





## Article

# Response of Warm Season Turf Grasses to Combined Cold and Salinity Stress under Foliar Applying Organic and Inorganic Amendments

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**Abstract:** Turfgrasses are considered an important part of the landscape and ecological system of golf courses, sports fields, parks, and home lawns. Turfgrass species are affected by many abiotic stresses (e.g., drought, salinity, cold, heat, waterlogging, and heavy metals) and biotic stresses (mainly diseases and pests). In the current study, seashore paspalum (*Paspalum vaginatum* Sw.) and Tifway bermudagrass (*Cynodon transvaalensis* Burttt Davy × *C. Dactylon*) were selected because they are popular turfgrasses frequently used for outdoor lawns and sport fields. The effect of the combined stress from both soil salinity and cold on these warm season grasses was investigated. Some selected organic and inorganic amendments (i.e., humic acid, ferrous sulphate, and silicon) were applied as foliar sprays five times during the winter season from late October to March. This was repeated over two years in field trials involving salt-affected soils. The physiological and chemical parameters of the plants, including plant height; fresh and dry weight per plot; total chlorophyll content; and nitrogen, phosphorus, iron, and potassium content, were measured. The results showed that all the studied amendments improved the growth of seashore paspalum and Tifway bermudagrass during this period compared to the control, with a greater improvement observed when using ferrous sulphate and humic acid compared to silicon. For seashore paspalum, the highest chlorophyll content in April was recorded after the application of ferrous sulphate at a level of 1000 ppm. The current research indicates that when grown on salt-affected soils, these amendments can be used in warm-season grasses to maintain turf quality during cold periods of the year. Further research is needed to examine any negative long-term effects of these amendments and to explain their mechanisms.

**Keywords:** *Cynodon transvaalensis*; diatomite; *Paspalum vaginatum*; seashore paspalum; Tifway bermudagrass; salt-affected soil



**Citation:** Taher, D.; Nofal, E.; Hegazi, M.; El-Gaied, M.A.; El-Ramady, H.; Solberg, S.Ø. Response of Warm Season Turf Grasses to Combined Cold and Salinity Stress under Foliar Applying Organic and Inorganic Amendments. *Horticulturae* **2023**, *9*, 49. <https://doi.org/10.3390/horticulturae9010049>

Academic Editor: Hakim Manghwar

Received: 30 November 2022

Revised: 20 December 2022

Accepted: 22 December 2022

Published: 3 January 2023



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## 1. Introduction

Turfgrasses have been utilized by humans for centuries in outdoor sports fields such as football fields and golf courts due to their low cost and safe recreational surface [1]. These grasses also have other important functions; they help to reduce surface water runoff and work as an eco-barrier that protects urban wildlife [2]. Among the 40 most important turfgrasses, two—Tifway bermudagrass (*Cynodon transvaalensis* Burttt-Davy × *C. Dactylon*) and seashore paspalum (*Paspalum vaginatum* Sw.)—were considered warm-season species. Tifway bermudagrass is a hybrid developed by Glenn Burton and released by the University of Georgia in 1960 [3]. It has a dark green color with medium-sized, fine-textured leaves and a high shoot density. Tifway bermudagrass is commonly grown in the tropics and

subtropics as a warm-season grass and provides an excellent surface for golf course fairways and athletic fields [4]. Seashore paspalum is native to tropical coastal environments. Despite its many desirable characteristics, including salinity tolerance [5] and turf quality [6], the species is less widely used on golf courses and sports fields. Turfgrass quality is particularly important for managers of recreation and sports facilities [7]. Turfgrass quality includes functional characteristics such as uniformity, density, leaf texture, leaf color, and ground cover [8]. Turfgrass species may vary in terms of appearance, use, requirements, and stress tolerance [9].

Abiotic stresses (e.g., drought, salinity, and cold stress) inhibit a plant's water uptake by creating osmotic imbalance or osmotic stress at the root–soil interface, and are considered to be the main factors that limit the growth and development of turfgrasses [10]. There are few studies published about the effect of combined abiotic stresses on turfgrasses (e.g., Torun et al. [11]), whereas the effect of individual stresses such as salinity stress is more widely investigated [10,12]. Cold temperature is a major abiotic stress affecting warm-season turfgrasses that ultimately leads to reduced growth and quality due to wilting and leaf firing [13]. Cold stress may also induce dormancy and affects CO<sub>2</sub> absorption during photosynthesis, thus promoting a decline in the photosynthetic production [14]. Warm-season grasses grow most actively within the temperature range 25–35 °C [1], whereas the optimal range for growth of cool-season grasses is 16–24 °C [15]. Turfgrasses of tropical and subtropical origin are susceptible to injuries at temperatures below 12 °C [16]. Plant nutrients (i.e., exogenous and indigenous) have beneficial effects on grass metabolism, including stimulating vital processes that result in greater turfgrass tolerance to abiotic and biotic stresses such as applied calcium [13] or foliar application of amino acids [17]. Thus, the application of amendments could benefit warm-season grasses during the winter in arid/semi-arid regions.

Agricultural production involves many abiotic and biotic stresses. These stresses can be ameliorated using many different organic materials (e.g., humic substances, organic matter, compost, etc.) and inorganic amendments (e.g., silicon sources, sulfuric compounds, selenium, etc.) [18]. These amendments have various roles in supporting crop production under stressful conditions [19]. Concerning silicon, it is the second-most abundant element in the earth's crust, and can be found in biological systems in the form of amorphous silica [20]. Silicon is considered a vital soil amendment which can improve resistance/tolerance to biotic/abiotic stresses in many plant species [20]. Silicon can improve the production of many crops under different stresses, such as water deficit [21], heavy metals toxicity [22], drought stress [23], and biotic stress [24]. Humic substances (mainly humic, fulvic, and acids) significantly impact plant cultivation by stimulating plant nutrient uptake, phytohormone signaling, enzyme antioxidants, and photosynthetic efficiency and regulating reactive oxygen species [25]. Humic substances can promote plant growth and development under different stresses such as salinity [25], soil remediation [26], drought and salinity [27], and biotic stress [28]. Sulfur compounds, which function like soil amendments or conditioners of salt-affected soils when oxidized into sulfuric acid, also have a vital role [29]. Sulfur is also effective at remediating alkaline/sodic soil by decreasing soil pH and increasing the concentration of sulfate anions [30].

Therefore, this study sought to answer the following specific research questions:

- (1) Which turfgrass is more tolerant to combined cold and salinity stress?
- (2) Which applied amendment is more effective in mitigating these previous stresses on the studied turfgrasses?
- (3) Which source of silicon and its applied dose is the best to ameliorate the growth and quality of the studied turfgrasses?
- (4) Which dose of the applied amendment can be used under salt-affected soil conditions?

