 3b). These values can negatively affect plant nutrition, since they can decrease the nutrient availability in the soil solution and can reduce organic matter mineralization, since neutral or slightly alkaline conditions favor bacterial growth [28].

Therefore, regarding the neutral soil, the rates of MSWC used can be higher. Conversely, soil pH values in acidic soil increased from 5.5 to average values of 6.8 and 7.3, respectively (Figure 3b). This range of pH can contribute to improving plant nutrition and decreasing exchangeable aluminum and manganese in soil solutions. This result also indicates that the addition of 30 t MSWC ha^{-1} to acidic soil was enough to increase soil pH to adequate values.

Conversely, as expected, the calcium nitrate addition did not increase soil pH values. This could be due to the increase in soil buffering capacity. On other hand, the nitrification of ammonium-N from fertilizer (15.5% $N\text{-}NO_3$, 1.1% $N\text{-}NH_4$, and 18.6% Ca) or ammonium uptake by plants can contribute to reducing soil pH [29].

Soil EC_e values in both soils increased with the rate of MSWC, supplemented or not with nitrogen addition (Figure 3c). Despite the increase, the EC_e values in both soils were very low, not exceeding 0.40 d*S* m^{-1} (Figure 3c). This value is much lower than the value that inhibited spinach plant growth (2 d*S* m^{-1}) or the growth of most crop vegetables or fruit crops [30,31]. These results indicate that despite the high EC_e of MSWC (5.4 d*S* m^{-1}), its addition to both soils, even at the highest rate (70 t), did not reach values that affect crop growth. This was also reported in soil cultivated with spiny chicory [32] and spinach [7], which was added at similar rates to compost with high EC_e .

The soil nitrate contents in both soils significantly increased with the rate of MSWC, but were not affected by the nitrogen addition (Figure 3d). This could be due to higher nitrogen uptake by the plants grown with MSWC plus nitrogen than those grown only with compost (Figure 5a) or the date of the last application of inorganic nitrogen (one week before spinach harvest).

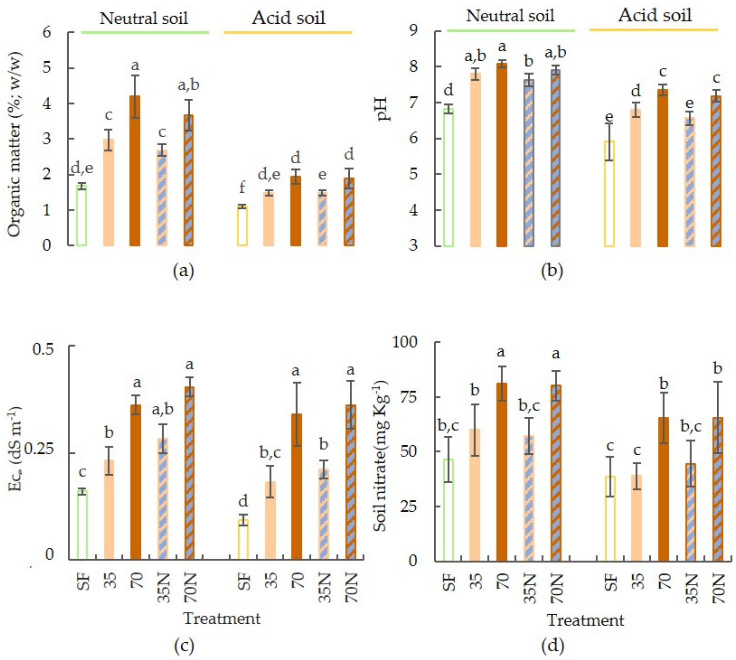

Figure 3. Effects of soil and MSWC supplemented or not with inorganic nitrogen on soil organic matter (**a**), pH (**b**), EC_e (**c**) and nitrate (**d**) values. Note: FS—fertilized soil; 35—35 t MSWC ha^{-1}; 70—70 t MSWC ha^{-1}; 35 + N—35 t MSWC ha^{-1}+ 92 kg N ha^{-1}; 70 + N—70 t MSWC ha^{-1} + 92 kg N ha^{-1}. Means with different letters are significantly different at $p < 0.05$. Each bar represents the mean of six replicates, and the error bars represent ±1SE.

3.2. Plant Growth and Yield

Shoot dry weight, foliar area, and yield (fresh yield) values were not affected by the interactions between treatments. The soil significantly influenced the yield ($p < 0.001$), which was higher in the neutral soil.