## 2. Materials and Methods

### 2.1. Experimental Design and Growth Conditions

Two turfgrasses (i.e., seashore paspalum and Tifway bermudagrass; hereby referred to in the text as SP and TB, respectively) were examined in field trials during winter months at the experimental farm of the Faculty of Agriculture at Kafrelsheikh University (31°05'54" N and 30°57'00" E). The daily minimum and maximum temperatures (at 2 m from the ground) during the experimental period ranged from 7 to 23 °C and 11 to 32 °C, respectively, in the 2018/2019 season and from 7 to 25 °C and 11 to 35 °C, respectively, in the 2019/2020 season. The soil used was salt-affected soil. The soil texture (0–20 cm) was classified as clay, with the particle size distribution being 19.7% sand, 25.0% silt, and 55.3% clay. The soil salinity (EC), pH, and sodium adsorption ratio (SAR) were 4.49 dS m<sup>-1</sup>, 8.65, and 19.0, respectively. The soil organic matter and soil cation exchange capacity (CEC) were 14.5 g kg<sup>-1</sup> and 40.5 cmol<sub>c</sub> kg<sup>-1</sup> soil, respectively, and the water table was at 90 cm from the soil surface. The available N, P, and K values were 30, 12, and 185 mg kg<sup>-1</sup>, respectively. The main soil moisture parameters, which were field capacity, wilting point, and available water, had values of 43.25, 23.11, and 20.14%, respectively. The previous soil parameters were determined according to the methods described by Sparks et al. [31] and Campbell [32]. In the current study, selected amendments, including both organic (humic acid) and inorganic amendments (sources of sulfate and silica), were tested. Compost (2.38 kg m<sup>-2</sup>) was applied to the research area on November 1st of each season. To both reduce the surface tension of the leaves and to avoid surface runoff, 0.1% Tween 20 (Polysorbate 20) was added into the sprinkler system.

The irrigation water used was a non-saline fresh water with pH and EC values of 7.71 and 225 ppm, respectively. The contents of available N and P in the irrigation water were 0.09 and 28 mg L<sup>-1</sup>, respectively. Irrigation was performed according to the recommendations of the Ministry of Agriculture and Land Reclamation of Egypt. Throughout the experiment, the turfgrasses were mowed at a height of 3 cm using the appropriate mowing machine 2–3 times a week during the warm months of the year and from 0 times to 1 time a week during the cold months. The plants were sprinkler-irrigated for 15 min three times a week and fertilized with NPK (20:20:20) once a week at a rate of 1.5 g L<sup>-1</sup> through the sprinkler irrigation system. The temperature during the study was recorded and the relevant data were provided as the average maximum and minimum per month (Figure 1).

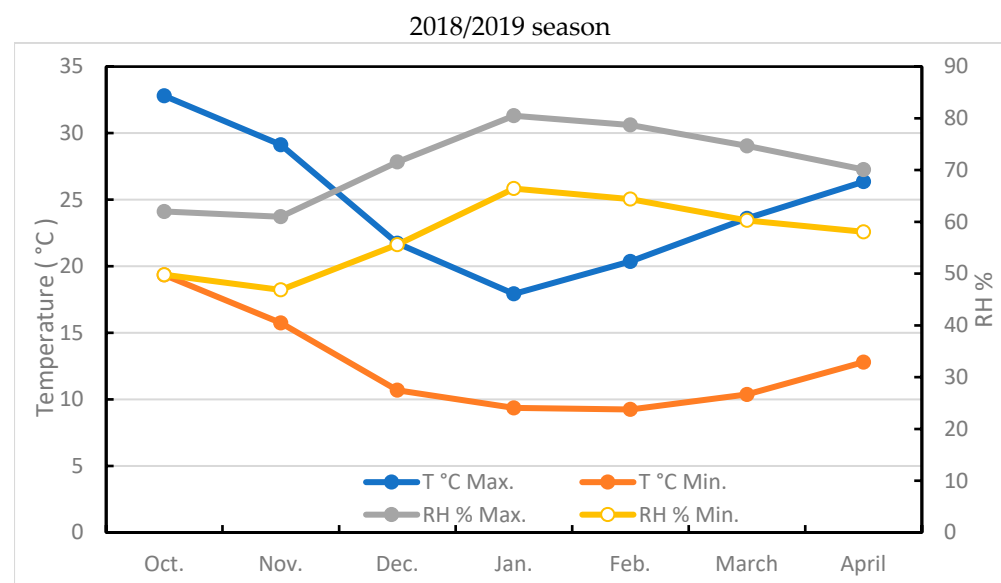
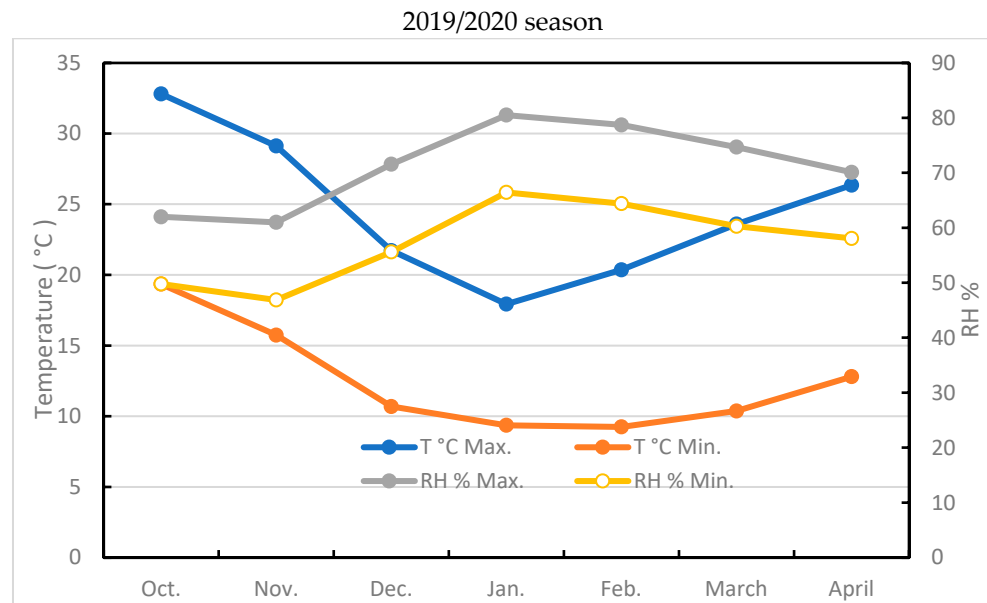


Figure 1. Cont.



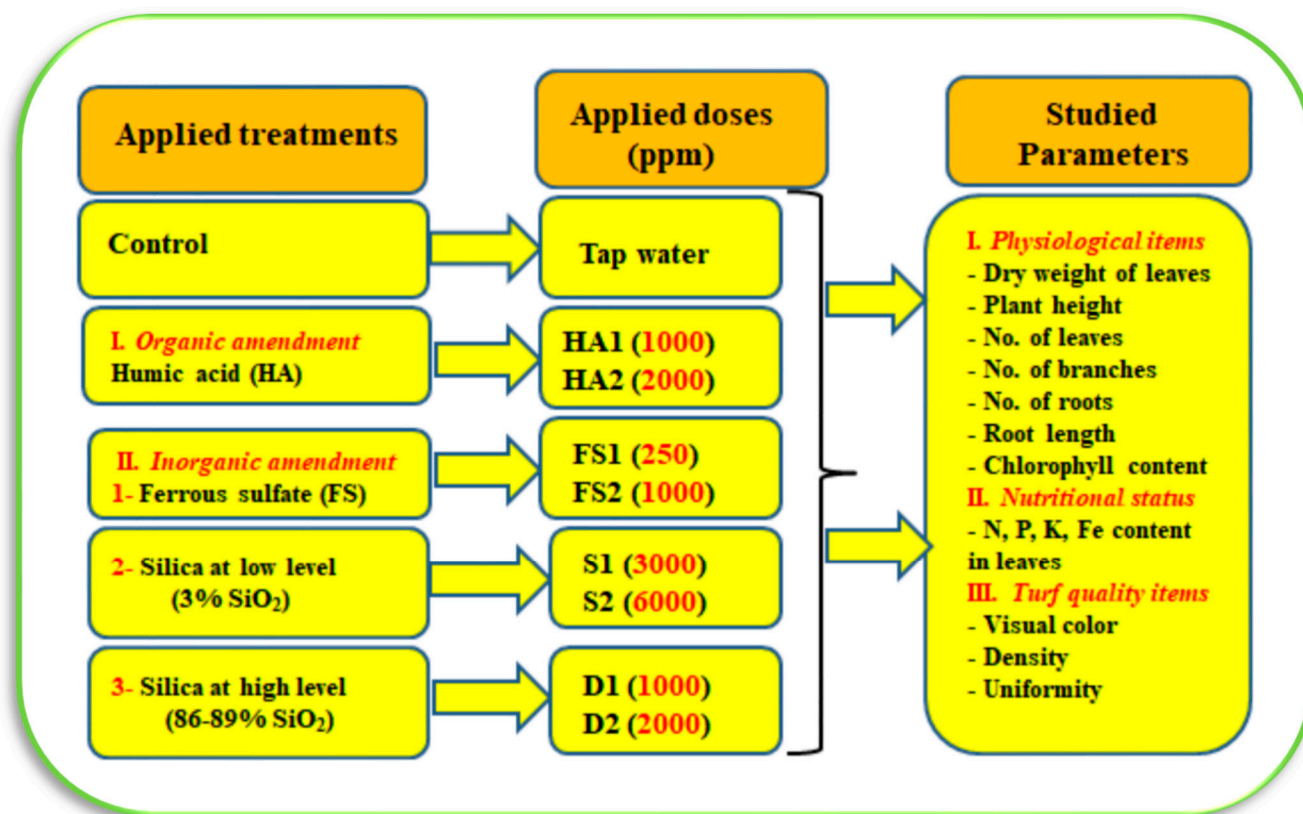
**Figure 1.** Average maximum and minimum temperature and relative humidity per month during the study in both seasons.

## 2.2. Treatments and Their Sources

The plant materials were obtained from the Horticulture Department at Kafrelsheikh University. A field study was run from October to April over two separate cold seasons. Some of the amendments that were investigated in the current study included humic acid (organic) and sources of iron and silicon (inorganic). Both types of grasses were treated with humic acid, ferrous sulphate ( $\text{FeSO}_4$ ), and two silica (S) sources (low level or Citrok Plus silica as 3%  $\text{SiO}_2$  and high level or diatomite silica as 86–89%  $\text{SiO}_2$ ) in two doses (Table 1 and Figure 2). Each experimental plot consisted of one grass unit with an area of  $10 \text{ m}^2$ . Each unit received five sprays every two weeks starting from 9 October until 9 December. After 9 December, the units were sprayed until the point of run-off at 20 days intervals. Treatments in both seasons were arranged according to a randomized complete block design (RCBD) with three replicates.

**Table 1.** Overview of the different treatments and their applied doses.

Code	Treatments (Applied Dose)	Details of the Amendments Used	Active Ingredient in the Applied Products
C	Control	Tap water	-----
HA1	Humic acid (1000 ppm)	Humic (20%) from GrowTech for Agricultural Development, Cairo, Egypt	Humic acid (0.02%)
HA2	Humic acid (2000 ppm)	Humic (20%) from GrowTech for Agricultural Development, Cairo, Egypt	Humic acid (0.04%)
FS1	Ferrous sulphate (250 ppm)	X-xtra Iron (10%) from Growth Products Ltd., White Plains, NY, USA	$\text{FeSO}_4$ (0.0025%)
FS2	Ferrous sulphate (1000 ppm)	X-xtra Iron (10%), Growth Products Ltd., White Plains, NY, USA, $1 \text{ cm L}^{-1}$	$\text{FeSO}_4$ (0.01%)
S1	Silica (3000 ppm)	Citrok plus (3% silica) from Novac Bio Science, El Mansurá, Egypt, 3%, $\text{SiO}_2$	$\text{SiO}_2$ (0.009%)
S2	Silica (6000 ppm)	Citrok plus (3% silica) from Novac Bio Science, El Mansurá, Egypt, 3%, $\text{SiO}_2$	$\text{SiO}_2$ (0.018%)
D1	Diatomite (1000 ppm)	Diatomite (86–89% $\text{SiO}_2$ ) from Shengmai Diatomite Functional Material Co. Ltd., Linjiang, China, 86–89% $\text{SiO}_2$	$\text{SiO}_2$ ( $0.875 \text{ g L}^{-1}$ )
D2	Diatomite (2000 ppm)	Diatomite (86–89% $\text{SiO}_2$ ) from Shengmai Diatomite Functional Material Co. Ltd., Linjiang, China, 86–89% $\text{SiO}_2$	$\text{SiO}_2$ ( $1.75 \text{ g L}^{-1}$ )



**Figure 2.** Overview of the experimental design including the main treatments, different applied doses of each treatment, and studied measurements.

### 2.3. Plant Physiological and Chemical Parameters

Different vegetative characteristics, including the dry weight of leaves; plant height; number of leaves, branches, and roots; and finally, the length of the longest root, were measured. The area of each plot used to measure these parameters was 100 cm<sup>2</sup>. For chemical characteristics, total chlorophyll was measured in fresh leaf samples using an SPAD instrument (SPAD 501 leaf chlorophyll meter, Minolta, Co., Ltd., Tokyo, Japan) according to the method described by Netto et al. [33]. Nitrogen (N) content was determined using the micro-Kjeldahl instrument (UDK 159, Velp Scientifica, Usmate, Italy), phosphorus (P) content was determined using a spectrophotometer (Libra S80PC, Biochrom, Cambridge, UK), and iron (Fe) and potassium (K) contents were measured using an inductively coupled plasma-optical emission spectrometry (ICP-OES) apparatus (Prodigy 7, Leeman Labs., Hudson, NH, USA) according to the method described by Sparks et al. [31]. Only plant height and total chlorophyll were recorded twice in each season during January and April; the remaining vegetative characteristics as well as the chemical composition were both measured only once in January.

### 2.4. Plant Quality Parameters

The main parameters of turfgrass quality were measured with the naked eye and included the visual turf color, turf density, and turf uniformity. The scorings were done according to the National Turfgrass Evaluation Program or NTEP guidelines [34]. The rating scale for most visual parameters (mainly turf color and density) ranged from 1 to 9, where 1 is the poorest or lowest value and 9 is the highest or best rating. In the winter, turf color is around a 1 on the visual rating scale if it is straw-brown or has not retained any color and is considered a 9 if it is dark green. All quality parameters were recorded only once in January.

### 2.5. Statistical Analyses

Statistical analyses were performed using the statistical software SAS (version 9.1; SAS Institute, Cary, NC, USA). One-way analysis of variance (ANOVA) and species-wise Duncan's multiple range tests were used to compare the mean values between the two seasons [35].

## 3. Results

### 3.1. Applied Amendments and Vegetative Growth

For each of the two studied turfgrass species, six vegetative parameters, including the dry weight of leaves (g); plant height (cm); number of leaves, branches, and roots; and finally, the length of the longest root (cm), were measured. The mean values of plant height and the number of branches and roots were tabulated in Tables 2 and 3, respectively, while the mean values for the dry weight of leaves, number of leaves, and the length of the longest root were presented in Figure 3. From the data in Table 2, it is apparent that the height of both turfgrasses was significantly influenced by the applied amendments in January and April. In both species and across both seasons, the measured mean values of plant height in January were lower than those in April. For each season, the highest respective values of plant height in January were 2.7 and 3.6 cm for SP and 2.7 and 3.5 cm for TB, whereas the highest respective mean values in April were 3.7 and 6.7 cm for SP and 3.6 and 5.6 cm for TB. For both plant species, it was noted that across both seasons, the previous highest values were recorded when ferrous sulphate was applied at a concentration of 250 or 1000 ppm. It was also noted that different sources of silicon were less effective compared to other amendments (humic and iron).