Plants grown with inorganic fertilization had greater shoot dry weight values than those grown with compost or compost plus nitrogen (Table 4). Shoot dry weight values increased with inorganic nitrogen addition to compost and with the rate of compost (Table 4). Nitrogen addition to the MSWC at both rates led to an increase in shoot dry weight by ≈100%.

Plants grown with the highest rate of compost plus nitrogen had greater leaf area than those grown with the other treatments (Table 4).

Table 4. Effects of soil and MSWC supplemented or not with inorganic nitrogen on shoot dry weight, foliar area, and fresh yield of spinach.

MSWC Treatments	**Shoot Dry Weight (g/plant)**		**Foliar Area (cm^2/plant)**		**Fresh Yield (kg m^{-2})**	
	Soil		**Soil**		**Soil**	
	Neutral	Acidic	Neutral	Acidic	Neutral	Acidic
FS	1.70 a [1]	1.44 a	215.20 b	240.98 b	3.97 a	3.60 a
35	0.59 d	0.52 d	101.24 d	82.80 d	1.61 d	1.23 d
70	0.72 d	0.71 d	126.83 c	124.08 c	2.03 c	1.90 c
35 + N	1.17 c	1.13 c	254.85 a	239.72 b	3.43 b	3.05 b
75 + N	1.44 b	1.42 b	258.30 a	255.25 a	3.86 a	3.68 a

[1] Means followed by different letters within a column are significantly different ($p < 0.05$). Note: FS—fertilized soil; 35—35 t MSWC ha^{-1}; 70—70 t MSWC ha^{-1}; 35 + N—35 t MSWC ha^{-1}+ 92 kg N ha^{-1}; 70 + N—70 t MSWC ha^{-1} + 92 kg N ha^{-1}.

Spinach fresh yield significantly increased with the rate of MSWC supplemented or not with nitrogen addition.

The addition of inorganic nitrogen to the 35 and 70 t of MSWC led to average increases in fresh yield of 147 and 90%, respectively. The increases in fresh yield due to inorganic nitrogen at both rates of MSWC and for both soils were similar.

The combined addition of inorganic nitrogen (43% of the inorganic nitrogen applied to fertilized soil) with the highest rate of compost addition increased the yield from the neutral (3.86 kg m^{-2}) and acidic soils (3.68 kg m^{-2}) to similar values to those obtained with inorganic fertilization (Table 4).

3.3. Shoot Nutrient Concentration and Uptake

Shoot N, P, K, Ca, and Mg contents were not influenced by the interactions between the treatments. Such behavior was also reported by Papafilippaki [32] in spiny chicory grown in soils with different MSWC rates. Shoot macronutrient concentrations, except for shoot N and Ca, were significantly affected by the soil, being higher in neutral soil.

The rate of MSWC combined or not with inorganic N had no significant influence on shoot N concentration (Figure 4a). Leaf N concentrations in plants grew only when MSWC was below the recommended level (2 to 4%) [33]. Calcium nitrate addition to compost increased shoot N concentrations (averaging 65% in both soils). Shoot N uptake followed the same trend, however in both soils the values were lower than those in plants grown in fertilized soil (Figure 5a).

Plants grown only with compost had higher shoot P concentrations than those grown only with conventional fertilization and compost plus nitrogen (Figure 4b). This increase has also been mentioned by other authors [7,14,34]. The addition of MSWC to the soil can increase shoot P uptake directly by supplying P and indirectly due to the addition of the humic substances (humic acids, fulvic acids, humins, etc.) and changes in the pH. Moreover, MSWC can also increase phosphorus uptake, since it can increase its diffusive flux and availability due to increases in soil moisture content (Figure 2) and microbial activity [35,36]. Calcium nitrate addition to compost significantly decreased shoot P concentrations (Figure 4b). Despite this, in both soils, the shoot P uptake of plants grown with the highest rate of compost plus calcium nitrate was equal to plants grown in the fertilized soil (Figure 5b). The shoot P concentration was within the required range (0.3–0.4%) [33]. This indicated that the combined application of the highest rate of compost plus nitrogen can contribute to reducing inorganic P application.

Plants grown only with compost at both rates and in both soils had higher shoot K concentrations (averaging 0.12% and 0.53% in neutral and acidic soils, respectively) than those plants grown using conventional fertilization (Figure 4c). This is consistent with other studies that also reported increases in plant tissue K contents in other crops [37–40] due to the addition of MSWC. This could be due to the fact that the K in MSWC is easily available to plants [10], since normally more than 75% of the potassium in compost is soluble [41]. In lettuce, another short-term crop similar to spinach, the use of MSWC also increased the leaf K content compared with plants grown with inorganic fertilizer [17]. Despite the difference in the K_2O exchangeable contents between soils (204 and 60 mg kg^{-1} in neutral and acid soils, respectively) (Table 1), shoot K uptake by the plants grown with the highest rate of compost plus inorganic N was equal (neutral soil) or higher (acidic soil) than in those plants grown in fertilized soil (Figure 5c). This shows that the addition of MSWC plus nitrogen could replace inorganic K fertilization to a significant extent.

Shoot Ca concentrations of the plants grown in fertilized soils were higher than those in the plants grown using the other treatments (Figure 4d). However, in these treatments, average shoot Ca values were equal to the lower end or within the sufficiency range (1–1.5%) [33] (Figure 4d).

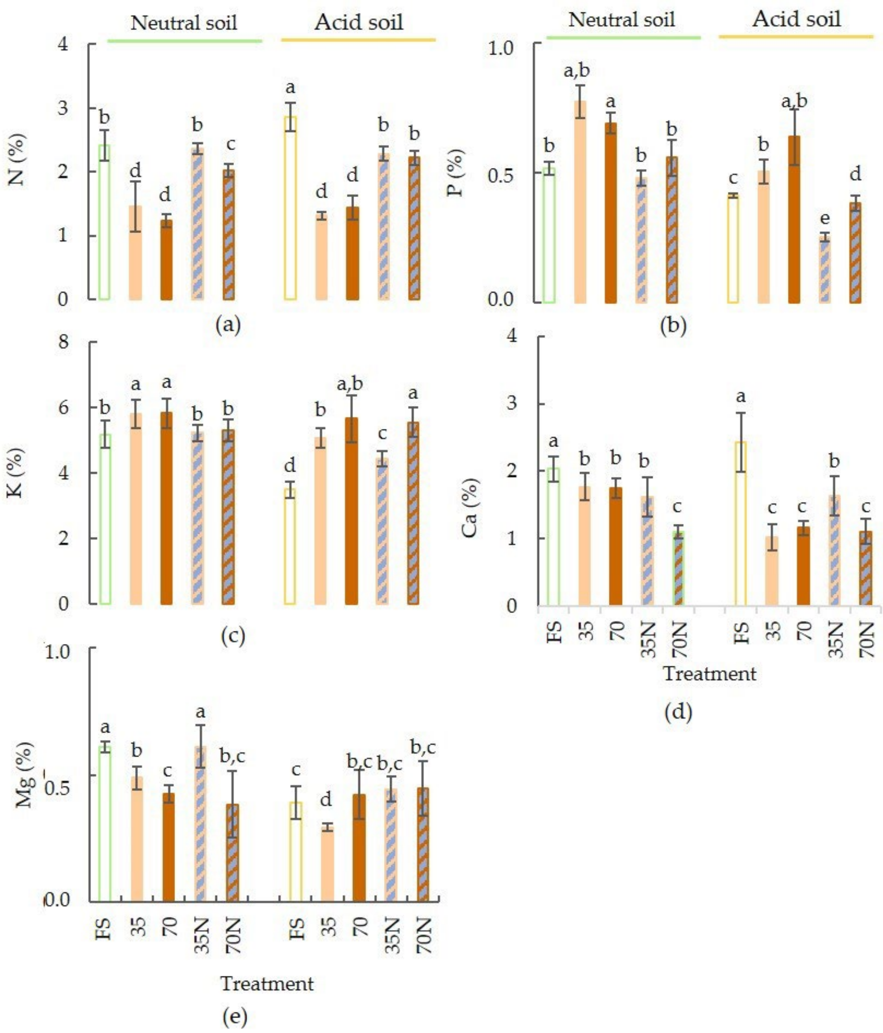

Figure 4. Effects of soil and MSWC supplemented or not with inorganic nitrogen on shoot N (**a**), P(**b**), K (**c**), Ca (**d**), and Mg (**e**) concentrations. Note: FS—fertilized soil; 35—35 t MSWC ha^{-1}; 70—70 t MSWC ha-1; 35 + N—35 t MSWC ha^{-1}+ 92 kg N ha^{-1}; 70 + N—70 t MSWC ha^{-1} + 92 kg N ha^{-1}. Means with different letters are significantly different at $p < 0.05$. Each bar represents the mean of six replicates, while the error bars represent ±1SE.

Despite the high concentration of Ca in MSWC (18.5 g Ca kg^{-1} on a dry weight basis), shoot calcium concentrations did not increase with the MSWC rate (Figure 4d). In basil (*Ocimum basilicum*, L.), it was also reported [38] that the addition of compost increased the soil calcium concentration but not plant Ca uptake. Calcium nitrate addition to the compost led to a decrease in shoot Ca concentration (Figure 4d). This was due to a dilution effect, since in both soils the addition of calcium nitrate to the compost led to an increase in shoot Ca uptake (Figure 5d).