**Table 2.** Measured mean values of plant height (cm) of seashore paspalum and Tifway bermudagrass treated with different amendments during January and April in two different seasons.

Treatments	Seashore Paspalum (SP)		Tifway Bermudagrass (TB)	
	Plant Height (cm) in the First Season			
	January	April	January	April
Control (water)	2.5 c	3.1 c	2.6 b	2.9 c
Humic acid (1000 ppm)	2.6 abc	3.4 b	2.6 b	3.2 bc
Humic acid (2000 ppm)	2.7 a	3.5 b	2.6 ab	3.5 ab
Ferrous sulphate (250 ppm)	2.7 abc	3.5 ab	2.7 a	3.6 a
Ferrous sulphate (1000 ppm)	2.7 ab	3.7 a	2.7 a	3.4 ab
Silicon (3000 ppm)	2.6 abc	3.3 b	2.6 ab	3.1 bc
Silicon (6000 ppm)	2.6 abc	3.5 b	2.6 ab	3.0 bc
Diatomite (1000 ppm)	2.6 bc	3.3 b	2.6 ab	3.3 ab
Diatomite (2000 ppm)	2.6 abc	3.4 b	2.6 ab	3.4 ab
	Plant height (cm) in the second season			
Control (water)	3.0 b	4.6 d	3.0 c	4.1 d
Humic acid (1000 ppm)	3.3 ab	4.7 d	3.2 bc	4.8 bc
Humic acid (2000 ppm)	3.2 ab	5.1 cd	3.3 ab	4.9 bc
Ferrous sulphate (250 ppm)	3.4 ab	6.0 b	3.5 a	5.6 a
Ferrous sulphate (1000 ppm)	3.6 a	6.7 a	3.5 a	5.4 ab
Silicon (3000 ppm)	3.3 ab	5.1 cd	3.4 abc	5.0 b
Silicon (6000 ppm)	3.5 ab	5.7 bc	3.4 bc	5.1 ab
Diatomite (1000 ppm)	3.3 ab	5.5 bc	3.3 abc	4.3 cd
Diatomite (2000 ppm)	3.4 ab	5.6 bc	3.3 abc	5.0 ab

Means within each column that have the same letters are not significantly different from one another according to the Duncan's Multiple Range Test (at  $p < 0.05$ ).

**Table 3.** Number of roots and branches of both species treated with different amendments during the studied seasons (area used for measuring was 100 cm<sup>2</sup>).

Treatments	Seashore Paspalum (SP)		Tifway Bermudagrass (TB)	
	No. of Roots	No. of Branches	No. of Roots	No. of Branches
	First season			
Control (water)	53 i	28 i	17 g	20 h
Humic acid (1000 ppm)	97 c	65 g	35 d	40 d
Humic acid (2000 ppm)	106 b	115 a	38 c	43 c
Ferrous sulphate (250 ppm)	119 a	103 b	41 b	46 b
Ferrous sulphate (1000 ppm)	87 e	80 e	40 a	49 a
Silicon (3000 ppm)	61 h	49 h	29 f	31 g
Silicon (6000 ppm)	93 d	88 d	33 e	36 e
Diatomite (1000 ppm)	80 f	72 f	30 f	35 e
Diatomite (2000 ppm)	78 g	95 c	29 f	33 f
	Second season			
Control (water)	60 i	30 h	21 h	23 i
Humic acid (1000 ppm)	106 e	72 f	37 d	45 d
Humic acid (2000 ppm)	125 c	117 a	42 c	49 c
Ferrous sulphate (250 ppm)	174 a	111 b	47 b	55 b
Ferrous sulphate (1000 ppm)	149 b	103 d	58 a	64 a
Silicon (3000 ppm)	140 h	54 g	28 g	33 h
Silicon (6000 ppm)	113 d	107 c	35 e	41 e
Diatomite (1000 ppm)	91 g	80 e	34 e	37 f
Diatomite (2000 ppm)	95 f	110 b	32 f	35 g

Means within each column that have the same letters are not significantly different from one another according to the Duncan's Multiple Range Test (at  $p < 0.05$ ).

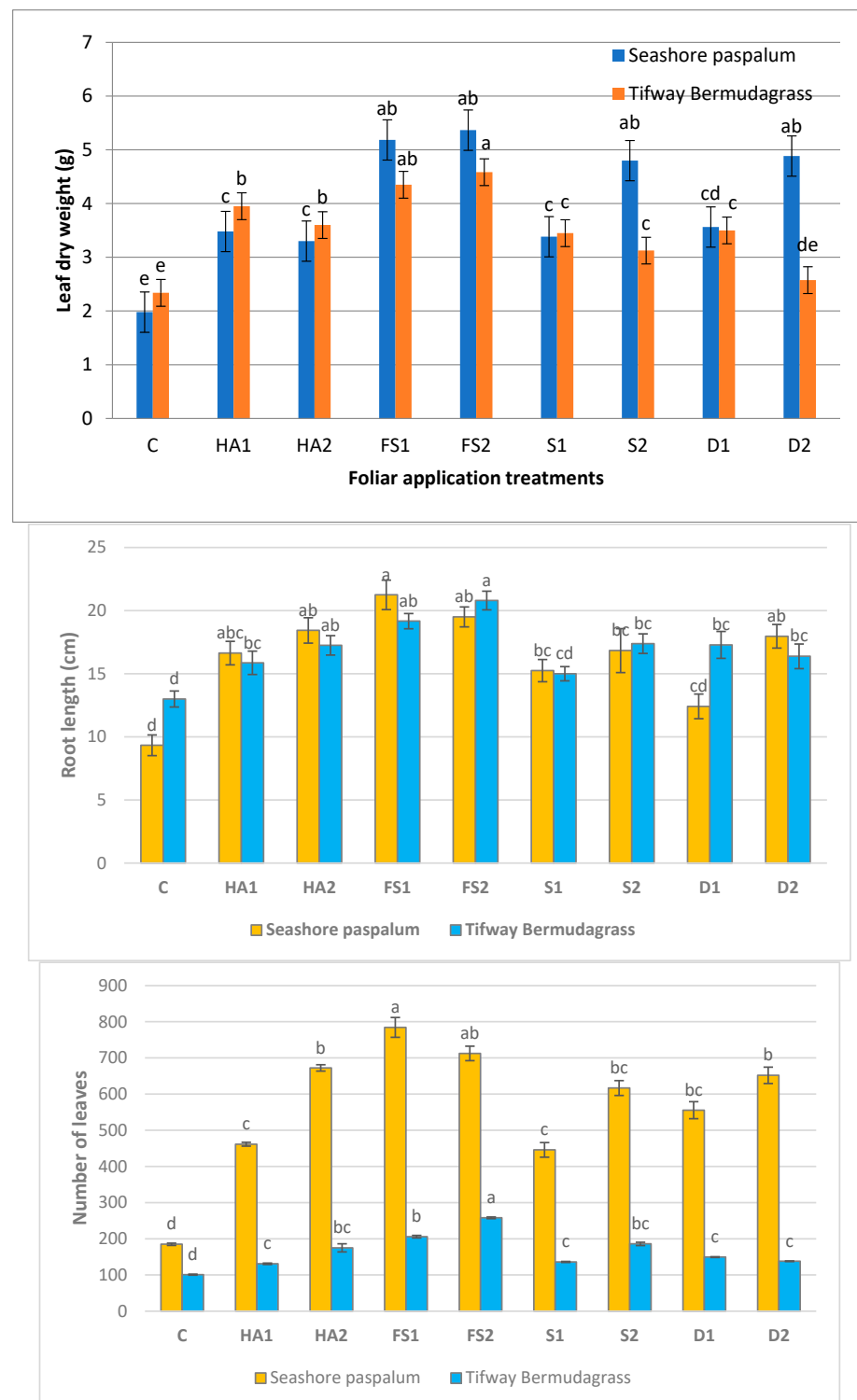
The most striking result to emerge from the data in Figure 3 (which presents values as an arithmetic mean of the two seasons) is that the mean values of the studied parameters of SP, including the number of leaves, root length, and dry weight of leaves, were higher than those of TB. Concerning the number of leaves, there were significant differences between the studied species for all the amendments that were studied. Compared to other amendments, the highest mean values of leaves were obtained after applying iron at 250 ppm, with the values obtained (near to 800 leaves per 100 cm<sup>2</sup>) being about four-fold greater than those of the control treatment. All amendments increased the root length compared to the control treatment. Although ferrous sulphate (both doses) produced the longest roots (about 22 cm) in both species, SP had the highest values, especially after humic or iron treatments were applied. Compared to the control treatment, the dry weight of leaves increased after applying each of the studied organic and inorganic amendments; the mean values of SP were higher compared to the other species. The inorganic ferrous sulphate still gave us the highest values (more than 5.2 g) of dry weight of leaves per 100 cm<sup>2</sup>, which represents more than a 2.5-fold increase compared to the control treatment.