Average shoot Mg concentrations in neutral and acidic soils ranged from 0.38 to 0.59% and from 0.29 to 0.44%, respectively (Figure 4e). These ranges of values were below or slightly higher than the lower end of the range considered to be sufficient (0.4 to 1%) [33]. This indicates that the plants may have been subject to Mg deficiency. However, none of the plants in the treatment groups showed visual symptoms of Mg deficiency. Shoot Mg concentrations in plants grown only with compost in the neutral soil decreased, while those in the acidic soil increased (Figure 4e). In lettuce [17] and in blueberry (V*accinium angustifolium*, L.) [42], shoot Mg concentrations also increased with MSWC rate. The addition of nitrogen to compost in both soils did not increase shoot Mg concentrations (Figure 4), but contributed to increasing shoot Mg uptake (Figure 5e).

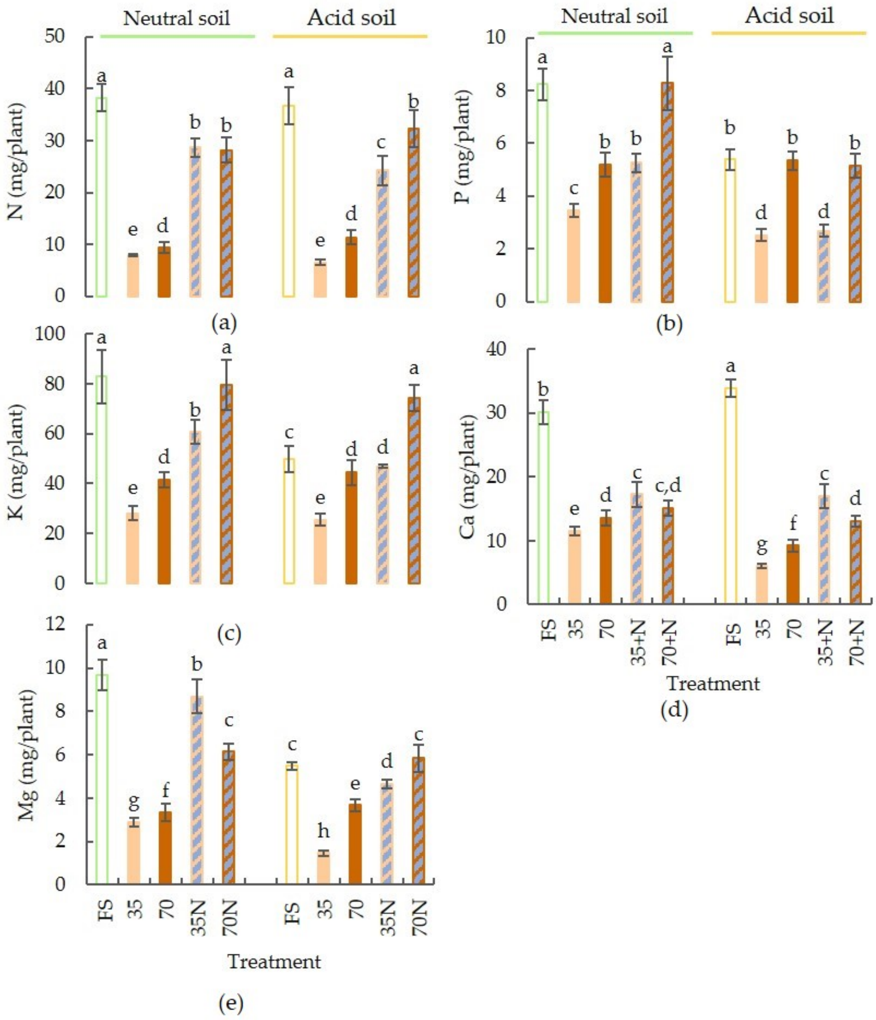

Figure 5. Effects of soil and MSWC supplemented or not with inorganic nitrogen on shoot N (**a**), P (**b**), K (**c**), Ca (**d**), and Mg (**e**) uptake rates. Note: FS—fertilized soil; 35—35 t MSWC ha-1; 70—70 t MSWC ha^{-1}; 35 + N—35 t MSWC ha^{-1}+ 92 kg N ha^{-1}; 70 + N—70 t MSWC ha^{-1} + 92 kg N ha^{-1}. Means with different letters are significantly different at $p < 0.05$. Each bar represents the mean of six replicates, while the error bars represent ±1SE.

Shoot micronutrient concentrations, except for Fe and Cu, were significantly affected by the interactions between the treatments. Shoot Fe and Cu were not significantly affected by soil or compost treatments. Therefore, the increase of the pH did not decrease shoot Fe contents, which may have been due to the Fe complexation by humic acids [43,44] in the compost (Table 2). Shoot Fe and Cu concentrations ranged from 89 to 135.8 μg g^{-1} and from 6 to 9.23 μg g^{-1}, respectively. These values were within the range considered to be sufficient for spinach [33].

Shoot Mn contents in both soil types were higher in plants grown in fertilized soil than those grown with the other treatments, particularly in the acidic soil (Figure 6a).

The addition of compost supplemented or not with calcium nitrate significantly decreased shoot Mn concentrations (Figure 6a), probably due to the increase in pH, while the exchangeable Mn availability decreased in the rhizosphere. Blueberry (*Vaccinium angustifolium*, L.) leaves also had lower Mn contents in samples from MSWC-treated soils when compared to control and fertilizer treatments [42]. This result indicates that the addition of MSWC is a way to eliminate or alleviate the Mn toxicity reported in this soil by [45].

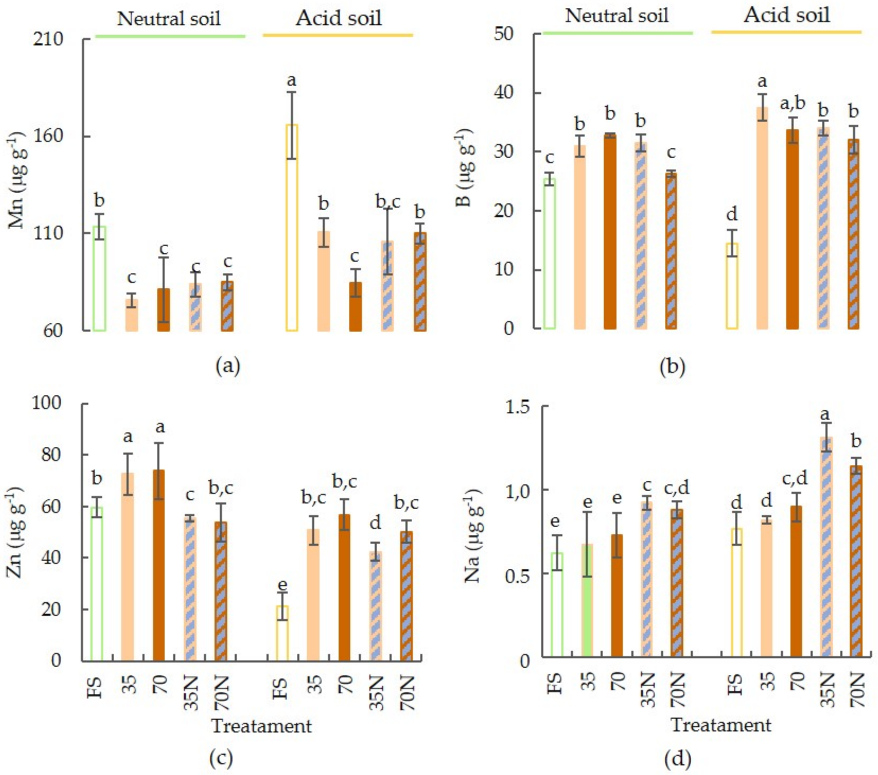

Figure 6. Effects of soil and MSWC supplemented or not with inorganic nitrogen on shoot Mn (**a**), B (**b**), Zn (**c**) and Na (**d**) concentrations. Note: FS—fertilized soil; 35—35 t MSWC ha^{-1}; 70—70 t MSWC ha^{-1}; 35 +N—35 t MSWC ha^{-1}+ 92 kg N ha^{-1}; 70 + N—70 t MSWC ha^{-1} + 92 kg N ha^{-1}. Means with different letters are significantly different at $p < 0.05$. Each bar represents the mean of six replicates, while the error bars represent ±1SE.

Despite the increase in soil pH, shoot Zn concentrations increased with the addition of MSWC, but did not increase more with increased rate of MSWC (Figure 6c). This may have been related to the increase of pH with the rate of MSWC (Figure 3b), which can contribute to reducing the availability of Zn in soil solution. The increase of shoot Zn with MSWC addition was also reported by Rajaie [46] in tomato and by Giannakis [17] in tomato and lettuce.