In both species, the highest numbers of leaves were retrieved with the application of ferrous sulphate. For SP, the highest numbers of branches (115 and 117 per 100 cm<sup>2</sup> in each season, respectively) were found for humic acid at 2000 ppm, followed by ferrous sulphate at 250 ppm. For TB, the highest numbers of branches (49 and 64 branches per 10 cm<sup>2</sup> in each season, respectively) were recorded after applying ferrous sulphate at 1000 ppm, followed by the same treatment at a dose of 250 ppm. In general, the mean values of number of roots of SP were some folds higher than the values of other species (TB), with a similar trend being observed for the number of branches (Table 3).

### 3.2. Applied Amendments and Turf Quality Parameters

Three different parameters of turf quality were evaluated: turf color, turf density, and turf uniformity. In terms of leaf color, all amendments resulted in darker green leaves than the control treatment, with the darkest colors being produced by ferrous sulphate, followed by humic acid. Visual scoring of the turf density showed the same pattern as described above, with the iron amendment resulting in the highest values. In general, the mean values of turf color and turf density of SP were higher than those of the other species, whereas the mean values of turf uniformity in both seasons were similar for both species

(Table 4). In both grasses and for both seasons, all treatments resulted in significantly higher turf quality (i.e., color, density, and uniformity) scores compared to the control treatment.



**Figure 3.** Mean values of leaf dry weight (g), root length, and number of leaves measured in January for the two species treated with different amendments obtained from combining the data from two seasons. C = control, HA = humic acid, FS = ferrous sulphate, S = silica, D = diatomite. For more details about the treatments used, please refer to Table 1. Values are presented as mean  $\pm$  standard error of the mean, with the letter(s) from the species-wise Duncan's multiple range tests indicated at the top of each bar. The area used for measuring the parameters was 100 cm<sup>2</sup>.



**Table 4.** Visual turf color score, turf density, and turf uniformity in seashore paspalum and Tifway bermudagrass treated with different amendments (area used for measuring was 100 cm<sup>2</sup>).

Treatments	Seashore Paspalum (SP)			Tifway Bermudagrass (TB)		
	Turf Color	Turf Density	Turf Uniformity	Turf Color	Turf Density	Turf Uniformity
	First season					
Control (water)	1.0 d	1.0 c	1.0 b	1.0 c	1.0 b	1.0 b
Humic acid (1000 ppm)	5.3 ab	3.0 a	2.0 a	5.0 ab	2.7 a	2.0 a
Humic acid (2000 ppm)	6.0 a	3.0 a	2.0 a	5.3 a	2.7 a	2.0 a
Ferrous sulphate (250 ppm)	6.0 a	3.0 a	2.0 a	5.0 ab	2.7 a	2.0 a
Ferrous sulphate (1000 ppm)	5.0 ab	2.7 ab	2.0 a	5.0 ab	2.7 a	2.0 a
Silicon (3000 ppm)	4.0 bc	2.3 ab	2.0 a	4.0 ab	2.3 a	2.0 a
Silicon (6000 ppm)	4.3 bc	2.3 ab	2.0 a	4.3 ab	2.3 a	2.0 a
Diatomite (1000 ppm)	3.3 c	2.0 b	2.0 a	4.0 ab	2.3 a	2.0 a
Diatomite (2000 ppm)	4.3 bc	2.3 ab	2.0 a	3.0 b	1.7 ab	2.0 a
	Second season					
Control (water)	1.0 d	1.0 c	1.0 b	1.0 b	1.0 b	1.0 b
Humic acid (1000 ppm)	5.7 a	3.0 a	2.0 a	5.0 a	2.7 a	2.0 a
Humic acid (2000 ppm)	5.7 a	3.0 a	2.0 a	5.3 a	2.7 a	2.0 a
Ferrous sulphate (250 ppm)	5.7 a	3.0 a	2.0 a	5.3 a	2.7 a	2.0 a
Ferrous sulphate (1000 ppm)	5.3 ab	3.0 a	2.0 a	5.0 a	2.7 a	2.0 a
Silicon (3000 ppm)	4.3 abc	2.3 b	2.0 a	4.3 a	2.7 a	2.0 a
Silicon (6000 ppm)	4.3 abc	2.3 b	2.0 a	4.3 a	2.3 a	2.0 a
Diatomite (1000 ppm)	3.7 c	2.0 b	2.0 a	4.3 a	2.3 a	2.0 a
Diatomite (2000 ppm)	4.0 bc	2.3 b	2.0 a	4.7 a	2.3 a	2.0 a

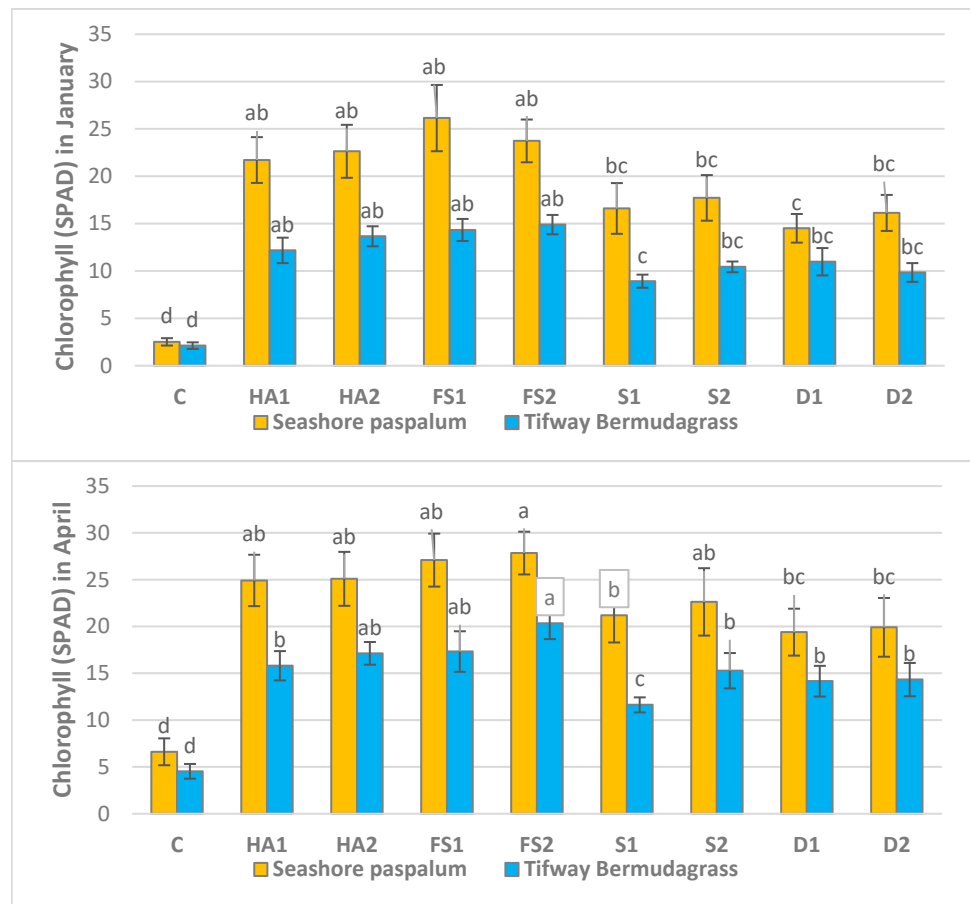
Means within each column that have the same letters are not significantly different from one another according to the Duncan's Multiple Range Test (at  $p < 0.05$ ).

### 3.3. Applied Amendments and Chlorophyll Content

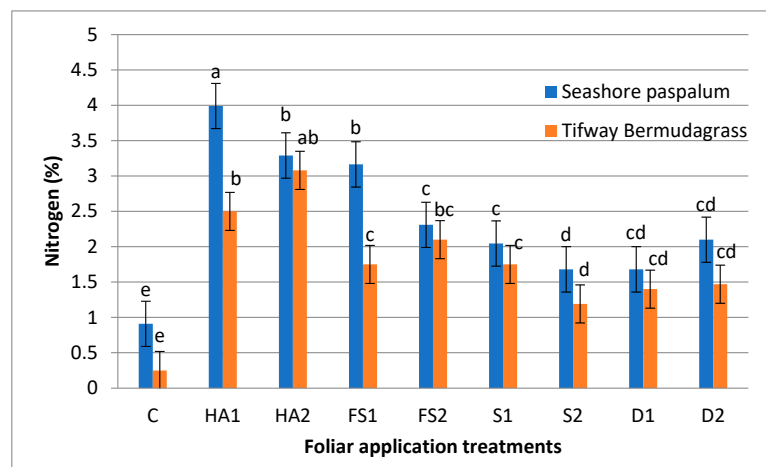
Foliar application of the amendments significantly increased the leaf total chlorophyll content (measured as SPAD values) in the two turfgrass species compared to the control treatment (Figure 4; values given as a mean of the two seasons) during January and April. In the case of the January measurements, both applied doses of ferrous sulphate gave the highest values for SP (27 and 23 for each dose, respectively). For TB, ferrous sulphate at 1000 ppm (mean of both seasons of 15) produced the highest values, followed by 250 ppm ferrous sulphate. In the case of April, all measured values were higher compared to the values in January for all the treatments (including the control). All the measured values of SP were higher in both January and April compared to the other species (TB). Dolomite treatments resulted in the lowest mean values of SPAD in both species, but these values were still higher than those of the control treatment. For both species, applying ferrous sulphate at 1000 ppm produced the highest values of SPAD for April, followed by ferrous sulphate at a dose of 250 ppm.

### 3.4. Applied Amendments and Chemical Composition of Leaves

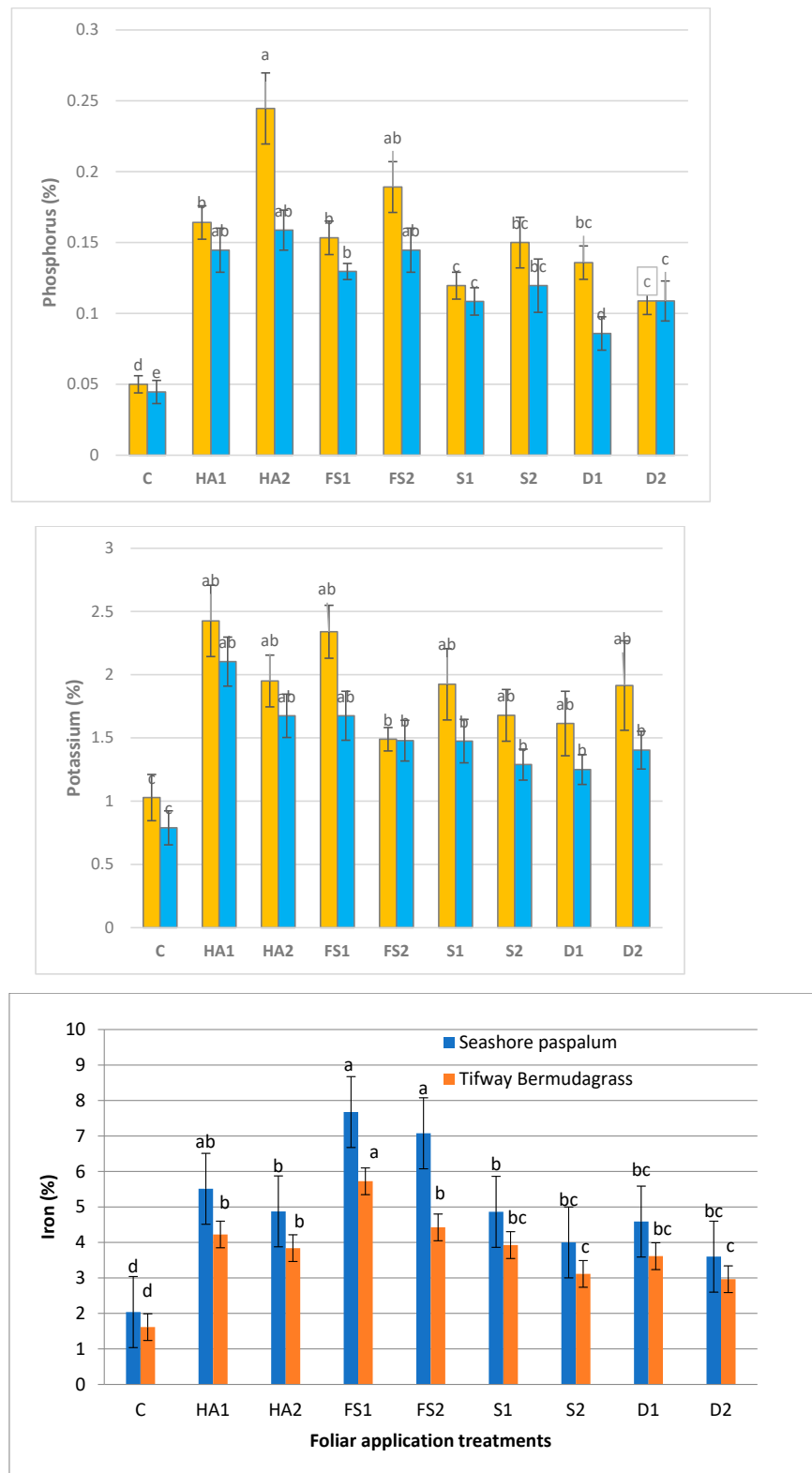
The data illustrated in Figure 5 show that the applied amendments significantly increased the content of nitrogen and other mineral nutrients in the two turf grasses. The mean values of all the studied nutrients (i.e., N, P, K, and Fe) were higher for SP compared to the other plant species for all treatments including the control. In general, for all the studied nutrients, the application of iron sources and humic acid led to the highest leaf nutrient contents in both species. For SP, the highest mean values of nutrient content of N, P, K, and Fe were 4, 0.24, 2.4%, and 7.6 mg kg<sup>-1</sup>, respectively. All these values resulted from the application of humic acid or iron sources, and the same trend was also noticed for TB. After the control treatment, application of silicon sources produced the lowest mean values of nutrients contents.