The addition of calcium nitrate to compost in both soils decreased shoot Zn contents (Figure 6c), which may have been due to their dilution, since shoot Zn uptake increased.

Average shoot Zn concentrations in both soil types and with both treatments ranged from 21 to 73.8 µg g^{-1} dry matter (DW). These values are lower than those that inhibit the growth of most plants (200–500 µg g^{-1} DM [47] and 100–700 µg g^{-1} DM [48,49], respectively). Indeed, average shoot Zn concentrations, except in the acidic, fertilized soil treatment (21 µg g^{-1} DW), were within the range considered to be sufficient for spinach (25–75 µg g^{-1}) [33].

Shoot B concentrations, except in the 70 + N treatment in neutral soil, were significantly higher in plants grown with MSWC supplemented or not with inorganic nitrogen than those grown in fertilized soil (Figure 6b). In these treatments, average shoot B concentrations ranged from 26.30 to 37 µg g^{-1}. These values were slightly below or within the ranges considered to be sufficient (30 to 50 µg g^{-1} DW [50] and 25 to 60 µg g^{-1} DW [33], respectively).

Despite the differences, the leaf micronutrient concentrations in the different treatments in both soils were always below toxic levels. Despite the differences, the micronutrient concentrations in the different treatments in both soils were always below toxic levels. Despite this, the low concentration of heavy metals in compost will be important in further studies to evaluate the influence of the application of MSWC on heavy metal concentrations in these soils.

Shoot Na concentrations were not affected by the interactions between treatments. Shoot Na concentrations were significantly higher in acidic soil. Shoot Na concentrations in plants grown only with compost at both rates (35 and 70 t) were not significantly different from the plants grown with conventional fertilization (Figure 6d). However, the addition of calcium nitrate to compost significantly increased leaf Na concentrations to values ranging from 0.90 and 1.3 μg g^{-1} (Figure 6d), which may have been due to an increase in the Na availability in the soil solution due to replacing Na in the soil exchange complex with Ca.

3.4. Photosynthetic Pigments

Leaf chlorophyll a and b and total chlorophyll (Chl a+b) contents in fresh weight were significantly affected by the interactions of the treatments ($p < 0.001$), indicating that the responses to the addition of MSWC supplemented or not with nitrogen differed among soils. However, Chl a contents in both soils were signifcantly higher in plants grown in fertilized soil than those grown using the other treatments (Figure 7a). In neutral soil, the Chl a decreased with MSWC supplemented or not with nitrogen. However, in acidic soil, the rate of compost had no influence on Chl a content (Figure 7a).

The Chl b content in neutral soil in plants grown with compost plus nitrogen was not significantly different from those plants grown in the fertilized soil. In acidic soil, Chl b was higher in plants grown with 35 t MSWC/ha than in those grown with the other treatments (Figure 7b).

The total chlorophyll content was highest in the neutral fertilized soil (50 mg/100 g FW) (Figure 7c). The increase in compost rate supplemented or not with nitrogen led to a decreased in total chlorophyll. This may have been due to a dilution effect, since plants grown with the highest MSWC rate had lower shoot dry weight percentages and high leaf area, or may have been due to differences in shoot nutrient concentrations or uptake (Figures 4–6). Chlorophyll synthesis is dependent on various nutrients [51], including micronutrients [52]. A nutrient deficiency strongly influences the photosynthetic apparatus structure and functions [53].

The decrease of the chlorophyll content may have also been due to the uptake of certain trace elements (metals) not measured in the present study that can negatively influence Chl a and b contents [54]. In acidic soil, the total chlorophyll content was not significantly affected by any treatment, with values ranging from 20 to 22 mg/100 g FW. These values are low compared to those recorded by Hussain (65.4 mg/100 g FW [55] and 96.2 to 301.8 mg/100 g FW) [56] and Machado (53 to 66 mg/100 g FW) [7]. Total chlorophyll was positively correlated with leaf Mg ($r = 0.65$ $p < 0.01$, data from both soils and treatments), and in both soils, as previously mentioned, plants grown with certain treatments may have been subject to magnesium deficiency. This indicates that in short-term crops it may be important to add some inorganic Mg to the compost to increase the leaf chlorophyll content.