**Figure 4.** Mean values of chlorophyll content in leaves in both January and April (SPAD value  $\pm$  SE) for seashore paspalum and Tifway bermudagrass treated with different amendments; values represent a mean of the two seasons. C = control, HA = humic acid, FS = ferrous sulphate, S = silica, D = diatomite. For more details about the treatments, please refer to Table 1. Data are presented as mean  $\pm$  standard error of the mean, with the letter(s) from the species-wise Duncan’s multiple range tests indicated at the top of each bar.



**Figure 5.** Cont.



**Figure 5.** Mean leaf content of nitrogen (%), phosphorus (%), potassium (%) and iron ( $\text{mg kg}^{-1}$ )  $\pm$  SE in seashore paspalum (orange bars) and Tifway bermudagrass (blue bars) treated with different amendments; values represent a mean of the two seasons. C = control, HA = humic acid, FS = ferrous sulphate, S = silica, D = diatomite. For more details about the treatments, please refer to Table 1. Data are presented as mean value  $\pm$  standard error of the mean, with the letter(s) from the species-wise Duncan’s multiple range tests indicated above each bar.

#### 4. Discussion

In this section, it is important to answer our previous questions, which we added as the main objectives of the current study:

Which turfgrass is more tolerant to combined cold and salinity stress? In the present study, the main foliar treatments or amendments applied on two turfgrass species (i.e., SP and TB) under combined cold and soil salinity stress included humic acid, ferrous sulphate, and two sources of silicon. All amendments were applied during the period from October to December, in two successive seasons, after which the plant samples were taken for physiological and chemical analyses. The turfgrass of SP was more tolerant to both cold and salinity stress compared to TB, as shown in all the studied vegetative parameters, namely, the leaf chlorophyll content, chemical composition of plant leaves, and quality characteristics (Tables 1–4, and Figures 1–3). This might be linked to the ability of this tolerant grass to grow under many environmental stresses, especially salinity stress [36], which may cause problems such as winterkill for bermudagrasses [37,38].

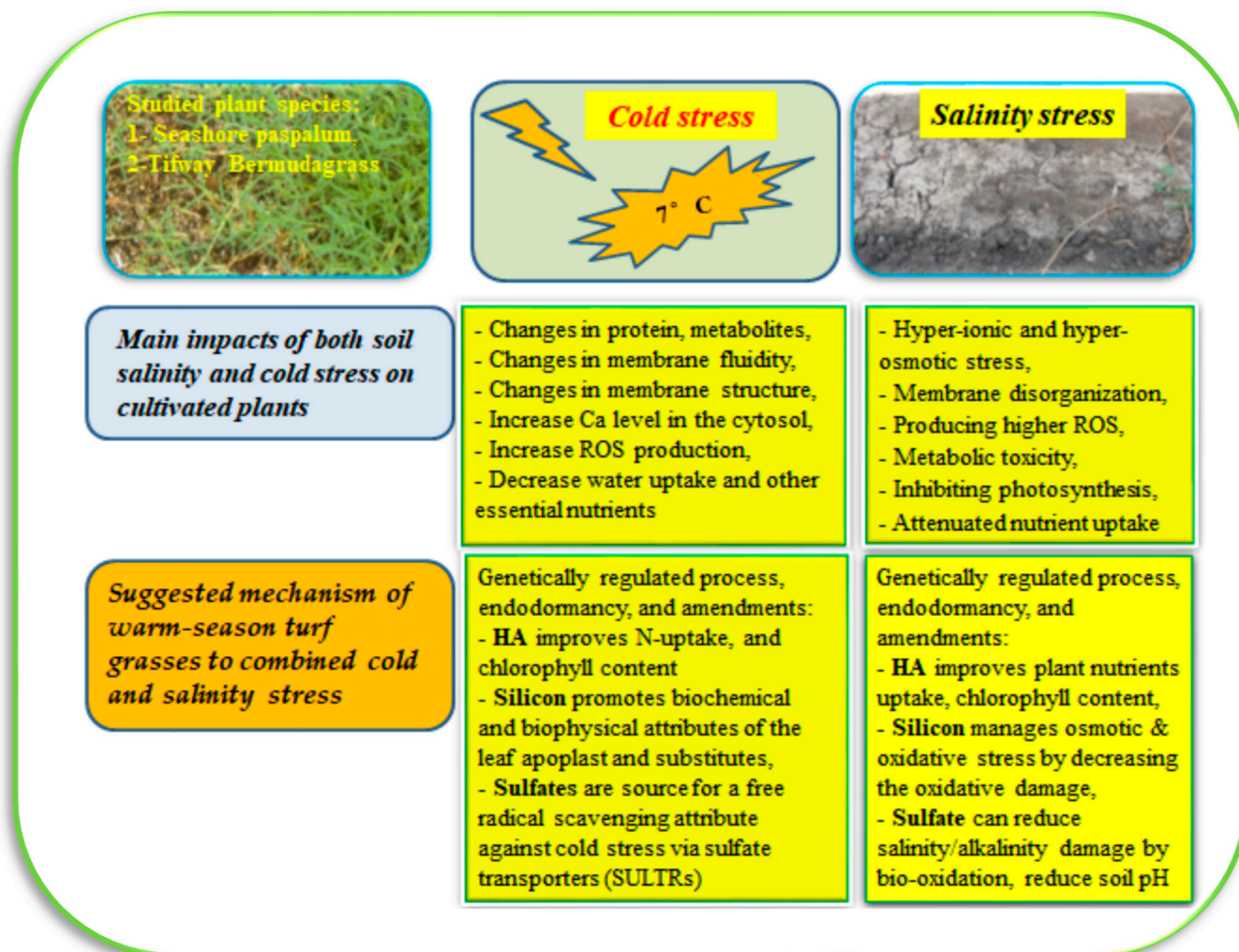
Which applied amendment is more effective in mitigating these previous stresses on the studied turfgrasses? In general, one could order the effectiveness of the applied amendments as follows: iron sources (ferrous sulphate in 250 and 1000 ppm), then humic acid, and finally, sources of silicon. For example, ferrous sulphate produced the highest mean values of N and Fe content in leaves, chlorophyll content, quality parameters and almost all the vegetative characteristics. What is the role of exogenous iron under turfgrass stress? Iron has distinct functions in different plant physiological processes like photosynthesis because it is essential for many enzymes involved in chlorophyll biosynthesis [39]. On the other hand, as a source of sulfate, ferrous sulphate reaches the cultivated soil via sprinkler irrigation. Once there, the sulfate can react with the  $\text{Na}^+$  and  $\text{Cl}^-$  ions present in saline/alkaline soil to sustain optimal  $\text{Ca}^{2+}/\text{Na}^+$  and  $\text{K}^+/\text{Na}^+$  ratios, leading to a reduction in soil pH and saving of nutrients for plant uptake [40]. This supporting role in nutrient uptake can indirectly enhance plant photosynthesis and other physiological attributes [40]. Under cold temperatures, iron fertilization has been shown to enhance recovery from winter desiccation injury in creeping bentgrass (*Agrostis palustris* L.). However, the amount of iron needed within plants can vary according to the local soil and plant conditions, and the response to iron fertilization can differ among species and varieties [41]. To the best of our knowledge, currently only one study on the growth of turfgrasses under cold and salinity stress has been published [42], and there are no publications on the effect of different amendments under these combined stresses. What is the main role of humic acid under plant stress? Humic acid can enhance phosphorus bioavailability and increase the availability of micronutrients and may also act as a plant growth stimulator by forming organo-iron complexes [43]. Similar results have been obtained in tall fescue (*Festuca arundinaceae* Schreb.) and creeping bentgrass [44].

Which source and dose of silicon is the best to ameliorate the growth and quality of the studied turfgrasses? Why did silicon treatments produce lower values compared to other treatments? In general, treatment with sources of silicon had a positive impact on the turfgrasses compared to the control treatment, but produced lower mean values compared to ferrous sulphate and humic acid. Some studies have shown beneficial effects of silicon (or silica) when it is used as a plant promotor against abiotic/biotic stresses such as salinity and cold stress, but the current results showed lower mean values with silicon compared to the other treatments. This could be because the higher applied doses of silicon used in this study might have had toxic effects on the turfgrasses. Concerning the applied doses of silicon for other crops, plant growth was promoted by applying 200 ppm under conditions of combined soil salinity and heat stress for cucumbers [45], 50–200  $\text{kg ha}^{-1}$  under salinity and drought stress for wheat [46], and 2000 to 4000 ppm under water deficit for squash [47]. The suggested mechanism for enhancing cucumber salt tolerance using silicon may involve increasing the accumulation of polyamine, enhancing antioxidants, and managing osmotic and oxidative stress to decrease the level of oxidative damage [48–50]. In our study, there was no clear trend regarding the use of different sources of silicon for all the measured

parameters. Thus, both the source of silicon and the applied dose that can best ameliorate the growth and quality of the studied turfgrasses are not clear. The applied Si-sources had different effects on the studied parameters, resulting in significantly higher values for many of the vegetative growth attributes, but no significant trend was observed in the case of turf quality. Silicon is considered to be a beneficial or quasi-essential element that can impact the plant–soil system by both protecting plants against biotic/abiotic stresses and optimizing soil fertility by improving the soil water status and maintaining the availability of nutrients to plants [20]. For the nutrient content, there was no clear difference between the silicon and diatomite treatments. Although many studies on the impact of different sources of silicon on some cultivated plants under stress, such as canola under water deficit [51], wheat under water deficit [52], and feverfew under drought [53], have been published, studies on turfgrass are rare (Figure 6).

Thus far, only a few studies have attempted to explain the production of warm-season turfgrasses under combined stresses. In one study, Liu et al confirmed that exposing turfgrasses to combined cold and salinity stress may cause more severe damage compared to growing turfgrasses under other conditions [42]. The results obtained in this study are in agreement with those of Chavarria et al. [49], who confirmed that turfgrass species and their cultivars differ in their tolerance to salinity stress. Concerning the role of humic acid under conditions of combined stress, its positive effects may be related to the enhancement of various antioxidants and subsequent scavenging of superoxide anions in the leaves, which protects the plant cell from damage caused by reactive oxygen and free radicals that are formed under stress. Our results are in agreement with the results of Abdel Fatah et al. [50], who concluded that the application of humic acid increased the content of nitrogen, phosphorus, and potassium in the leaves of bermudagrass. In the current study, it seems that treatment with iron sources not only increased the iron content in the leaves, but also increased the content of other nutrients. The stimulatory effects of humic acid on the nitrogen, phosphorus, and potassium contents of leaves have been observed in other studies. In addition to enhancing the bioavailability of phosphorus, humic acid has also been shown to increase both the availability of micronutrients and the phosphorus content of leaves [54,55] and improve nutrient uptake by plants.

After presenting the suggested mechanisms in Figure 6, it is clear that the selected organic and inorganic amendments have different modes of action. The specific mode of action for each amendment depends on how the amendment can contribute to tolerance against combined cold and salinity stress in cultivated plants. These differences between the studied amendments may lead to several questions including the following one: which applied dose of each studied amendment can be used? Currently, more studies under salt-affected soils are needed before the recommended dose of each applied amendment can be determined, this is especially true for treatments that use sources of silicon. The interaction between these different amendments also requires further investigation, including additional research using different soil application methods and under salt-affected conditions.



**Figure 6.** An overview of the suggested main damage to studied plants resulting from both cold and soil salinity and the mechanisms suggested to contribute to tolerance of these stresses (sources: Pompeiano et al. [56], Prokopiuk et al. [57], Sharma et al. [58] Bello et al. [40], Hajiboland et al. [59], Nguyen et al. [60]).

## 5. Conclusions

This research has shown that the foliar application of humic acid, ferrous sulphate, and to some extent, silicon, on warm season turfgrasses (i.e., SP and TB) is beneficial for the turf growth and quality during the cold season in salt-affected soils. Ferrous sulphate and humic acid were the best options and worked at both applied doses. From an economic point of view, lower doses might be sufficient, but further research is needed to fine-tune the application regimes. Under the studied conditions, seashore paspalum had the highest values of all parameters of growth and quality compared to Tifway bermudagrass, which was due to its tolerance to salinity stress. A lot of well-defined evidence, which is clearly reflected in all the tables and figures, was collected in this study. Such evidence verified that seashore paspalum is superior to Tifway bermudagrass in many studied measurement (e.g., chlorophyll content, turf quality, nutrient contents, and vegetative parameters). The most striking result is that in both turfgrasses, the effect of silicon was lower than that of humic acid and ferro sulphate; this was due to the use of high applied doses, which may have negatively impacted plant growth. This study offers some important insights into the cultivation of warm-season turfgrasses under combined cold and soil salinity stress, which we have shown can be regulated by increasing nutrient uptake, chlorophyll content

in leaves, and turf quality. More studies using lower applied doses of silicon sources and different combinations of applied amendments are needed.

**Author Contributions:** Conceptualization, E.N., M.H., M.A.E.-G. and H.E.-R.; methodology D.T., E.N., M.H., M.A.E.-G. and H.E.-R.; investigation, D.T.; data curation, D.T.; writing—original draft preparation, D.T.; writing—review and editing, S.Ø.S.; visualization, D.T. and S.Ø.S.; supervision, E.N., M.H., M.A.E.-G. and H.E.-R. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Data Availability Statement:** Not applicable.

**Acknowledgments:** We would like to thank the institutions involved for providing administrative and technical support needed to conduct the field trials and analysis.

**Conflicts of Interest:** The authors declare no conflict of interest.

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