Leaf carotenoid (Cc) contents were not significantly affected by the interactions between treatments or by soil type. The Cc was higher in the FS treatment (Figure 7d), this can be because of high nitrogen application. In spinach [7] and kale [57], the Cc content increased with nitrogen application. Indeed, the Cc content was positively correlated with leaf N content ($r = 0.651$ $p < 0.01$). The rate of compost without nitrogen supplementation in both soils did not significantly affect the Cc contents. However, Cc contents only increased significantly in plants grown with the highest compost rate supplemented with nitrogen (Figure 7d). Despite the differences in Cc levels, the values measured (ranging from an average of 22 to 40 mg/100 g FW (Figure 7d)) were within or above the value ranges reported by Borowski and Michalek (17 to 32 mg/100 FW) [58] and Machado (21.5 to 31.1 mg/100 g FW) [7].

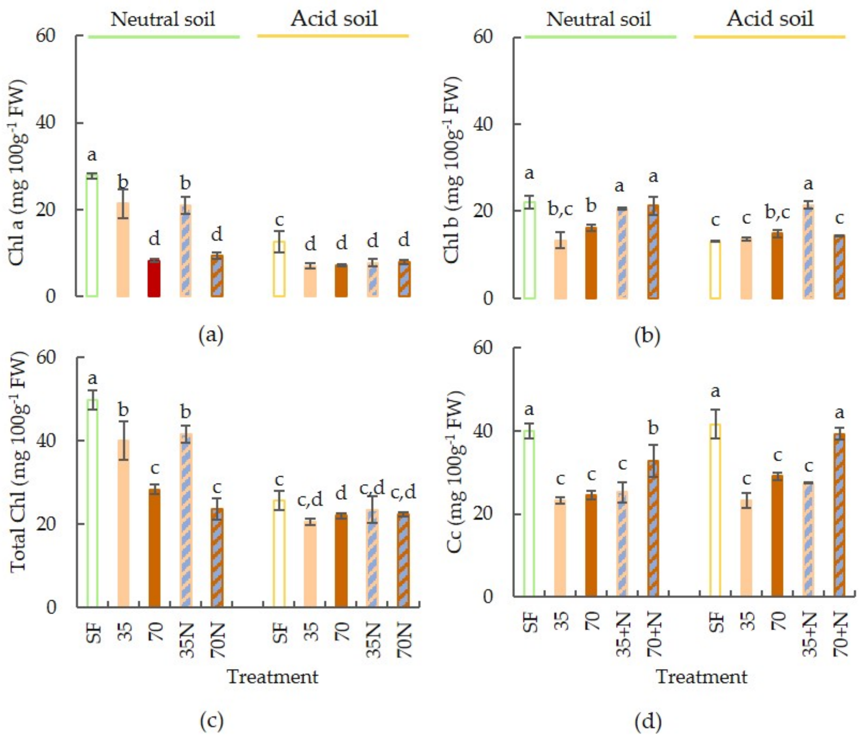

Figure 7. Effects of soil and MSWC rates supplemented or not with inorganic nitrogen on levels of photosynthetic pigments [chlorophyll a (**a**), chlorophyll b (**b**), total chlorophyll (Chl a+b) (**c**)], and carotenoids (**d**). Note: FS—fertilized soil; 35—35 t MSWC ha^{-1}; 70—70 t MSWC ha^{-1}; 35 + N—35 t MSWC ha^{-1}+ 92 kg N ha^{-1}; 70 + N—70 t MSWC ha^{-1} + 92 kg N ha^{-1}. Means with different letters are significantly different at $p < 0.05$. Each bar represents the mean of six replicates, while the error bars represent ±1SE.

3.5. Nitrate

Shoot NO_3^- concentrations were significantly influenced by the interactions between the treatments. However, in both soils and with the different treatments, shoot nitrate concentrations always below the maximum value allowed by the European Union for fresh spinach (3.5 mg g^{-1} fresh weight) [59] (Figure 8). This result indicates that the addition of MSWC at either rate, supplemented or not with inorganic nitrogen, does not represent an issue for NO_3^- concentrations in spinach.

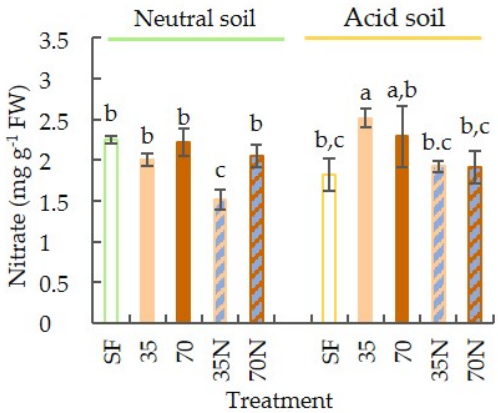

Figure 8. Effects of soil and MSWC supplemented or not with inorganic nitrogen on shoot nitrate concentrations. Note: FS—fertilized soil; 35—35 t MSW compost ha^{-1}; 70—70 t MSW compost ha^{-1}; 35 + N—35 t MSWC ha^{-1} + 92 kg N ha^{-1}; 70 + N—70 t MSWC ha^{-1} + 92 kg N ha^{-1}. Means with different letters are significantly different at $p < 0.05$. Each bar represents the mean of six replicates, while the error bars represent ±1SE.

3.6. Antioxidant Activity (DPPH)

Leaf spinach antioxidant activity (DPPH) levels were not significantly affected by the interactions between treatments or by soil type. Leaf DPPH levels in both soils, regardless of the applied rate, were lower in treatments where MSWC was applied but not combined with nitrogen (Figure 9).

Overall, leaf DPPH levels increased with the addition of nitrogen to the compost (Figure 9). This may have been due to the increase in yield, which consequently increased nutrient plant uptake, which may have led to the occurrence of nutrient deficiency, as apparently occurred with magnesium.

As apparently occurred with magnesium, the antioxidant activity increased with nutritional stress, deficiency, imbalance, and specific toxicities. For example, Mg deficiency increased the activity of antioxidant enzymes and the concentrations of antioxidant molecules in beans [60] and pepper [61].

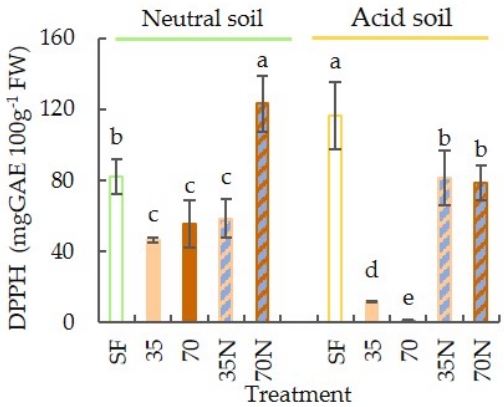

Figure 9. Effects of soil and MSWC supplemented or not with inorganic nitrogen on shoot antioxidant activity (DPPH) levels. Note: FS—fertilized soil; 35—35 t MSW compost ha^{-1}; 70—70 t MSW compost ha^{-1}; 35 + N—35 t MSWC ha^{-1} + 92 kg N ha^{-1}; 70 + N—70 t MSWC ha^{-1} + 92 kg N ha^{-1}. Means with different letters are significantly different at $p < 0.05$. Each bar represents the mean of six replicates, while the error bars represent ±1SE.

In treatments where nitrogen was applied, DPPH levels (ranging from an average of 45 to 123 mg GAE/100 g FW (Figure 9)) were similar to or above the values reported by Galani (100 mg 100 g^{-1} FW) [62] and Machado (20.54 to 31.1 mg 100 g^{-1} FW) [7].

Thus, further studies should evaluate the influence of MSWC supplemented with inorganic nitrogen and magnesium on plant growth, photosynthetic pigments, and oxidative stress markers such as ROS-scavenging enzymes (e.g., peroxidases, catalases) and secondary metabolites (e.g., phenols, ascorbate, glutathione).

4. Conclusions

The addition of MSWC to soil increased soil organic matter and pH values in both soils. Regardless of the rate of MSWC added to acidic soils, pH increased to adequate values for plant growth (close to neutral). Plant growth in both soils increased with the addition of inorganic nitrogen to the compost and with the rate of compost added. The supplementation of the highest rate of MSWC (70 t of MSWC) a fresh yield similar to those obtained in the fertilized soils, and substantially reduced the amount of inorganic nitrogen applied. Weekly addition of inorganic nitrogen to MSWC increased levels of shoot N, P, K, Ca, and Mg uptake. The addition of nitrogen to the highest MSWC rate increased shoot P and K to levels similar to those grown with inorganic fertilization treatment. MSWC addition reduced the shoot Mn concentration considerably. Regardless of the MSWC rate or addition of N, leaf tissue concentrations of Zn, Fe, Mn, and Cu did not reach toxic levels. Shoot NO3 concentrations were also lower than the maximum allowed by the European

Union for fresh spinach. The supplementation of the 70 t rate of MSWC with inorganic nitrogen increased leaf antioxidant activity in both soils.

Author Contributions: R.M.A.M. conceived and designed the experiments; performed the experiments; analyzed and interpreted the data; contributed reagents, materials, analysis tools, and data; and wrote the paper. M.R. performed the experiments and analyzed the data. I.A.-P. and R.F. performed the experiments; analyzed and interpreted the data; contributed reagents, materials, analysis tools, and data; and wrote the paper. All authors have read and agreed to the published version of the manuscript.

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